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FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

TRACKING THE ACQUISITION OF EATING HABITS IN CHILDREN AND ITS EFFECTS ON BEHAVIOURS RELATED TO APPETITE AND ON ADIPOSITY

ANA SOFIA MOURÃO VILELA
TESE DE DOUTORAMENTO EM
SAÚDE PÚBLICA APRESENTADA
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Tracking the acquisition of eating habits in children and its effects on behaviours related to appetite and on adiposity

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“Education is not the learning of facts, but the training of the mind to think. Anyone who has never made a mistake has never tried anything new”

“The important thing is never stop questioning”

Albert Einsten

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I hereby declare that I have actively contribute to the definition and operationalization of the hypothesis tested in the paper, in addition to the data analysis and interpretation. I have participated in the process of handling data and checking databases related to dietary intake in G21, and I have participated in the development of the project IAN-AF, along with the training of the interviewers. I wrote the first draft of all manuscripts included, with the exception of the paper III (in which I have the role of supervisor) and actively participated in the preparation of the final versions.

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Abstract

Background

Specific dietary habits acquired during childhood can have a relevant impact on immediate and later children's health. An understanding of the stability or tracking of children's eating habits is crucial for formulating policy and interventions to improve nutrition-related health outcomes such as obesity. Research on the influence of early eating habits on appetite-related eating behaviours in a prospective approach is scarce, especially in children. The examination of specific characteristics of eating habits (e.g. meal patterns and diet variety) and its influence on eating behaviours will provide insights on the establishment of appetite-related eating behaviours. In addition, the consideration of different approaches, such as time-of-day energy and macronutrient intake, to evaluate the influence of eating habits on adiposity is scarce in literature and requires more in-deep research.

Objectives

The main aim of this thesis was to assess the tracking of eating habits from preschool to school age and its effects on eating behaviours related to appetite and weight status at 7 years of age. The following specific objectives were defined:

I. To test and calibrate a non-quantitative food frequency questionnaire in 4 and 7-year-old children;
II. To evaluate the tracking of dietary intake in childhood and its effects on diet quality;
III. To estimate the influence of early life factors (pregnancy characteristics and neonatal/infancy characteristics) and socio-economic characteristics on tracking of diet quality during childhood;
IV. To evaluate the effect of tracking diet variety from 4 to 7 years of age on eating behaviours related to appetite at 7 years of age;
V. To assess the effect of frequency and type of meals at 4 years on eating behaviours related to appetite and weight status at 7 years of age;
VI. To assess the effect of time-of-day energy and macronutrients intake at 4 years on eating behaviours related to appetite and weight status at 7 years of age.

Participants and Methods

This thesis includes data from the previously assembled birth cohort Generation XXI (G21) of 8647 children born in Porto in 2005/2006 and from the most recent National Food, Nutrition and Physical Activity Survey of the Portuguese general population (IAN-AF 2015-2016).

Regarding G21 children, at 2 years of age, a subsample of 855 children was re-evaluated, 705 of whom were also evaluated 2 years later. At 4 and 7 years of age a reevaluation of the entire cohort was performed, with a participation rate of 86% and 80%, respectively. Similar procedures were adopted at baseline and at all follow-up assessments. Data were collected, in face-to-face interviews, conducted by trained interviewers using structured questionnaires that gathered information on sociodemographic, clinical and behavioural characteristics. Anthropometric evaluation was also performed by the trained examiners.

In the IAN-AF, data were collected in two interviews (time interval was set at 8 to 15 days) by trained interviewers using Computer-Assisted Personal Interviewing, during 12 months distributed over the four seasons and including all days of the week. A total of 5811 participants aged between 3 months and 85 years completed two interviews and 6553 completed only the first one. From the total children (<10 years), 1329 had complete data for the two face-to-face interviews. For the present thesis, we included data from children aged 3-9 years of age (n=604).

Children's dietary intake was evaluated using a food frequency questionnaire (FFQ) in G21 and food diaries in both G21 and IAN-AF. The tracking of children's eating habits was performed using different approaches: specific food groups, *a priori* dietary patterns to defined diet quality and diet variety, and eating frequency based on different definitions described in the literature. A *posteriori* meal patterns of time-of-day energy and macronutrient intake identified by principal component analysis were also assessed.

The studied outcomes were children's eating behaviours related to appetite and weight status. Children's eating behaviours related to appetite was assessed through a parental report psychometric instrument previously validated in Portugal: the Children's Eating Behaviour Questionnaire (CEBQ). Eight sub-dimension of the scale were included: 'Food Responsiveness', 'Enjoyment of Food', 'Emotional Overeating' and 'Desire to Drink', as food-approach appetitive traits, and 'Satiety Responsiveness', 'Slowness Eating', 'Emotional Undereating' and 'Food Fussiness' as food-avoidance appetitive traits.

Children's weight status was assessed through objective measures of weight and height and subsequent calculation of age- and sex-specific body mass index (BMI) standard deviation scores defined according to the World Health Organization.

Results

In comparison with 3-day food diaries, and after a calibration process, the non-quantitative FFQ used to evaluate dietary intake among 4 and 7-year-old children performed reasonably well and seems to be a useful instrument for assessing a wide range of food groups and key nutrient intake in these children [paper I]. The consumption of energy-dense foods (EDF) increased from 2 to 4 years of age and a higher consumption at 2 years was associated with a higher consumption at 4 years. An inverse association was found between higher intake of EDF and later fruit and vegetables (F&V) consumption, but not with other foods groups. Weekly and daily consumption of EDF at 2y was associated with a lower healthy eating score at 4y (incidence rate ratio (IRR)=0.75, 95% CI: 0.58; 0.96 and IRR=0.56, 95% CI: 0.41; 0.77, respectively) [paper II]. From 4 to 7y, some negative changes occurred, namely, an increase of sweets and soft drinks intake, and a decrease in consumption of dairy. After adjustment for potential confounders, higher scores at 4y influenced positively diet quality at 7y (β=0.329, 95% CI: 0.295; 0.363). Higher maternal education (β=0.044, 95% CI: 0.016; 0.071) and higher maternal dietary score (β=0.175, 95%CI: 0.143; 0.207) were positively and independently associated with a higher adherence to the healthy eating index score at 7y. No

significant associations were found between early life factors, namely pregnancy characteristics, child's birth weight and duration of breastfeeding and tracking of diet quality between 4 and 7 years [paper III].

From 4 to 7 years, a more varied diet predicted a fair to moderate tracking, with a high tracking for F&V. Compared to maintaining a low variety, increasing diet variety was inversely associated with the 'Desire to Drink' (β=–0.090, 95%CI: –0.174; –0.006) and 'Satiety Responsiveness' (β=–0.119, 95%CI: –0.184; –0.054) subdimensions and positively with 'Enjoyment of Food' (β=0.098, 95%CI:0.023; 0.172) and 'Emotional Overeating' (β=0.073, 95%CI:0.006; 0.139). Those classified as either increasing or maintaining a high diet variety, in comparison with maintaining a low variety, had lower scores of 'Food Fussiness' [paper IV].

The tracking of eating occasion frequency (main meals and snacks) between 4 and 7 years was moderate and statistically significant. Using a prospective analysis, a bidirectional relationship was described between eating frequency and children's eating behaviours related to appetite. The stronger association found was between snack frequency at 4 years and the eating behaviours at 7 years of age. A higher frequency of snacks at 4y was independently associated with higher scores in 'Desire to Drink' at 7y (β=0.05, 95%CI: 0.00; 0.09). Cross-sectionally, a higher number of main meals had a positive association with 'Enjoyment of Food' and 'Food Responsiveness', while, a higher number of snacks had a positive association with 'Satiety Responsiveness' and 'Slowness in Eating', but an inverse association with 'Enjoyment of Food' and 'Food Responsiveness' [paper V]. In Portuguese children aged 3 to 9-years, after adjustment for potential confounders, and considering only plausible reporters of energy intake, having less than three snacks per day (OR=1.98, 95% CI: 1.00; 3.90) was positively associated with being overweight or obese [paper VI].

A higher intake of carbohydrates at the main meals but lower at mid-afternoon at 4y was associated with lower scores in 'Food Fussiness' and 'Satiety Responsiveness', but higher scores in 'Enjoyment of Food' at 7y. Additionally, a higher intake of energy, protein and fat at lunch and dinner was positively associated with 'Enjoyment of Food'. Having a higher energy intake at lunch and supper (but not at dinner) (OR=1.19, 95%CI: 1.05; 1.34) or at mid-afternoon (OR=1.18, 95% CI: 1.05; 1.34) at 4y was associated with higher odds of developing overweight or obesity at 7y. A higher intake of fat at lunch was positively associated with later children's BMI (OR=1.17, 95%CI: 1.03; 1.32). These associations were independent of the effect on children's eating behaviours related to appetite [paper VII].

Main conclusions

Using different approaches, we found some level of stability of eating habits throughout childhood, however, there is still scope for behavioural change during this period of life. Tracking of diet quality and variety can be considered positive, whereas, tracking of less healthy eating habits, such as energy-dense foods should be targeted to interventions. Better socioeconomic circumstances, as well as healthy maternal behaviours, ensure the establishment of healthy eating habits since early ages and their maintenance

throughout childhood. From 4 to 7 years of age, maintain or increase a high varied diet, higher eating frequency, and a higher proportion of energy and macronutrients intake at the main meals, were associated with healthier eating behaviours related to appetite, namely lower scores in desire to drink, food fussiness and higher scores in enjoyment of food. Moreover, a lower daily frequency of snacks was positively associated with overweight and obesity. The beneficial effect of a higher intake of energy and macronutrients at main meals on children's weight status seems to be independent of the effects on appetite control.

Strategies for the establishment of healthy eating habits in childhood should be implemented early in life, such as a high variety of healthy foods, a higher number of daily meals (maintaining the energy intake stable) and a higher distribution of energy and macronutrients at the main meals, to ensure healthy eating behaviours and a healthy body weight on the long term.

Resumo

Introdução

Determinados hábitos alimentares adquiridos na infância podem ter um relevante impacto na saúde das crianças a curto e a longo prazo. Uma melhor compreensão da estabilidade ou manutenção destes hábitos ao longo da infância é crucial para a formulação de políticas e intervenções orientadas para a prevenção de problemas de saúde relacionados com o estado nutricional, nomeadamente a obesidade. É escassa a investigação sobre a influência de hábitos alimentares precoces nos comportamentos alimentares relacionados com apetite usando uma abordagem prospetiva, especialmente em população pediátrica. O estudo de características específicas de hábitos alimentares (ex. padrão das refeições e variedade alimentar) e a sua associação com comportamentos alimentares fornecerá evidência sobre o desenvolvimento de comportamentos alimentares relacionados com o apetite. Além disso, o uso de diferentes abordagens para avaliar a influência dos hábitos alimentares, como a distribuição da ingestão energética e de macronutrientes ao longo do dia, no desenvolvimento da adiposidade é insuficiente na literatura e requer investigação mais aprofundada.

Objetivos

O objetivo principal da presente tese foi avaliar a estabilidade/manutenção de hábitos alimentares entre a idade pré-escolar e a idade escolar e os seus efeitos nos comportamentos alimentares relacionados com o apetite e no peso corporal aos 7 anos de idade. Os seguintes objetivos específicos foram definidos:

I. Testar e calibrar um questionário de frequência alimentar não-quantitativo aos 4 e 7 anos de idade;
II. Avaliar a estabilidade do consumo alimentar e a sua associação com a qualidade alimentar;
III. Estudar a influência de fatores precoces na vida (características da gravidez, neonatais e primeira infância) e características socioeconómicas na estabilidade da qualidade alimentar durante a infância;
IV. Avaliar o efeito da estabilidade da variedade alimentar dos 4 para os 7 anos nos comportamentos alimentares relacionados com o apetite aos 7 anos de idade;
V. Estimar a associação entre a frequência e o tipo de refeições diárias aos 4 anos de idade e os comportamentos alimentares relacionados com o apetite e a adiposidade aos 7 anos;
VI. Avaliar o efeito da distribuição da ingestão energética e de macronutrientes ao longo do dia aos 4 anos nos comportamentos alimentares relacionados com o apetite e a adiposidade aos 7 anos;

Participantes e Métodos

Esta tese utilizou informação recolhida no âmbito da coorte de nascimento Geração XXI (G21), previamente estabelecida e constituída por 8647 crianças nascidas no Porto em 2005/2006, e do mais recente Inquérito Alimentar Nacional e de Atividade Física (IAN-AF 2015- 2016).

Em relação ao projeto G21, aos 2 anos de idade, uma subamostra de 855 crianças foi reavaliada, das quais 705 também foram avaliadas 2 anos depois. Aos 4 e 7 anos de idade, foi realizada uma reavaliação de toda a coorte, com uma proporção de participação de 86% e 80%, respetivamente. Procedimentos similares foram adotados para todas as avaliações. A informação foi recolhida através de entrevistas presenciais, realizadas por entrevistadores treinados usando questionários estruturados sobre características sociodemográficas, clínicas e comportamentais. A avaliação antropométrica também foi realizada por examinadores treinados.

No estudo IAN-AF, a informação foi recolhida através de duas entrevistas (intervalo de tempo entre 8 a 15 dias) durante 12 meses, distribuídos pelas quatro estações do ano e incluindo todos os dias da semana, através de entrevistadores treinados com recurso à metodologia de inserção da informação assistida por computador (CAPI). Um total de 5811 participantes entre os 3 meses e 84 anos completaram duas entrevistas e 6533 completaram apenas a primeira. Do total das crianças avaliadas (<10 anos), 1329 apresentaram informação completa nas duas entrevistas. Para a presente tese foi incluída e analisada a informação de crianças de 3 a 9 anos de idade (n=604).

O consumo alimentar das crianças foi avaliado através de um questionário de frequência alimentar (QFA) na coorte G21 e diários alimentares na G21 e IAN-AF. A avaliação da estabilidade dos hábitos alimentares das crianças foi realizada utilizando diferentes estratégias: grupos de alimentos específicos, padrões alimentares para definir qualidade e variedade da alimentação, e frequência de refeições diárias baseada em diferentes definições descritas na literatura. Os padrões de refeição foram também avaliados de acordo com a ingestão de energia e macronutrientes ao longo do dia, e identificados por análise de componentes principais.

Os desfechos (*outcomes*) estudados incluem os comportamentos alimentares relacionados com o apetite e a adiposidade na infância. Os comportamentos alimentares foram avaliados através de um instrumento psicométrico respondido pelos pais e previamente validado em Portugal: o *Questionário de Comportamento Alimentar da Criança* (CEBQ). Oito sub-dimensões da escala foram incluídas: 'Resposta à Comida', 'Prazer em Comer', 'Sobre Ingestão Emocional' e 'Desejo de Beber', como traços relacionados com uma maior ingestão alimentar, e 'Resposta à Saciedade', 'Ingestão Lenta', 'Sub Ingestão Emocional' e 'Seletividade', como traços relacionados com uma menor ingestão alimentar.

A adiposidade da criança foi avaliada através de medições objetivas de peso e altura com cálculo subsequente do Índice de Massa Corporal (IMC) baseado nos desvios-padrão específicos para sexo e idade, definidos de acordo com os critérios propostos pela Organização Mundial da Saúde.

Resultados

Em comparação com diários alimentares de 3 dias, e após um processo de calibração, o FFQ não-quantitativo usado para avaliar o consumo alimentar das crianças aos 4 e 7 anos de idade mostrou ser um instrumento útil para estimar a ingestão de um amplo número de grupos de alimentos e nutrientes chave nestas crianças [artigo I]. O consumo de alimentos de elevada densidade energética (AEDE) aumentou entre os 2 e os 4 anos e um maior consumo aos 2 anos associou-se com um maior consumo aos 4 anos. Uma associação inversa foi encontrada entre uma maior ingestão de AEDE e posterior consumo de frutas e vegetais (F&V), mas não com outros grupos de alimentos. O consumo de AEDE aos 2 anos associou-se com uma menor pontuação no índice de alimentação saudável aos 4 anos (razão de taxas de incidência (RTI)=0,75, IC 95%: 0,58; 0,96 e RTI=0,56, IC 95%: 0,41; 0,77, respetivamente para consumo semanal e diário) [artigo II]. Entre os 4 e 7 anos de idade, foram registadas mudanças negativas no consumo alimentar, nomeadamente, um aumento de doces e refrigerantes e uma diminuição no consumo de lacticínios. Após ajuste para potenciais confundidores, uma maior pontuação aos 4 anos influenciou positivamente a qualidade alimentar aos 7 anos (β=0,329, IC 95%: 0,295; 0,363). Uma maior educação materna (β=0,044, IC 95%: 0,016; 0,071) e uma maior pontuação no índice alimentar materno (β=0,175, IC 95%: 0,143; 0,207) associaram-se positivamente e independentemente com uma maior adesão da criança ao índice de alimentação saudável aos 7 anos de idade. Não foram encontradas associações significativas entre os fatores em fases precoces da vida, nomeadamente características da gravidez, peso ao nascimento e a duração da amamentação e a estabilidade da qualidade da alimentação dos 4 para os 7 anos [artigo III].

Entre os 4 e os 7 anos de idade, o consumo de uma alimentação variada mostrou ser relativamente estável, sobretudo para o consumo de F&V. Em comparação com manter uma baixa variedade, um aumento da variedade alimentar relacionou-se inversamente com as sub-dimensões 'Desejo de Beber' (β=–0,090, IC 95%: –0,1774; –0,006) e 'Resposta à Saciedade' (β = –0,199, IC 95% : –0.184; –0.054) e positivamente com 'Prazer em Comer' (β=0.098, IC 95%: 0.023; 0.172) e 'Sobre Ingestão Emocional' (β=0.073, IC 95%: 0.006; 0.139). As crianças que aumentaram ou mantiveram uma variedade alimentar alta, em comparação com manter uma variedade baixa, apresentaram menores pontuações de 'Seletividade' [artigo IV].

Entre os 4 e os 7 anos, verificou-se uma estabilidade moderada e estatisticamente significativa no número de refeições diárias (refeições principais e lanches/*snacks*). Usando uma abordagem prospetiva, uma relação bidirecional entre o número de refeições e os comportamentos alimentares relacionados com o apetite foi encontrada. A associação mais forte foi observada entre a frequência de lanches/*snacks* aos 4 anos e os comportamentos alimentares aos 7 anos de idade. Uma maior frequência de lanches aos 4 anos associou-se com maiores pontuações em 'Desejo de Beber' aos 7 anos (β=0.05, IC 95%: 0.00; 0.09). Na análise transversal, um maior número de refeições principais associou-se positivamente com 'Prazer em Comer' e 'Resposta à Comida'. Por outro lado, um maior número de lanches apresentou uma associação direta com 'Resposta à Saciedade' e 'Ingestão Lenta', mas uma associação inversa com 'Prazer em Comer' e 'Resposta à Comida' [artigos V]. Em crianças portuguesas de 3 a 9 anos de idade, após ajuste para potencial confundidores e considerando indivíduos com declarações plausíveis de ingestão energética, fazer menos de três lanches/*snacks* por dia (OR = 1,98, IC 95%: 1,00; 3,90) associou-se diretamente com ter excesso de peso ou obesidade [papel VI].

Uma ingestão superior de hidratos de carbono nas refeições principais, mas inferior a meio da tarde, aos 4 anos de idade, associou-se com menores pontuações nas sub-dimensões 'Seletividade' e 'Resposta à Saciedade', mas maiores pontuações em 'Prazer em Comer' aos 7 anos. Adicionalmente, uma maior ingestão de energia, proteína e gordura ao almoço e jantar associou-se positivamente com 'Prazer em Comer'. As crianças com uma ingestão superior de energia no almoço e na ceia (mas não no jantar) (OR=1,19, IC 95%: 1,05; 1,34) ou a meio da tarde (OR=1,18, IC 95%: 1,05; 1,34) aos 4 anos apresentaram maior probabilidade de desenvolver excesso de peso ou obesidade aos 7 anos. Uma maior ingestão de gordura ao almoço também se associou diretamente com o IMC posterior da criança (OR=1,17, IC 95%: 1,03; 1,32). Estas associações foram independentes do efeito dos comportamentos alimentares das crianças relacionados com o apetite [artigo VII].

Conclusões principais

Usando diferentes abordagens metodológicas, verificou-se algum nível de estabilidade dos hábitos alimentares durante a infância. No entanto, parece existir ainda alguma margem para a mudança comportamental durante este período de vida. A manutenção da qualidade e variedade alimentar elevada pode ser considerada positiva, enquanto a manutenção de hábitos alimentares considerados menos saudáveis, como o consumo de alimentos com elevada densidade energética, deverá ser combatido e alvo de intervenções. Um melhor ambiente socioeconómico, bem como comportamentos maternos saudáveis contribuem para o desenvolvimento de hábitos alimentares saudáveis desde idades precoces e para a sua manutenção durante a infância. Entre os 4 e os 7 anos de idade, manter ou aumentar a variedade da alimentação, uma maior frequência de refeições diárias, e uma maior proporção de ingestão de energia e macronutrientes nas refeições principais, associaram-se com comportamentos alimentares relacionados com o apetite mais saudáveis, nomeadamente, menores pontuações nas sub-dimensões 'Desejo de Beber', 'Seletividade' e maiores pontuações em 'Prazer em Comer'. Adicionalmente, uma menor frequência diária de lanches/*snacks* aumentou a probabilidade de excesso de peso e obesidade nas crianças. O efeito benéfico de uma maior ingestão de energia e macronutrientes nas principais refeições no peso das crianças parece ser independente dos efeitos no controlo do apetite.

Estratégias para o estabelecimento de hábitos alimentares saudáveis na infância, tais como aumentar a variedade de alimentos saudáveis, maior frequência de refeições diárias (mantendo estável a ingestão energética) e uma maior distribuição de energia e macronutrientes nas refeições principais, devem ser implementados desde idade precoces para assegurar melhores comportamentos alimentares e um peso corporal mais saudável a longo prazo.

Abbreviations

ALSPAC	Avon Longitudinal Study of Pregnancy and Childhood
ARC	Arcuate Nucleus
BMI	Body Mass Index
BMI z-scores	Age- and sex-specific Body Mass Index Standard Deviation Scores according to the World Health Organization
CEBQ	Children Eating Behaviour Questionnaire
DDS	Dietary Diversity Score
eAT24	Electronic Assessment Tool for 24-hours recall
EDF	Energy-dense foods
EO	Eating occasion
FBC	Food-Based Classification
FFQ	Food Frequency Questionnaire
FD	Food Diaries
F&V	Fruit and Vegetables
G21	Generation XXI birth cohort
HDVI	Healthy Diet Variety Index
IAN-AF Survey	National Food, Nutrition and Physical Activity Survey of the Portuguese general population
NDNS	National Diet and Nutrition Survey
PCA	Principal Component Analysis
PHCUnit	Primary Health Care Units
SSB	Sugar-sweetened beverages
UK	United Kingdom
US	United States
WHO	World Health Organization

INTRODUCTION

1. Eating habits in childhood

Healthy eating habits have a key role in childhood since they promote an optimal health, growth and intellectual development (1, 2). Young children who engaged in a healthy dietary pattern had a better lean mass, cognition and behaviour compared to children with worse dietary patterns (3-5). On the other hand, children with an energy-dense, low fibre, high-fat diet had higher fat mass and greater odds of excess adiposity in childhood (6). Children's eating habits include, but are not limited to, food and nutrient intake, and also the combination of foods at dietary pattern level. Other dimensions of eating habits, including not only what but also how and when food is consumed have emerged in literature as important constructs to study in relation to health-related outcomes. Taking the example of less healthy eating habits, such as skipping breakfast, eating away from home, consumption of fast food and overeating, they were all previously linked to obesity and other diet-related diseases in childhood or adolescence (7, 8). Moreover, early diet influences not only the immediate health of the child but can also impact later in health outcomes (9). In a prospective study with a long follow-up period, a dietary pattern reflecting more health-conscious food choices in childhood or adolescence was inversely associated with cardiovascular risk factors 21 years after (10).

In particular, obesity has been described by the WHO as a "global epidemic" due to its high and increasing prevalence (11). Globally, over 340 million children and adolescents aged 5-19 are classified as overweight or obese and among preschool children (<5 years of age), the global prevalence of excessive weight increased from 4.2% in 1990 to 6.7% in 2010. This trend is expected to reach 9.1%, representing 60 million children, in 2020 (12). Nevertheless, obesity itself is not the main source of concern but, instead, the morbidities that arise associated with it. Childhood obesity is a complex disease with several adverse consequences, including psychological, social and health consequences (13, 14). Poor self-esteem, depression and eating disorders were described more in obese children than in their normal weight peers (15-17). Furthermore, obese children are more susceptible to cardiovascular disease risk factors such as hypertension, dyslipidaemia, chronic inflammation, endothelial dysfunction and insulin resistance (18-22). One of the most important long-term consequence of childhood obesity is its persistence into adulthood (23). It is estimated that 50-80% of obese adolescents will remain obese in adulthood (24, 25). Many of the health complications linked to obesity seems to be more severe if the obesity has been present for a long time; early onset obesity was suggested as a risk factor for morbidity and mortality later in life, especially in the development of chronic illnesses, i.e. cardiovascular disease, cancer and type 2 diabetes (26). Research has suggested that infancy and early childhood may be a particularly sensitive period for predicting obesity later in life (27) and focus should be on early life prevention of overweight and obesity.

1.1. Children's dietary intake: secular trends

Over the past few decades, significant changes have taken place in eating habits and home environments, leading to a world dominated by highly processed diets (28, 29). Dietary patterns have changed across all age groups in the modern societies and seem to be converging to a diet high in animal fat and sugar but low in fibre (30, 31). This has been the result of a decreased consumption of fruit and vegetables (F&V) and an increased consumption of sweetened and highly processed foods (32, 33). All the changes in diet and lifestyles (including increases in energy intake and sedentary lifestyles) that occurred in the past decades are believed to be a major driving force for the current obesity epidemic (28, 34, 35).

Figure 1.1 and 1.2 depict US 2-6-year-old children's mean caloric intake of foods from the top ten with the greatest absolute changes in per capita consumption between 1989 and 2008 (36). Overall, during this 20-year period, total daily energy increase by 109 kcal (36), with a marked increase in pre-schoolers' consumption of foods high in added sugar, solid fat and sodium intake (Figure 1.1).

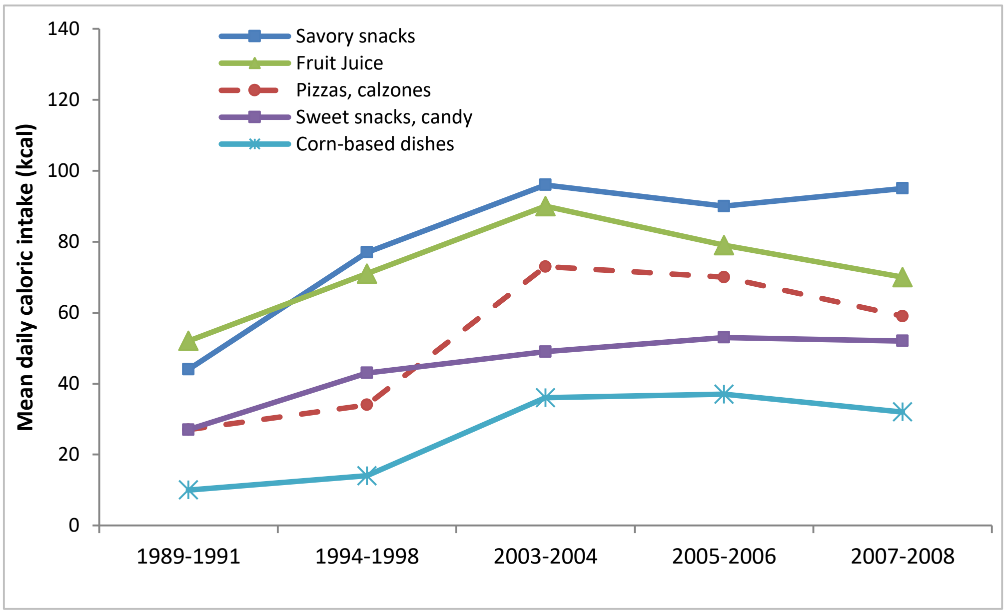

Figure 1.1. *Trends in per capita energy from energy-dense foods US children aged 2-6 years [source: adapted from reference (36)].*

Daily consumption of savoury and sweet snacks, pizzas, calzones, corn-based dishes, and fruit juice increased by a combined 148 kcal per capita, while consumption of fruit by any source increased by only 24 kcal (Figure 1 and 2). Children's consumption of starchy vegetables (-22 kcal) and nuts & seeds (-19 kcal) has decreased during this 20-year period (Figure 1.2).

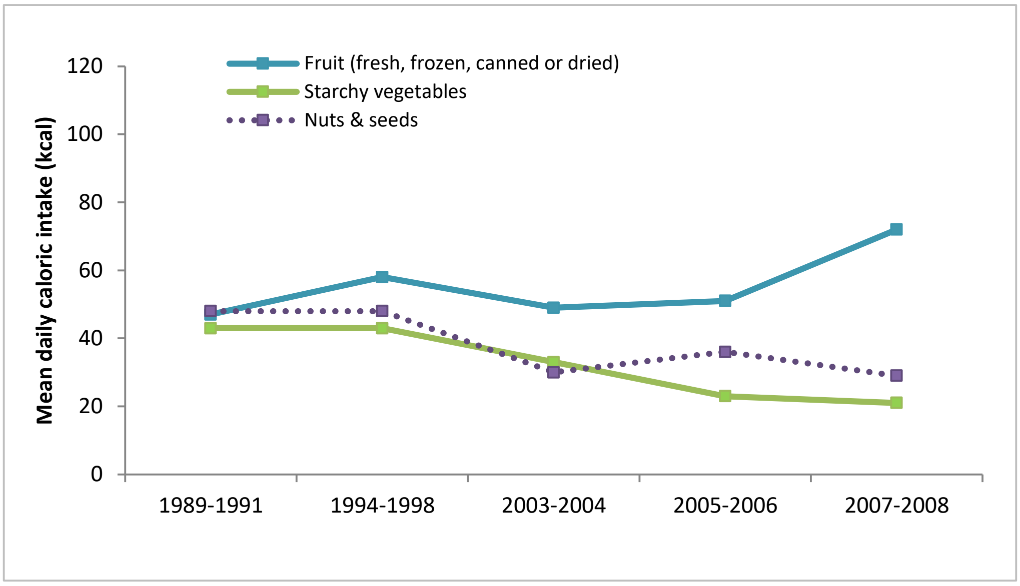

Figure 1.2. *Trends in per capita energy from fruit, starchy vegetables and nuts & seeds in US children aged 2-6 years [source: adapted from reference (36)].*

Data from the UK National Diet and Nutrition Survey (NDNS) shows that the consumption of F&V started to decrease from age 7, reaching its lowest intake during adolescence, mainly by a reduction in fruit intake (37). In the last evaluation of the NDNS the median vegetable intake was 0.9 portions (around 70g/d) per day by boys and 1.3 portions/d (100g/d) by girls, and the median of the total intake of fruit was 1.9 portions/d (153g/d) for boys and 2 portions/d for girls (160g/d) (37).

In Portugal, at the national level, there is no update and regular evaluation of individual dietary intake that would allow studying trends. One alternative to analyzing trends within a country is the Food Balance Sheet, although it is only possible to obtain food availability of the all population and not real individual consumption (38). In the most recent Portuguese Food Balance sheet (2012-2016), it was described a decrease in the availability of milk and dairy products (-7.1%), fruit (-1.8%), dry pulses (-3.1%) and cereals (-1.5%), and an increase in sugar availability (+2.9%), in comparison with the period 2008-2011 (39). Although the availability of vegetables has increased (+14.4%), the trend was not enough to achieve the recommended value per capita. The same is valid for fruit, dairy and dry pulses (39). On the other hand, the availability of fat and oils was above the recommendations (39). This method did not allow, however, specifying the availability trends for children.

Considering data from the most recent Portuguese National Food, Nutrition and Physical Activity Survey (2015-2016) (40) the inadequacy of F&V consumption, as well as sugar-sweetened beverages (SSB), seems to be more problematic among children and adolescent.

In Portuguese children results from the population-based birth cohort Generation XXI (G21) showed that at 4 years of age, less than half of the children were consuming vegetables on a plate, and 92% were consuming vegetable soup every day (41). The daily intake of sweet snacks and SSB was high (41). The consumption of salty snacks, including pizza, burgers and crisps, was done one to four times a week by 73% of the children (41). Moreover, children having one-quarter of their meals out-of-home (e.g. restaurants or coffee shops) had a higher intake of salty snacks, cakes, soft drinks and fruit juice, and a lower intake of vegetables and dairy products, resulting in a lower dietary adequacy index (42).

Focus on children's dietary intake and trends in childhood and adolescence should be studied, taking into account factors influencing intake, aiming to improve children's quality diet.

1.2. Children's eating habits: influencing factors

Determinants of eating habits are complex, may interact and could also change throughout life, especially during childhood. In early years of life, parents have a central role in shaping the family's food environment and eating experiences (43). As providers of children's food, family members are also relevant role models and help to establish rules and norms related to food and eating practices. Caregiver influences can be transmitted directly through the offered food, and indirectly, through transmission of behavioural models and social norms to the child (44, 45). Older children, in contrast, have greater autonomy over their food intake and progressively start making their own choices (46). Previously, higher levels of maternal age, education and occupation and also a higher household income were all inversely associated with the consumption of energy-dense foods among 2-year-old Portuguese children (47). In addition, at 4-years of age, a lower socioeconomic position of the mother when she was 12 years of age was positively associated with unhealthier dietary patterns; maternal education at child's delivery was also directly inversely associated with the odds of children following these patterns (48). Noteworthy, maternal behaviours were associated with children's dietary intake mainly through their one diet (48).

Research from the British Avon Longitudinal Study of Pregnancy and Childhood birth cohort (ALSPAC) also showed that 3-year-old children with higher scores in a dietary pattern characterized by high consumption of snack foods and high-fat foods were the ones with younger and less educated mothers and with a lower household income (49). Additionally, at 4 and 7 years old, a component describing a diet based on junk type foods was positively associated with low levels of maternal education and age (50). Low maternal education and unfavourable working conditions were all associated with a higher intake of energy-dense foods among Flemish children (51).

A systematic review (52) on the topic of predictors of children's dietary intake according to the socioeconomic position (SEP) showed that the SEP, mainly defined by parent education, influences children's nutrition knowledge, parent modelling, home food availability and accessibility. Also, data from the ALSPAC cohort described that the mother's educational attainment was a strong determinant of the children's dietary patterns extracted using three different statistical methods; higher educational attainment was associated with healthier, more nutrient-dense dietary patterns (53). All, these results enhance the prominent role of mothers in shaping the children’s eating habits since early life.

In addition, parents who are overweight or who are concerned about their children’s risk for excessive body weight may adopt inadequate child-feeding practices (54, 55). Previous research has linked some parenting practices, such as parental restriction and pressure to eat with higher consumption of palatable snacks (56-58) and lower consumption of F&V (57, 59). Moreover, factors in early life, such as the relationship between birth weight and gestational age were linked with chronic diseases later in life, such as obesity, cardiovascular diseases and type-2 diabetes (60-63). Early diet could partly explain these associations as low or high birth weight could influence early food preferences or change the way parents feed their children. Additionally, a previous study (64) showed an inverse association between birth weight and saturated fat intake among 43-month-old children. Breastfeeding is another factor influencing the establishment of children’s eating behaviours (65). For instance, among European children, the breastfeeding duration was positively associated with later F&V intake (66, 67).

In order to improve the effectiveness of interventions to change children's eating habits, it is necessary to replicate and accumulate evidence, improving the understanding of its determinants. A better understand how children's eating habits are acquired and if they are influenced by early life factors, including intrauterine and extrauterine factors is essential in developing strategies to improve the quality of children's dietary intake.

2. Specificities in measuring children's dietary intake

For the majority of studies, the assessment of the long-term diet, rather than the assessment of intake on a specific day or on a small number of days, is the conceptually relevant exposure parameter (68). In that way, prospective cohort studies with real-time assessment of the diet offer the best opportunity to gather valid and reliable individual information on dietary intake (69). Particularly among the paediatric population, the dietary assessment methods commonly used include respondent-based methods, such as dietary recalls (e.g. 24h recall), food diaries, and food frequency questionnaires (FFQ) (70).

2.1. Dietary recalls and food diaries

Overall, the 24h recalls and the food diaries collected information on foods and beverages consumed by an individual (including quantities) on one or more specific days. Both methods are based on current intake and might be used to estimate absolute food or nutrient intake. They also allow gathering information on other aspects of eating habits, such as the time of eating and place of consumption (71).

The completion of a food diary requires the training of each participant for them to be able to accurately describe the foods and quantities consumed, including the preparation methods, recipes and portion sizes (72). Therefore, a high level of motivation is necessary as well as literate respondents (72). This might result in a high participation burden and, in addition, the recording process may change the usual eating habits. In the particular case of young populations, generally, parents are the one reporting for their child.

On the other hand, using the 24h recall method results in a lower burden of participation as is a trained interviewer who collected the dietary data. Nevertheless, it relies on respondents' memory, which also increases the difficulty in estimating accurately the portion sizes. For this dietary assessment method, is the interviewer who must be well-trained, as the recall customarily is conducted by interview using probing questions (73). Contrary to the food diary methods, the potential for the dietary assessment to interfere with dietary habits is lower due to the recording process occur after the food has been consumed. Both methods may require multiple days of dietary collection (71).

2.2. Food Frequency questionnaire (FFQ)

Comparing to the method of doubly labelled water, the 24h multiple pass recall for children aged 4 to 11 years and weighted food diaries for children younger than 4 years, provides the best estimation for total energy intake (74). However, at present, FFQs are the favourite dietary assessment method used in most epidemiologic studies, investigating links between diet and disease in both adults and children (75-78). This can be justified mainly because FFQs are easy and simple to administrate, relatively inexpensive and have a low respondent burden. In addition, this method also allows capturing foods eaten rarely. On the other hand, the FFQ is limited by the foods included, it requires an estimation of portion sizes (by the responder or *a posteriori* by the researcher), needs to be validated, has low precision at the individual level, does not capture day to day variations and has a possible misreporting (71). However, the issue of misreporting was also described in others respond-based dietary assessment methods (74).

An FFQ typically includes a list of foods and beverages which responders are asked to report the frequency of consumption and, in some cases, portions sizes. For young children, the reference period usually ranges from the past month to the past year. The portion size assessment is one of the main components of the adaptation of an FFQ for a given population. A quantitative FFQ collected data on portion size for all foods; a semi-quantitative FFQ collected this information using typical portion sizes (e.g. milk glasses) or use a closed category of response (higher, equal or lower than a portion predefined). On the non-quantitative FFQs, portion sizes are not asked, and the nutrient estimation is usually based on standard portion sizes. If the chosen portion sizes are not adequate, this can lead to significant inaccuracy in food and nutrient intake estimation. Moreover, the lack of portion size estimation in an FFQ can be considered as a missing data problem. The classical method that uses a 'standard' portion applied equally to all responders might reduce sensitivity if portion sizes vary within the population (79).

In comparison with other dietary methods, there is a general trend for dietary intake overestimation using the FFQ in both adults and children (80-84), regardless the nationality or age group, as it is summarized in Table 2.1 for children. Such overestimation has been explained by the large number of foods asked in the FFQ, providing a broader selection of options as compared to other methods or by an inaccurate reporting of the consumption frequency of commonly consumed foods. However, it is possible that the portion sizes assumed in the FFQ are incorrectly too high or that increasing frequency could result in decreasing the portion size. Young children seem to self-regulate their energy intake by adjusting their portion sizes depending on the number of eating occasions per day (85). On the other hand, the caregiver could decrease the portion size given to the child with increasing frequency. Therefore, it is possible that children change portion size depending on the frequency of intake. Assuming a standard portion equal to all frequency categories could result in less accurate dietary estimation.

Taking this into consideration, researchers have tested other methods for handling missing portion size in FFQ, in both adults and adolescents, such as, stochastic methods (86) or combination with data from food diaries or 24h recall (87-89). Even tough, when using a non-quantitative FFQ, new methods to calibrate portion size data are necessary to be tested in children population. The reduction of the exposure measurement error will increase the accuracy in the estimation of diet and its associations with health outcomes.

Table 2.1 – Overall validation of FFQ among adolescent and children population

Reference	Country	Participants	Method	Main results
Günes EF, 2016 (90)	Turkey	7-12y (n=50)	138-item FFQ vs. 3d food diary	Compared to the mean of DRs, the mean of FFQs overestimated energy and macronutrients intake.
Vioque J, 2016 (91)	Spain	4-5y (n=169)	105-item FFQ vs. 3x24h recall	The mean nutrients and energy intake derived from the three 24h recall were, in general, about 15–20% smaller than those estimated by the two FFQ.
Marcinkevage J, 2015 (92)	Guatemala	6-11y (n=145)	108-item FFQ vs. 3x24h recall	The mean values for the majority of nutrients obtained from the 24h recall were lower than for those obtained from the FFQs.
Bel-Serrat S, 2014 (82)	8 European countries	2-9y	43-item FFQ vs. 2x24h recall	The FFQ provided higher intake estimates for most of the food groups than the 24h recall.
Mulasi-Pokhriyal U, 2013 (93)	USA (Hmong)	9-18y (n=335)	Block Kid's FFQ vs. 2x24h recall	Among all children, Block FFQ nutrient estimates for vitamin A, vitamin C and food group servings for vegetables and fruits were significantly higher than those assessed through the 24h recalls ($P < 0.001$).
Matos SM, 2012 (83)	Brazil	4-11y (n=108)	98-item FFQ vs. 24h recall	The energy and nutrient intake estimated using the FFQ was significantly higher than what was obtained using the 24h recall.
Kobayashi T, 2011 (94)	Japan	3-16 (n=89)	75-item FFQ vs. 4d weighted record	There was some over/ underestimation of nutrients in the FFQ.
Bertoli S, 2005 (95)	Italy	6-10y (n=18); 16-20y (n=19)	136-item FFQ vs. 7d weighted record	The FFQ seemed to overestimate the nutrient intake when compared with 7d weighed records.
Andersen LF, 2004 (96)	Norway	2y (n=187)	Semi-quantitative FFQ vs. 7d weighted record	The average intake of all micronutrients, except for calcium, was overestimated by the FFQ.
Vereecken CA, 2003 (97)	Belgium	11-18y (n=7072)	FFQ vs. 24h food behaviour checklist (FBC)	Only for cereals, diet soft drinks and other milk products were considerably higher food frequencies than expected found from the FBC.
Vereecken CA. 2003 (97)	Belgium	11-12y (n=101)	FFQ vs. 7d food record (FD)	Comparison of the FFQ with the FD showed overestimation for all but three food items (cheese, soft drinks and chips).

More recently, short FFQs are being investigated as a useful instrument to evaluate children's total diet. A previous systematic review (98), published in 2013, included short (less than 50 items) tools that measure whole-of-diet intake of young children (0-5 years). A total of 15 tools were included in the review (8 tools for children aged 2-5 years old), being the majority FFQ (varying from 6 to 47 food items). The authors highlighted the need to have more brief, valid and reliable short instruments to evaluate the total diet in young children.

The evaluation of the performance of dietary intake assessment methods among children is essential in order to obtain high-quality data on the dietary intake of this population. For that, it is necessary to develop validated, reliable and age-specific instruments that are both practical and suited to the needs of the research. Although there is no absolute gold standard for dietary assessment, usually the measurement of the validity of a dietary assessment instrument is based on the comparison with another dietary technique, ideally with independent errors. Multiple 24h recalls and food diaries are usually used for the evaluation of the validity of an FFQ. Briefly, the validity of an FFQ is defined as the degree to which its results match the results obtained through the validity technique. It is recommended that the validity of FFQs be tested using multiple statistical tests, with different tests reflecting different facets of validity (99).

When available, biomarkers may be an alternative or supplementary reference method for the validation of some nutrients intakes since their measurement errors are independent of those of the FFQ (100). Dietary biomarkers are desirable for their ability to more accurately assess nutritional intake versus self-reported methods. Biomarkers can be categorized into short-term (reflecting intake over past hours or days), medium-term (reflecting intake over weeks or months) and long-term markers (reflecting intake over months or years). Although dietary biomarkers generally provide a more proximal measure of dietary intake, genetic variability, lifestyle factors (e.g., smoking), dietary factors (e.g., nutrient-nutrient interaction), biological sample and analytical methodology may affect the utility of a dietary biomarker to properly reflect dietary exposures (100). Also, biomarkers do not exist for many dietary factors (100).

A combination of methods, self-reported methods and biomarkers (for specific nutrients), improve the process of validation of an FFQ and has been used in several large prospective studies (91, 101, 102).

3. Tracking eating habits – Do early habits persist later in childhood?

In epidemiology, the term "tracking" is defined as the stability or maintenance of a given characteristic during a period of time (103). The behaviour patterns acquired during childhood and adolescence are likely to track into adulthood (104). Diet tracking might represent the maintenance of eating habits, food or nutrient intake over time. The continuity of these habits can be assumed as a positive or negative behaviour, depending on the eating habits. In particular, early life dietary intake seems to influence the dietary intake a few years later (105, 106).

Understanding how children's eating patterns are acquired is essential in public health planning, supporting strategies to improve the quality of children's diet. Dietary habits have mainly been tracked in adults or between adolescence and adulthood, while few studies have tracked diet during childhood (107-109). These studies have suggested that eating patterns are somewhat established early in life. For example, results from the ALSPAC birth cohort (53) indicated that the dietary pattern of the child at 7 years of age was a strong determinant of children's dietary patterns at 10 and 13 years. Using principal component analysis (PCA) and categorizing dietary patterns' scores into quintiles, the highest agreement was between 4 and 7 years of age, however, periods of change were described between the ages of 3 and 4 and between 7 and 9 years (107). In general, previous studies assessing the stability of dietary patterns had mixed results with only moderate (107, 108, 110, 111) or poor tracking (109, 110, 112). Moreover, a review of the literature described a weak to moderate tracking of dietary patterns between childhood and adolescence (113). Research suggests that a single measurement of dietary intake during all childhood may not be sufficient to represent children's individual dietary patterns.

Portuguese results, using G21 data, has described three distinct dietary patterns that children adopted at 4 years of age: high in energy-dense foods (EDF), low in foods typically consumed at main meals and intermediate in snacks (Snacking), and higher in vegetables and lower in EDF (Healthier) (114). These dietary patterns tracked between 4 and 7 years, and girls following the EDF dietary pattern at 4 years of age had a significantly higher adiposity 3 years later, comparing to girls following the healthier pattern (114).

Other aspects of children's eating habits, such as the quality and variety of diet as well as eating frequency have been less studied, particularly assessing the tracking of these eating dimensions from pre-school to school age.

3.1. Variety of diet

Dietary recommendations from the WHO highlight the consumption of a higher variety of foods originated mainly from plants in detriment of animals sources (115). A diet with higher variety comprehends a higher consumption of different foods or foods groups over a given reference period. In that way, a varied diet is a resourceful approach to achieve an optimal nutritional status and to meet nutrients' recommendations.

Beyond the nutritional benefits, diet variety also influences other dimensions of eating, such as the pleasure of eating (116). In a given meal, being exposed to different types of food, with different sensory experiences, might prevent the onset of sensory-specific satiety. This effect is defined as the decline in pleasure derived from consuming a known type of food in comparison to exposure to a new flavour or food (117). Being exposed to different types of foods might also retard the habituation process and delay the end of the meal (118-120). Considering this, a great diet variety might have a perverse result; in the current Western obesogenic environment a supply of highly palatable and food variety might stimulate appetite, delay satiety and promote excessive energy intake.

A recent meta-analysis of observational studies (121), examining the link between dietary diversity score (DDS) and obesity, failed to find any statistical association between DDS and body mass index (BMI) status. Several limitations were pointed out to justify these null associations. First, the authors stressed that there were no prospective studies assessing the relationship between DDS and BMI, and so, a causal relationship cannot be inferred. Second, most of the included studies did not adjust for important confounders such as energy intake and socioeconomic status. Lastly, measuring usual intake is required, as well as a consistent use of similar approaches evaluating DDS. The use of an FFQ was recommended as the most appropriate tool to measure long-term dietary variety (121). Nevertheless, several studies have supported a beneficial effect of diet variety, through an increase of variety of nutrient-dense foods within and across food groups, on health outcomes (122-126). Key messages should focus on a greater variety of healthy foods, which were previously associated with a lower prevalence of excessive weight, rather than energy-dense foods which were associated with higher adiposity (127).

Early diet variety shows tracking in childhood: the introduction of a greater variety of foods as early as in the complementary feeding period is positively associated with the children's diet variety later on (128, 129). This tracking shows effects as late as early adulthood (130). Eating a variety of foods every day were evaluated in the Ewha Birth and Growth Cohort when the children were 7 and 9 years of age (108). Food variety was consistently associated with children's healthy intake at both ages and 58% of the children showed a stable eating variety while 27% increase it from 7 to 9 years, resulting in a weighted kappa of 0.43 (moderate tracking) (108). Diet variety can be evaluated in different ways and there no consistent validated method (131). In that way, the assessing and tracking of the overall food variety throughout childhood, using the same validated instrument, is lacking in the literature.

3.2. Eating patterns

3.2.1. From a reductionist to a more holistic approach

In the past decades, a reductionist approach has dominated nutrition research, with the focus on individual dietary constituents, such as essential nutrients, and their relationship with specific physiologic effects (132). This reductionist paradigm consists of explaining a complex phenomenon by studying their separate entities' functional properties (133). This approach has led to a better understanding of how nutrients, or other bioactive compounds, affect human metabolism. For instance, the discovery of the role of vitamins had a huge impact on people's health, especially in developing countries where hypovitaminosis is prevalent

(134). However, the increasing prevalence of diet-related chronic diseases, such as obesity and cardiovascular disease worldwide (135, 136) has pushed forward researchers to explore new ways to disentangle these phenomena, with real improvements in the public health.

Research so far demonstrates that organisms are much more than a simple sum of the parts and the behaviour of complex physiological processes cannot be explained by solely investigate its parts (132). In that way, the reductionist approach seems to fail in describing adequately the diversity of metabolic effects on the whole organism, and it appears that the focus on only nutrients is in many ways counterproductive (132, 137). The very nature of diet is complex, as is the result of a mixture of foods, which in turn are composed of several chemicals. So, the food compounds may act in synergy and interaction (or antagonism) between nutrients, and other components, cannot be ruled out (137).

As explained by Fardet *et al* (134) a holistic approach “represents a top-down method, which begins with a more general view and develops into a specific reductionist approach for explaining a particular point when necessary”. This approach considers that the whole is more than the sum of its parts (102). So recently, researchers have been encouraged to explore complex issues through a holistic view before addressing any specific question to explain the whole. In that way, strategies such as assessing dietary patterns rather than isolated food compounds or nutrients, or evaluating different dimensions of eating habits (e.g. diet variety and quality) are encouraged. Moreover, the examination of food combination at the meal level provides an interesting approach, following this point of view, by expressing cumulative and interaction effects of foods and nutrients, overcoming the limitations of the study of nutrients or isolated foods.

3.2.2. Eating frequency – Is there a consensus for a universal definition?

A lack of public health recommendations on eating frequency or temporal distribution of meals is common among European authorities, especially in the young population (138). Eating behaviours can influence the diet quality and health outcomes, being the ‘skipping breakfast’ the most reported eating behaviour associated with a worse diet quality (139, 140) and with poor cardiometabolic health outcomes, including a higher BMI (141, 142). Nevertheless, evidence for other types of eating behaviours, such as eating frequency, has been less consistent (7). One explanation is the inconsistency in definitions of eating behaviour, which might hamper data interpretation and comparison between studies (7). Accordingly, differences in characterization of eating behaviours, namely those related to meal patterns, may affect the direction and magnitude of the association between diet and health outcomes (143).

The terms ‘eating occasion’ (EO) or ‘eating event’ are usually used to describe any occasion where food or drink is consumed, and thus includes all meal types. However, there is no consensus on a universal definition of what constitutes an EO and how to differentiate main meals from snacks (144). The definition of snacking varied across studies: some researchers defined snack events as eating between meals (145-147), other defined it as consuming small food portions or packaged food (148, 149), others defined snacking as eating specific foods (e.g. salty foods, sweets or cakes) (150-152), or according to time of consumption (153, 154). Although, there is a lack of a universal definition differentiating main meals and snacks (144), snacking events have been described as increasing in recent years, particularly among children (155, 156).

This differentiation between a main meal and a snack requires attention, as people tend to give a different perception to an EO, which in turn could influence significantly their dietary intake. Experimental studies showed that adults who define a particular EO as a main meal, based on some eating cues (i.e. use of dishes and utensils and being seated at a table), may reduce caloric intake later in that day (157). In an intervention study, undergraduate students reported feeling less satiated by a snack than a meal, even when the two EO were isocaloric (158). These peculiarities highlight that simply the label of an EO as meal or snack may influence food choice, satiety, and daily energy intake (159). Hence, researchers have advised to avoid the word 'snack' when collecting information on individuals' eating patterns and to simply ask participants, for example, to record meals and food and drinks consumed between meals (159).

A variety of approach has been described in the literature to define an EO, main meals and snacks, namely time-of-day, participant-identified, food-based classification (FBC) and neutral (143). Each classification system has its own strengths and limitations, and can potentially influence the outcome and interpretation of results. These definitions are discussed below.

Time-of-day

This approach defined meals based on the time-of-day in which food or drinks were consumed. The main meal may be defined as the largest EO (e.g. higher percentage of energy) occurring between specific time intervals, while smaller EOs and any EOs occurring outside these intervals are considered as snacks. While this approach is easy to apply and understand, it does not capture meals eaten at unusual times, such as among shift workers and it requires a measure of time of eating. In addition, a bias towards traditional eating patterns might occur, as these time frames might be influenced by local or cultural factors, limiting comparison across different countries or cultural groups. Moreover, a definition of meals based on time might become more complex on weekend days or during holidays periods (143).

Participant-identified

This definition implicates that participants are asked to report their own EO, distinguishing between main meals and snacks, usually using a list of pre-specified meal labels (e.g. breakfast, lunch, dinner, or snacks). This approach avoids the use of a complex criterion to classify main meals and snacks; however, the definition cannot be standardized due to participant's subjectivity (143). This definition relies on individual's perception of a meal or snack which might vary, depending on using the terms 'a snack', 'snacks foods' or 'snacking' (159). Moreover, the researcher must decide how to treat the EOs that are not clearly defined as a main meal or a snack by the participant and whether EOs occurring close in time should be combined using additional time criteria. The meal labels might also introduce a researcher bias.

Food-based classification (FBC)

In this approach, foods are categorized into food categories based on their nutritional profile. The type of EO, ranging from a complete meal (high nutrient density) to a low–quality snack (low nutrient density), is based on food category combinations. Another variation of the FBC is to distinguish main meals and snacks based on core and non-core foods. This approach intended to encompass both qualitative and quantitative aspects of meal patterns, however, due to its complex categorization is use is limited. Additionally, differences in criteria to classify meals and snacks (e.g. nutrient profiles vs. energy density of foods) may limit comparison across studies and generalizability of this classification (143).

Neutral

The neutral approach defines an EO as any occasion when foods or drinks are consumed. The advantage of this definition is that it can be standardized and results are more comparable over time and across studies. Additional criteria have been used to define EO using the neutral definition, such as the time intervals between EO (e.g. 15min, 30min or 45min), a minimum of energy per EO (e.g. 210 kJ) and whether beverage-only EO are included or not. Noteworthy, these criteria have also been used in other definitions, namely the time-of-day and participated-identified, in order to define a separate meal and/or snack (143).

A previous research (160) has examined the influence of eight different definitions of EO on the characterization of eating patterns among adults and the extent to which they predict the proportion of variance in the total energy intake. It was the neutral definition, with a 15-min time interval plus a 210kJ (50 Kcal) energy criterion, which best predicted the variance in total energy intake. One of the advantages of using this definition is that it can be standardized and comparable over time and across different studies (143). Overall, the study showed that different approaches to the definition of EOs affect how eating patterns are characterized (160).

It seems that to optimize the definition of a main meal or a snack more than one criterion should be used, in order to avoid ambiguities about what constitutes a main meal and what constitutes a snack. A more consistent approach to the definition of EO would facilitate appropriate interpretation of present and future research, improving the knowledge of the role of eating frequency on health. Nevertheless, even in the absence of a universal definition, a clear description of what is considered to constitute an EO should always be provided in manuscripts investigating the topic of EO (161).

3.2.3. Eating frequency and obesity

Research at the 'meal' level has shown that specific meal characteristics, such as the nutrient composition, eating frequency or regularity of meals, could influence adverse outcomes in health, such as obesity, insulin resistance, and metabolic syndrome, independently of other risk factors (162-168). For example, increased eating frequency might actually lead to a higher exposure to energy-dense foods, such as fast food and soft drinks, and large-portion-size foods, resulting in an increased hunger, excess energy intake, and ultimately unhealthy body weight gain (169-171). Nevertheless, some studies among adults showed that a greater meal frequency is associated with a healthier weight status (172, 173). Indeed, a recent systematic review of observational studies among adults (169), found fourteen studies that reported an inverse association between eating frequency and body weight or body composition, and seven studies reporting a positive association. Although, in men a potential protective effect was consistently described, the authors concluded that there was not enough evidence for establishing a clear association between eating frequency and body weight in adults.

The association between eating frequency and adiposity measures in children is also not consistent, as a large number of cross-sectional studies among young populations have shown an inverse relationship (174-179) but other showed a null or the opposite relationship (180, 181). A previously meta-analysis (182) found an overall inverse association between eating frequency and the likelihood of being overweight or obese in children and adolescents. However, when stratified by sex, the effect remained significant only in boys. The presence of publication bias and heterogeneity of studies might in part explain these differences, and further research was advised. More recently, in centrally obese children, eating frequency was associated with a higher BMI and no association was found between physical level and eating frequency (181). Among very young children, using a longitudinal approach, the daily eating frequency was not associated with the current or subsequent change in BMI (183). The authors justified, in part, these null associations due to a potential improvement of internal energy self-regulation by young children, in comparison with older children (183). Among healthy weight children aged 9-10 years, increased eating frequency was positively associated with levels of physical activity and improved composition of snacks and breakfast in terms of F&V, fat, fiber and carbohydrates (181).

Overall, the results between eating frequency and BMI should be interpreted with caution due to potential methodological limitations associated with the different studies. Besides the difficulty in interpretation of previous research related to the fact that there is no universal definition about what constitutes an EO, main meals or a snack, assessment of eating frequency has often been based on self-reported questionnaires (176-178, 184-188), while just a few have assessed EF using information on actual dietary habits (using e.g. food diaries or 24h recall) (180, 181, 183, 189, 190). Another issue is the potential for underreporting by obese or overweight individuals (191, 192) that might confound the association between eating frequency and adiposity.

A review of studies that have examined snacking patterns among children and adolescents described that although more frequent snacking has been associated with a higher total energy intake and a higher proportion of energy from added and total sugar, the regular consumption of snacks also contributes to the intake of key nutrients (193). Moreover, the majority of studies included in the review either found no evidence of a relationship between snacking patterns and weight status or found an inverse relationship, indicating that children or adolescences who more often consumed snacks were less likely to be obese (193). However, a few studies have described a deleterious effect of frequency of snacking or percentage of energy consumed at snacks (including energy-dense snacks foods) on children's weight status (193). The multiple different criteria used by the studies to define a 'snack occasion' might in part explain these mixed results (194).

4. Eating behaviours related to appetite

A healthy eating behaviour is characterized by eating when feeling hungry and at regular moments, allowing an adequate physiological growth (195). However, the prevalence of problematic eating behaviours is increasing, varying from picky eating (selective eating which can lead to malnutrition) to overeating and disinhibited eating, including a high food responsiveness, excessive hunger, avid appetite, rapid eating, eating without hunger, which ultimately can lead to excess adiposity (196-198).

A fussy/picky eating behaviour is characterized by a limited diet variety due to the frequent rejection of familiar and unfamiliar foods (199), which might have a negative impact on the children's nutrition status. These children tend to eat small meals, eat slowly, be less interested in food, to accept a limited number of foods and to have a limited intake of vegetables and other foods (200, 201). Parents are frequently concerned about their children's fussy behaviour, due to a possible lack of adequacy of children's diet, which in turn, might lead the parents to pressure the child to eat (202). On the other hand, overeating is defined as consuming large quantities of food during a specific period of time (even in absence of hunger), eating more rapidly than normal and eating until feel uncomfortable (203, 204).

Feeding problems that manifest early in childhood may persist as maladaptive eating later in life, compromising their future health (205). Fussy behaviour has been associated, for example, with anorexia nervosa in adolescence (206). On the other hand, overeating may be linked with excessive weight and development of binge eating in adolescence (207). These eating behaviours are the product of both genetic and environmental factors (208, 209) and hence they are modifiable behaviours that potentially reduce children's risk of developing an unhealthy body weight in the short and long terms.

The relation between several aspects of diet and eating behaviours related to appetite has been less explored in previous research and some inconsistencies between their findings are yet to be clarified. Certain dietary components have been shown to influence mechanisms of appetite control, namely those related to satiety (210, 211). For example, protein intake can induce satiety, increasing secretion of gastrointestinal hormones and diet-induced thermogenesis (212). Previous evidence has also suggested that characteristics of the meals, such as nutrient composition (162-164) and frequency of meals (167, 168, 183, 213), could influence outcomes in health, such as obesity, insulin resistance, and metabolic syndrome, independently of other risk factors. It has been hypothesized that regular eating frequency could modify metabolism, by increasing satiety and improving glucose and insulin metabolism (166, 167). On the other hand, controlled feeding studies in adults have shown that reduced eating frequency negatively influence appetite control, by increasing the perceived appetite and reducing the perceived satiety (167). Palatable foods can also exert a positive influence over feeding behaviour (214). Long-term studies are needed to understand the impact of specific dietary components in the regulation of appetite.

To better understand the behavioural aspects of children's appetite, some underlain biological mechanism are described below.

4.1. Physiological regulation of appetite

Appetite is generally described as the desire to consume food and is experienced as perceived hunger (or the subjective 'desire to eat' or 'urge to eat'). Alternatively, satiety can be described as the feeling of being satisfactorily full and unable to eat any more food (215). Appetite can also be described as a trait-like characteristic (216). The appetite control is the result of the perceived appetite and satiety sensations that ultimately lead to whether food is or not consumed.

Eating regulation is a complex neurophysiological process influenced by both environmental, including food availability, knowledge, emotional state and experience of the individual, and biological mechanisms (217, 218). The brain, and in particular the hypothalamus, is believed to be the master coordinator of appetite and body weight. The majority of biological models explaining this coordination suggest a relationship between homeostatic and hedonic pathways. The homeostatic system ensures that when energy levels are perceived as adequate, the drive to eat is low and when energy level decrease, the motivation to eat increases (219). The hedonic system, in turn, regulates the pleasure and reward aspect of eating, increasing the desire to consume highly palatable foods, overcoming the homeostatic pathway (220). Eating is a highly reinforcing behaviour that not only provides nutrients (and energy) needed for survival, but that also induce feelings of gratification and pleasure.

Homeostatic system

Numerous hormones and neuropeptides have been implicated in the chronic and acute regulation of appetite satiety in humans (219, 221). The predominant appetite regulatory site, the arcuate nucleus (ARC), receives metabolic signals from peripheral tissues such as the liver, pancreas, adipose tissue, gut and muscle (figure 4.1). At least two populations of neurons in the ARC acts on food intake: medial ARC orexigenic [neuropepetide Y (NPY) and agouti-related protein (AgRP)] and lateral ARC anorexigenic neurons [proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART)] (222). Orexigenic and anorexigenic neurons stimulate and suppress food-seeking behaviours, respectively. These neurons project to other hypothalamic nuclei and to the nucleus of the solitary tract in the brain stem to control multiple aspects of the homeostatic regulation of energy balance. They are the first-order neurons on which metabolic hormones, including orexigenic (ghrelin and orexins) and anorexigenic peptides (cholecystokinin, polypeptide YY, glucagon-like peptide-1, oxyntomodulin, leptin and others) primarily act (221, 222).

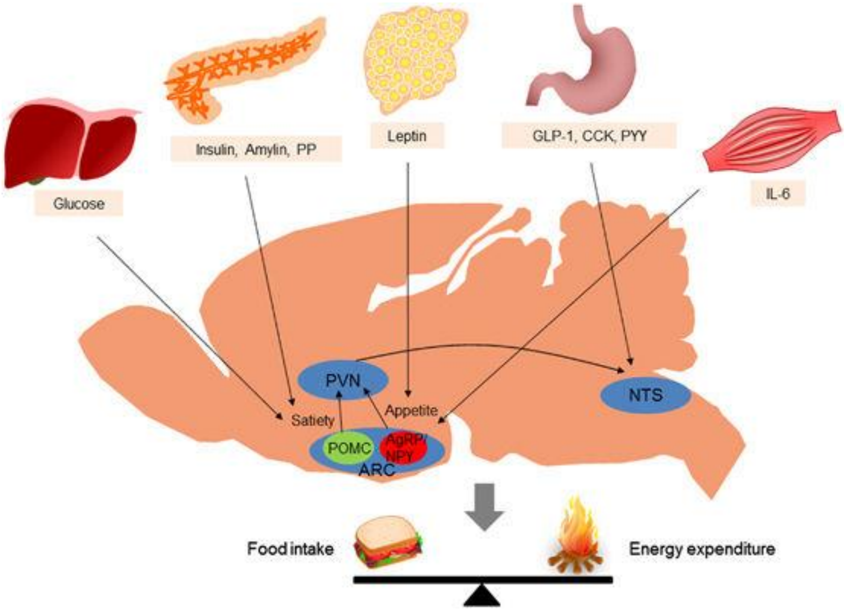

Figure 4.1. *Integration of peripheral metabolic signals and the central nervous system in control of energy homeostasis. ARC, arcuate nucleus; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; IL-6, interleukin-6; PP, pancreatic polypeptide; PVN, paraventricular nucleus; PYY, peptide YY.* [Source: (221)]

Specifically, the adipose tissue, which is spread throughout the body, produces several paracrine and endocrine factors, including adiponectin, TNF-α, resistin, interleukin-6, angiotensinogen, and acylation-stimulating protein (223). Leptin is an adipocyte-derived protein with impact in the regulation of appetite that signals information to the hypothalamus about the degree of adiposity and nutrients. This process control ingestive behaviour and energy expenditure. Leptin levels are proportional to adiposity energy stores, suppressing appetite and acting on receptors in the ARC to promote satiety and energy expenditure (223).

Other peripherally hormones that contribute to central regulation are ghrelin and cortisol. Ghrelin is produced in the gastrointestinal tract and increase hunger, decreases energy expenditure, and stimulates cortisol release. In turn, cortisol stimulates the mobilization of glucose to ensure that the body has sufficient energy to respond to an acute stressor. However, chronically elevated cortisol can lead to an attenuated leptin's response, increasing the desire for energy-dense foods and the accumulation of abdominal fat (217).

Hedonic system

In the modern societies, feeding behaviour often occurs even in the absence of hunger. This 'hedonic' mechanism is not regulated by metabolic feedback but related to cognitive, reward, and emotional pathways (224). Food consumption is a potent natural rewarding *stimulus* and human's pleasurable response to food largely overlaps with the brain 'score reward circuits'. This mechanism is triggered by food-related cues, particularly taste and smell, which increase motivation to eat, through learning and memory (225). Like other addiction behaviours, the mesolimbic dopaminergic pathway is involved in hedonic feeding (226). The mesolimbic dopamine system encodes subjective "liking" and "wanting" of palatable foods, which is subjected to modulation by the hindbrain and the hypothalamic homeostatic circuits and by satiety and adiposity hormones. The presentation of highly palatable foods induces a potent release of dopamine, acting on the motivation to achieve food rewarding (220). The levels of dopamine are correlated with the level of pleasure obtained through food intake (227).

In that way, food intake can be disrupted due to pathological functioning of either the homeostatic system or the hedonic system. Examples of appetite dysregulation can be found in obesity, anorexia nervosa, bulimia nervosa and binge eating disorder (217). Moreover, both homeostatic and hedonic system interact in the regulation of food intake (220); peripheral hormones related with energy homeostasis such as leptin, insulin, and ghrelin can modulate the activity of the mesolimbic dopamine system (228). Leptin can decrease the basal secretion of dopamine as well as the feeding-stimulated dopamine release, whether administration of ghrelin stimulates the release of dopamine into the striatum. This interaction could explain in part the recent obesity epidemic, whereby the 'hedonic' system seems to interacts with the current obesogenic environment overriding the homeostatic mechanism and leading to an increase in body weight (229).

In similar environmental conditions, not all individuals develop weight gain, suggesting that the hedonic system may play an important role in influencing food intake and subsequent unhealthy weight gain. Another reason might be the development of leptin resistance identified in many obese people (230). This phenomenon occurs with chronically high circulating leptin levels due to excess adipose tissue. The response from the brain becomes blunted and the leptin no longer produces the same degree of satiety after food intake. The dopamine release is also decreased, resulting in a diminished sense of reward with ingestion.

4.2. Measuring of appetite control and related eating behaviours

Appetite is a subjective and multidimensional construct and, so, there is no universally accepted method to quantify it. Several methods have been used to quantify appetite control, including both biological and behavioural methods. First, biological methods included the examination of key appetite-suppressing and appetite-stimulating gastrointestinal hormones, previously discussed, that are secreted in response to energy intake (231, 232). Gastric emptying can also be evaluated as another mechanism affecting appetite control (231, 232). However, there are no reliable biological markers for quantifying appetite (231).

Behaviours methods, including laboratory and questionnaires instruments, measure indirectly appetite. These methods include the assessment of perceived hunger or satiety through the use of visual analogue scale questionnaires (233), or indirectly by measuring subsequent food intake. Psychometric instruments have been also developed to detect individual differences in eating among adults and children. More recent observational studies on children's appetitive behaviours have used the Dutch Eating Behaviour Questionnaire (DEBQ) (234), the Baby's Eating Behaviour Questionnaire (BEBQ) (235), and the Children's Eating Behaviour Questionnaire (CEBQ) (216).

The DEBQ was developed to measure three eating styles: 'emotional eating' (eating in response to internal emotional factors, such as fear, anger and anxiety), 'external eating' (eating in response to external stimuli such as the sight and smell of food, without regard to internal signals of hunger or satiety) and 'restraint' (consciously restrain of food intake to control body weight). The BEBQ was developed for completion by mothers of 0–3 months old infants, during the breast/bottle-feeding period, or retrospectively, after complementary feeding. It was based on the CEBQ, and covers four aspects of appetite, namely, 'Enjoyment of Food', 'Food Responsiveness', 'Slowness in Eating' and 'Satiety Responsiveness'. The CEBQ is the most widely used instrument to assess children's eating behaviours related to appetite and is described in detail below.

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

The CEBQ was developed by Jane Wardle and colleagues for measuring eating behaviours among UK pre-school aged children (216) but has been examined in children up to age 12 (236-238). This questionnaire is considered as the most comprehensive instrument to assess children's eating behaviour and it has been tested with high reliability and validity in several countries (37, 38), including Portugal (238, 239). It aims to capture the differences between aspects (sub-dimensions) of eating behaviours that theoretically contribute to malnutrition or overweight. The included dimensions were obtained from previous literature on eating behaviour of children and adults, followed by qualitative interviews with parents about their child's eating behaviours (216).

The CEBQ is a parent-report instrument of 35 items, rated in a 5-point Likert frequency scale (never, seldom, sometimes, often and always), scored 1 to 5. It was developed to assess eight sub-dimensions of eating behaviours in children: 'Food Responsiveness', 'Enjoyment of Food', 'Emotional Overeating' and 'Desire to Drink' as 'food-approach' appetitive traits, and 'Satiety Responsiveness', 'Slowness in Eating', 'Emotional Undereating' and 'Food Fussiness' as 'food-avoidance' appetitive traits (216). Each of the eight sub-dimensions contained 3 to 6 items. Table 4.1, described the eight sub-dimensions as well as the items included in each of them.

Table 4.1 - Description and items of each CEBQ sub-dimensions

Dimension	Sub-dimension	Definition		Original items
Food Approach Eating Behaviours	Food Responsiveness	Measures eating in response to environmental food cues (e.g. sight or smell of highly palatable food - hedonic pathway) (236).	12	My child's 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.
	Enjoyment of Food	Represents a general interest in food and captures the extent to which a child finds eating pleasurable and desires to eat (236).	1	My child loves food.
			5	My child is interested in food.
			20	My child looks forward to mealtimes.
			22	My child enjoys eating.
	Emotional Overeating	Represents increased eating in response to negative emotions (e.g. anger and anxiety) (237).	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.
	Desire to Drink	A general desire to have a drink (236).	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	Satiety Responsiveness	Represents the degree to which a child regulates food intake, based on their perceived fullness (homeostatic pathway) (236).	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.
	Slowness in Eating	Measures the speed of eating during the course of a meal; A higher score represents a reduction in eating rate as a consequence of lack of enjoyment and interest in food (237).	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.
	Emotional Undereating	Represents decreased eating in response to negative emotions (237).	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.
	Food Fussiness	Reflects a lack of interest in food and rejection of familiar and new foods (food neophobia), leading to the consumption of an inadequate variety of foods (237).	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.

In general, the 'food-approach' traits have been positively associated with children's weight, whereas the 'food-avoidance' traits have been negatively associated with children's weight (236, 240). However, when studying each of the sub-dimensions these associations are not so clear. While higher scores in 'Satiety Responsiveness' and 'Slowness in Eating' have been linked with a lower risk of overweight (241), previous studies have also found an association between higher scores in these eating behaviours and higher consumption of sweets (242) and lower preferences for F&V (243). Children with these appetitive traits may not only be consuming less of overall, but also eating disproportionally fewer nutrient-dense foods. On the other hand, higher scores in 'Enjoyment of Food' (a food-approach trait) have been associated with higher intake of F&V (242, 243). However, as these eating behavioural traits (a general interest in food or eating in response to environmental food cues) were previously related to a higher weight in children (241), these children might benefit from interventions to avoid excessive food consumption, especially of noncore foods. Higher scores in 'Desire to Drink' seem to have different effects on food consumption depending on age. While higher scores in 'Desire to Drink' was associated with a higher consumption of SSB in pre-adolescent (244), in younger children (6-8 years) this trait was associated with a higher consumption of fat-containing milk and lower consumption of skimmed milk (242).

5. A conceptual framework for establishment of eating habits and its associations with appetite and obesity

Eating is a complex social and cultural practice, influenced by many factors from individual factors to macro-level social, physical and economic factors (245). At the social level, parents at early ages and peers in later childhood/adolescence seem to have a particular impact on children's eating habits. The social environment may influence children's food choices and eating habits through direct mechanisms such as offering specific foods to the child or indirectly by role modelling, social support, and social norms (44, 45). The physical environment also influences which foods are available to eat (e.g. meals at school), while at macro-level (e.g. food production and marketing) the influence is more distal but with a substantial impact on people's dietary intake (245).

This thesis will focus on modifiable individual eating behaviours and influences at the individual and social level while acknowledging that these behaviours occur and are impacted by the wider context of the social, economic and cultural environment (figure 5.1). Specific eating habits acquired during childhood could have an important impact on immediate and later health of children. Given that dietary intake has previously shown some degree of stability over time, we hypothesized that other dimensions of eating habits, such as meal patterns and varied diet may also track from preschool age to school age. Despite the fact that this is an important area of research, no published studies so far has addressed these issues.

Overall, eating behaviours related to appetite established early in life and seems to have an important genetic influence (209). However, environmental impact cannot be ruled out leaving us space to modify less healthy behaviours (208, 209). Research on the influence of early eating habits on appetite-related eating behaviours, using a prospective approach is scarce and usually focuses only on breastfeeding or complementary feeding

(246, 247). The majority of previous studies focused on a unidirectional relationship between eating behaviours related to appetite and dietary habits, but a bidirectional relationship cannot be discarded. One of the few prospective studies on this thematic suggested that following a less healthy dietary pattern early in life increases the risk of disordered eating behaviours later in childhood (248). The exploration of other characteristics of eating habits (e.g. eating frequency and diet variety) and its influence on eating behaviours will increase clarification on the establishment of appetite-related eating behaviours. In addition, the consideration of different approaches to evaluate influence of eating habits (meal patterns and time-of-day food intake) on adiposity is scarce in literature and requires more in-deep research.

Figure 5.1 summarizes the conceptual framework developed for this research for assessing early life determinants of children's eating habits and associations between different dimensions of children's eating habits and appetite and obesity.

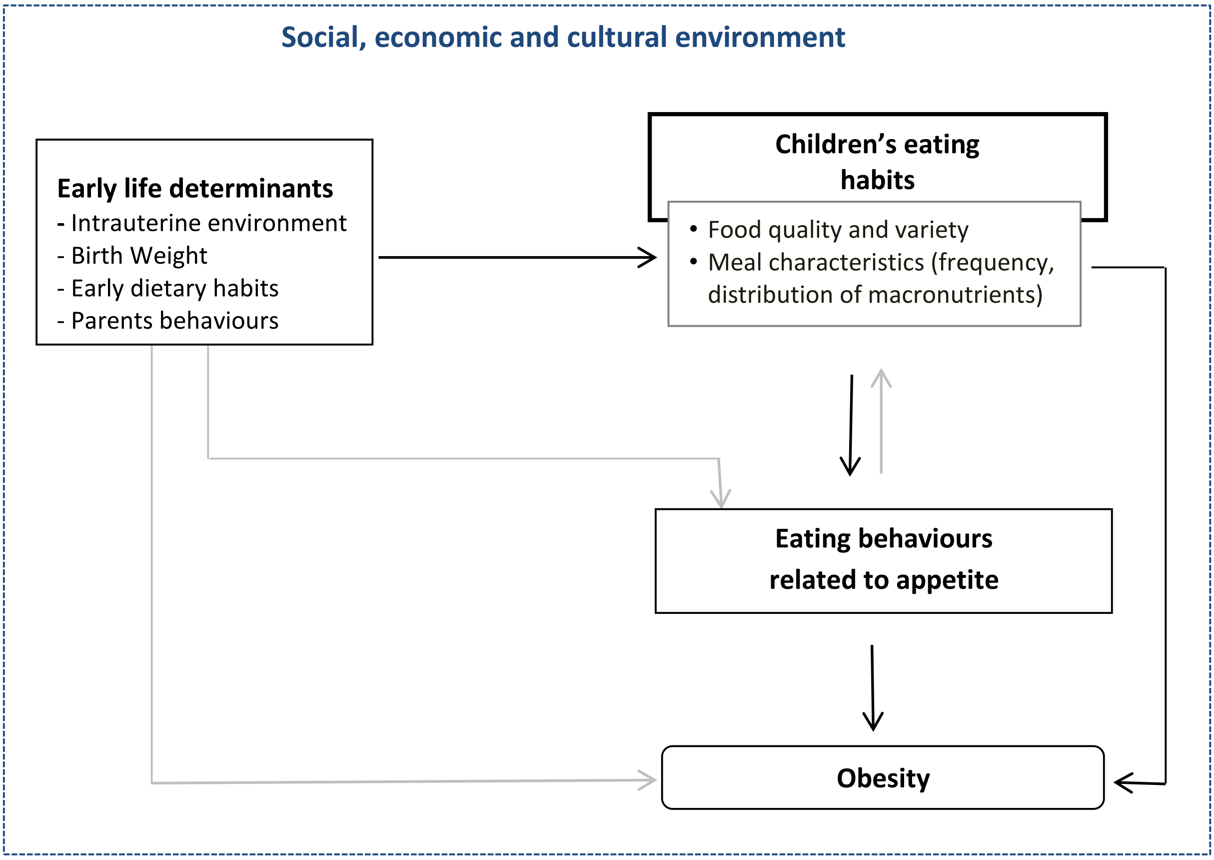

Figure 5.1. *Conceptual framework for evaluating determinants of children's eating habits and associations between diet and children's eating behaviours related to appetite and obesity.*

OBJECTIVES

The main aim of this thesis was to examine the tracking of eating habits from preschool to school age and the effect of different characteristics of diet on eating behaviours related to appetite and weight status at 7 years of age. We planned to answer these questions through the following specific objectives:

I. To test and calibrate a non-quantitative food frequency questionnaire in 4 and 7-year-old children. [paper I]

II. To evaluate the tracking of dietary intake in childhood and effects on diet quality. [papers II and III]

III. To estimate the influence of early life factors (pregnancy characteristics and neonatal/infancy characteristics) and socio-economic characteristics on tracking of diet quality during childhood. [paper III]

IV. To evaluate the effect of tracking diet variety from 4 to 7 years of age on eating behaviours related to appetite at 7 years of age. [papers IV]

V. To assess the effect of frequency and type of meals at 4 years on eating behaviours related to appetite and weight status at 7 years of age. [paper V and VI)]

VI. To assess the effect of time-o-day energy and macronutrients intake at 4 years on eating behaviours related to appetite and weight status at 7 years of age. [paper VII]

RESEARCH METHODS

1. Participants

This thesis used data from the population-based birth cohort Generation XXI (G21) assembled in the metropolitan area of Porto (2005-2006) (249), and data from the National Food, Nutrition and Physical Activity Survey of the Portuguese general population, 2015-2016 (Portuguese acronym: IAN-AF 2015-2016 Survey) (250). From these two datasets, papers in this thesis use prospective data from G21 collected at the ages of 2, 4 and 7 (G21), and cross-sectional data from the IAN-AF 2015-2016 Survey Portuguese children aged between 3 and 9 years. A general description of the participants and data collection of both studies is provided below. The selection of participants eligible for each analysis depends on the specific objectives of the research and is described in detail in the methods sections of the individual manuscripts

1.1. The birth cohort Generation XXI (G21)

The G21 is the first prospective Portuguese population-based birth cohort, assembled in Porto. The project G21 aims to characterize the prenatal and postnatal growth and development, identifying its determinants to better understand the state of health in childhood and later in life (249). Recruitment was conducted between April 2005 and August 2006 according to the following eligibility criteria: mothers living in one of the six municipalities of the metropolitan area of Porto (figure 1); delivering at the public hospitals covering those municipalities; and giving birth to live babies with gestational age >24 weeks. These maternity units were responsible, at enrolment, for 91.6% of the deliveries in the entire catchment population. Mothers were invited to participate 24 to 72 hours after delivery, and of the invited mothers, 91% accepted to participate. A total of 8647 children and 8495 mothers were enrolled at baseline.

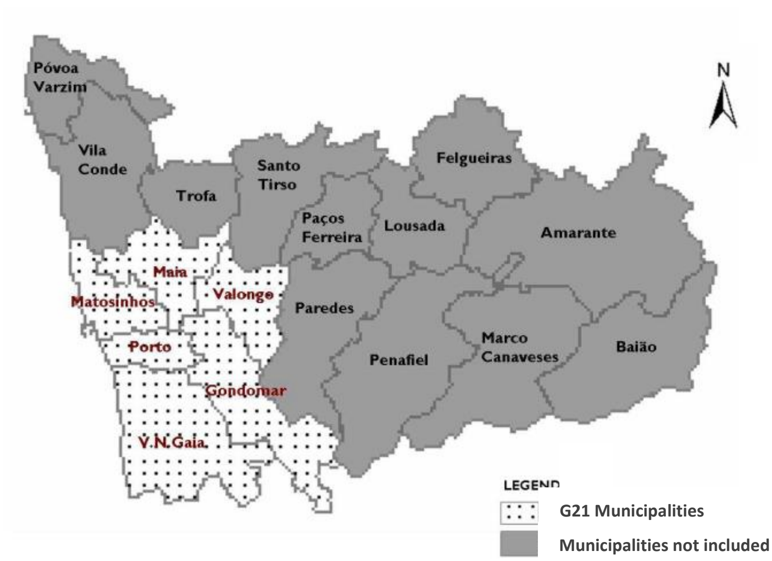

***Figure 1**. Porto Municipalities include in the G21 study*

When compared the puerperal women from the catchment area, delivering in 2005 and 2006, women participating in the cohort were slightly younger but similar in marital status. The prevalence of caesarean section was slightly higher than the overall prevalence in Portugal in 2004. The cohort had a similar gender proportion but a higher proportion of multiple foetuses as compared to the national percentages. When compared to the Portuguese deliveries in 2005 and 2006, the cohort presented a higher proportion of preterm and low birth weight newborns, probably because all the maternities enrolled had highly differentiated perinatal support (251).

Subsamples of the cohort were evaluated at the ages of 6, 15 and 24 months (n=1555, n=1043 and n=855 respectively). At 2 years of age, a subsample of children was re-evaluated in two phases: those completing 2 years of age between April and August of 2007 were invited to participate in the first phase; later, all children born in January 2006 were invited to a similar evaluation in January 2008. In the present thesis we used data from the sub-sample evaluation at 2 years and from re-evaluations of the entire cohort at 4 years of age (2009-2011, 86% participation), and at 7 years of age (2012-2014, 80% participation). The evaluations were performed by face-to-face interviews or, for those families not able to participate in-person (20% and 15% at 4 and 7 years, respectively), it was performed by telephone using a shorter version of the questionnaire.

Similar procedures were adopted at all evaluations. Data were collected by trained interviewers using structured questionnaires that gathered information on sociodemographic, clinical and behavioural characteristics. Anthropometric measures were also performed.

1.2. National Food, Nutrition and Physical Activity Survey of the Portuguese general population (IAN-AF 2015-2016)

The IAN-AF 2015-2016 (hereby referred to as IAN-AF) aimed to collect nationwide data on eating habits and physical activity and to evaluate the relation of these dimensions with health determinants (252). Data were collected by two interviews in a representative sample of the Portuguese general population, aged between 3 months and 84 years of age, selected from the National Health Registry, by multistage sampling, in each of the seven Statistical Geographical Units of Portugal (NUTII) and weighed according to sex and age groups (252). In the first step, sampling was based on the random selection of the Primary Health Care Units (PHCUnit) stratified by the seven NUTS II, weighted by the number of individuals registered in each PHCUnit. The second step of sampling was based on the random selection of registered individuals in each PHCUnit, according to sex and age groups. Participation was independent of the regular attendance to the National Health System. Figure 2 shows the spatial distribution of the PHCUnits, randomly selected by the seven regions (NUTS II), weighted by the number of registered individuals.

Abbreviations: NUT, Statistical Geographic Units of Portugal

Figure 2. *Spatial Distribution of the Primary Health Care Units, Randomly Selected by the Seven Statistical Geographic Units of Portugal (NUTS II), weighted by the Number of Registered Individuals – The IAN-AF 2015-2016 Survey (source:* (252)*)*

The sample selection was performed in consecutive recruitment waves in order to use the most updated versions of the National Health Registry lists. Criteria for exclusion were living in collective residences or institutions, living in Portugal for less than 1 year (non-applicable to infants), non-Portuguese speakers, with diminished physical and/or cognitive abilities that hamper participation (e.g. blind, deaf, with diagnosed dementias), and dead.

A total of 5811 participants completed the two interviews, and 6553 completed only the first one. Approximately 23% of the sample was children under age 10 years. The participation rate among eligible individuals was 33.4% (6553/19,635), considering the first interview and 29.6% (5811/19,635) considering completion of the two interviews. The participation rates were higher in children and adolescents (approximately 40%) and much lower in the elderly group (approximately 20%).

Participants' characteristics were compared with participants who refused to participate but have accepted to answer a short refusal questionnaire by phone. Individuals who refused to participate were older (over 65 years: 22% vs. 13%) and less educated (over 12 years: 19% vs. 27%). Regarding variables representing the main research areas of the survey, the differences between participants and refusals were considered of small magnitude: F&V consumption (≥5 portions/day: 18.6% vs 18.1%), practice of regular structured physical activity (33% vs. 39%), and prevalence of obesity (12.4% vs. 12.7%).

The estimates driven by the IAN-AF study include the weighting of the sample data. The weighted sample represents the number of individuals from the national general population that are represented by each individual in the study sample. This weighting includes: initial weights taking into consideration different probability of the sampling units selection; a second weight to overcome the different probability of individuals being selected in each Unit, by sex and age and correction of these initial weights for non-response bias (252).

2. Ethical considerations

All the phases of this research complied with the Ethical Principles for Medical Research involving Human Subjects expressed in the Declaration of Helsinki. Written agreements from the parents or legal representatives were required for children. All documents with identification data were treated separately and stored in a different dataset.

The G21 study was approved by the University of Porto Medical School / S. João Hospital ethics committee and a signed informed consent according to the Declaration of Helsinki was required for all participants.

For the IAN-AF project, ethical approval was obtained from the National Commission for Data Protection, the Ethical Committee of the Institute of Public Health of the University of Porto and from the Ethical Commissions of each one of the Regional Administrations of Health.

3. Data collection

3.1. Generation XXI (G21)

At baseline, trained interviewers were allocated to the five hospitals and were responsible for presenting the project G21, and subsequent participation invitation, to the eligible mothers, between 24-72 hours after delivery. Data were collected, in a face-to-face interview, using structured questionnaires designed by a multidisciplinary team. Anthropometric evaluation of parents and babies were performed by the same interviewers. Clinical records were also reviewed at birth to retrieve data on prenatal care, pregnancy complications, delivery and neonatal characteristics. Data on demographic and social characteristics, lifestyle behaviours (e.g., diet, physical activity), and medical history were also collected.

3.1.1. Social and demographic characteristics (G21)

Data on socioeconomic characteristics at child's birth considered in this thesis were the maternal age in completed years (used as continuous); the maternal education as the number of completed schooling years (used as continuous) and the maternal work status [not working (unemployed, student, seeking the first job, retired and housewife) or working (part or full-time and working-student)].

Family characteristics at child's 4 years of age were also considered. Siblings' age was used to define a categorical variable (older, younger, and no siblings), and child's main day-time caregiver was defined as family (parent or another family member) or non-family (kindergarten or babysitter). Also for some analyses, the maternal age, estimated as the difference between the date of birth and the date at the 4-year interview, and maternal education evaluated at the 4-year-evaluation were included.

3.1.2. Maternal behaviours (G21)

As described elsewhere (253), mothers were considered as having gestational diabetes or hypertension during pregnancy when recorded on obstetrical records as a diagnosis during the current pregnancy. Information on maternal smoking during pregnancy was asked at baseline. For each trimester mothers were classified as no-smoking during that period or smoking (including less than 1 cigarette per day or at least 1 cigarette/day).

Maternal diet was assessed at the 4-year-evaluation by a food frequency questionnaire (FFQ) adapted from a previous one validated for the general adult population (254) and for pregnant women in a subsample of the G21 cohort (255). The FFQ included 18 items and assessed consumption in the previous year with 9 response options ranging from 'never' to '4 or more times per day'. Frequencies of consumption were converted into daily frequencies. A maternal food index (*a priori* dietary pattern) previously developed was used to evaluate the influence of maternal food intake on the child's food intake (48). Briefly, the dietary score considers eight food items/groups: milk, fish, red meat, bread, fruit, vegetables, energy-dense foods (EDF) and sugar-sweetened beverages (SSB). For each item/food group, quartiles of frequency of consumption were obtained and a score was assigned with a range from 1 to 4 points, according to the increase in the quartile of consumption (milk, bread, F&V) or decrease in quartiles of consumption (red meat, EDF and SSB). Points assigned were summed up, resulting in a dietary score that reflects maternal dietary quality, with a higher score representing a better diet.

3.1.3. Children's dietary intake (G21)

Breastfeeding

Information on breastfeeding considered in this specific research was collected retrospectively when the child was 4 years of age. The type of breastfeeding and the duration were recorded and a variable of duration of non-exclusive breastfeeding was defined, being categorized into never or less than 16 weeks, between 16 weeks and 20 weeks, and more than 20 weeks.

Child's diet – evaluated by the food frequency questionnaire (FFQ)

At the 2 years follow-up evaluation from the G21 cohort, a 17-item-FFQ was developed to evaluate the consumption of energy-dense foods, not so often consumed. Response options were in six-point frequency scale ranging from never to every day, then transformed into average daily frequencies. Energy-dense foods and beverages were defined by summing up daily frequencies of sugar-sweetened beverages (carbonated and non-carbonated), crisps, pizzas, hamburgers, cakes, chocolates, sweets and candies.

Dietary intake at 4 and 7 years was assessed by two FFQs aiming to assess the overall frequency of consumption in the previous six months. Response options were collected using nine categories: ≥ 4 times/day, 2-3 times/day, once a day, 5-6 times/week, 2-4 times/week, once a week, 1-3 times/month, <once a month, and never. At 4 years, additional food items were added to the previous FFQ based on the information collected from the 2-year food diaries, to allow an estimation of the total diet of these children. The food items added included questions regarding frequency of dairy (6 items), meat (2 items), fish, bread, cookies (2 items), vegetables (3 items), fruit, pasta, rice and potatoes, and added fat, achieving a total of 35 items. This decision was based on the low participation of parents in the completion of the food diaries, comparing to the FFQ. At 7 years, a few other items were added, namely milk with chocolate, breakfast cereals, fresh fruit juice; separation between salty pastry based on fish or meat, all meat was grouped together (red and white meat) as well as yoghurts with or without sugar. We decided not to separate the

yoghurts due to its low consumption at 4 years and the difficulty of parents to identify the type of yoghurts. The 7-year-FFQ had 38 food items.

Child's diet – evaluated by food diaries

When children were 4 and 7 years of age, parents or other main caregivers were asked to complete a 3-day food diary, 2 weekdays and 1 weekend day (non-consecutive days), previously to the face-to-face interview. Oral and written instructions were given for the correct completion of the food diaries and for the quantification of food portions. Caregivers were asked to provide detailed descriptions of each food and drink consumed by the child, including the method of preparation, recipes and place of consumption, whenever possible. They also recorded the day, time and location for each eating occasion in an open section. In relation to the prepared dishes, instructions were given to provide recipe details, including ingredients and cooking methods. Parents were taught how to use household measures and standard units to quantify the food portions. It was advised to let children follow their usual diet and to ask for the help of other caregivers in the case the child was out of the home during the day.

The food diaries' codification process was conducted a posteriori by a team of trained nutritionists, using an age-specific food-coding manual previously developed by our research team. Nutrient intake was estimated using the software Food Processor SQL (2004-2005 ESHA Research, Salem, Oregon), based on the Food Composition Table of the United States of America Department of Agriculture (256). For typically Portuguese foods or culinary dishes, new codes were created with national nutritional information, as previously described (257, 258).

Information from food diaries were used first to calibrate and validate the dietary intake obtained through the FFQ at 4 and 7 years [paper I] and, second, to assess specific dimensions of children's eating habits not evaluated by an FFQ, such as eating frequency [paper V] and time-of-day energy and macronutrient daily distribution [paper VI].

Meal definition

Two approaches were used to define an eating occasion (EO), main meals and snacks (figure 3), using food diary data. First, a neutral approach was used: an EO was any occasion when food or drink was consumed separated by 15 or 30min and provided a minimum of 50kcal. A sensitivity analysis was performed using the 15 and 30 min definitions, and as we did not find significant differences on the associations and to avoid an artificially high number of EO we opted for the cut point of 30 min. Second, a time-of-day approach was used. In food diaries, caregivers did not distinguish the meals; they only recorded time and place of consumption, and therefore in the codification process the team of trained nutritionists classified and distinguished between meals (e.g breakfast, lunch, mid-day) according to the type of foods, periods of time and place of consumption. Using this variable, a decision tree was applied to define the best period of time for breakfast, mid-morning, lunch, mid-afternoon, dinner and supper. After that, for the period of time defined as breakfast, lunch and dinner, the EO with higher energy content (>=50kcal) was defined as the main meal and all other EO separate by 30 minutes, were considered as snacks

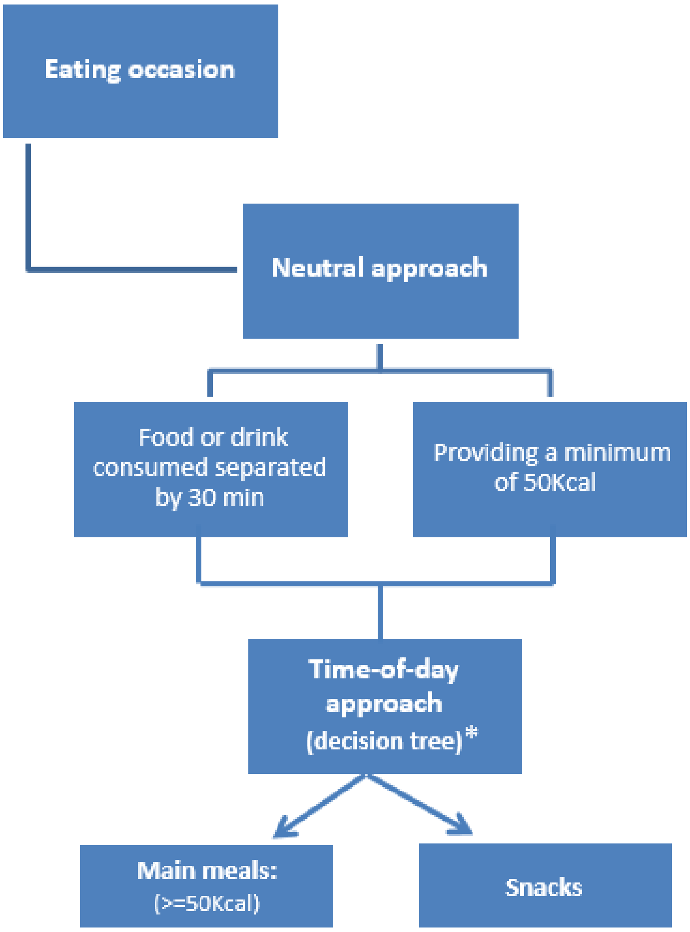

Figure 3. *Flowchart for the definition of eating occasions and differentiation between main meal and snacks, at 4 and 7 years of age, using data from G21.*

**Main meal was considered the eating occasion with the higher energy intake at breakfast (between 6-9.30 am), lunch (between 11 am-2.30 pm) and dinner (between 7-9.30 pm). The other periods of time were considered for mid-morning, mid-afternoon and supper, and all meals were classified as snacks.*

3.1.4. Children's physical activity and sedentary behaviours (G21)

At 4 years of age, the practice of regular physical exercise was assessed through a structured question asking type and duration (minutes per week) of physical exercise, then converted into a qualitative variable of regular practice of structured physical exercise (non-practitioners vs. practitioners). Daily screen time was also assessed by a structured question asking average daily minutes spent in front of a screen (television, computer or game devices) during both week and weekend days. The average of daily screen was calculated and categorized into less than 120 minutes versus 120 minutes or more per day, as less than 2 hours per day of screen time is recommended (259).

3.1.5. Anthropometrics (G21)

The weight gain during pregnancy was obtained by subtracting the self-reported final pre-delivery weight by the pre-pregnancy weight and categorized as 'less than or equal to 10kg', 'between 10 and 20kg', higher than 20kg. At the 4-year-evaluation, objective measures of maternal weight and height were performed by a team of trained examiners, according to standard procedures. The height was measured to the nearest 0.1 cm with a wall stadiometer (SECA®, Hamburg, Germany) and weight was measured to the nearest 0.1 kg with a digital scale (Tanita®, Arlington Heights, Illinois, USA). The BMI was computed as weight in kg divided by height in square meters. This variable was used either as a continuous or categorized as normal or underweight (<25 kg/m2) and overweight or obese ≥25 kg/m2), according to WHO cut-off points.

A detailed description of newborns' anthropometrics is described elsewhere (260). Briefly, birth weight was abstracted from medical records by trained interviewers at baseline evaluation. During the hospital stay, trained examiners weighed babies to the nearest 1g using infant scales. The birth weight adjusted for gestational age were computed as a continuous variable according to the Canadian reference (261). Accordingly, children were categorized as small for gestational age (children with birth weight below the 10th percentile for their gestational age and sex), appropriate for gestational age or large for gestational age (children weighing above the 90th percentile for their gestational age and sex) (261).

At the 2-year-evaluation, the recumbent length was measured to the nearest 0.1 cm with a length measuring board or, when children were able to stand alone, height was measured to the nearest 0.1 cm with a wall stadiometer (SECA®, Hamburg, Germany). At 4 and 7 years of age, a wall stadiometer was also used to measure children's height. Height was measured from the top of the head to the bottom of the feet without shoes.At the ages of 2, 4 and 7 years, weight was measured to the nearest 0.1 kg using a digital scale (Tanita®, Arlington Heights, Illinois, USA). Moreover, weight was measured in light clothing and without shoes. Age- and sex-specific BMI standard deviation scores (BMI z-scores) were computed at the three ages according to the WHO's child growth standards (262).

3.2. IAN-AF

Procedures of data collection in the IAN-AF survey were based on the European Food Safety Authority (EFSA) guidelines in view of the EU Menu methodology (73).

Overall, the IAN-AF survey evaluated the following dimensions:

- Dietary and nutritional intake including eating habits and dietary supplementation use (24h recall, food diaries and food propensity questionnaire);
- Food insecurity;
- Physical activity [International Physical Activity Questionnaire, diaries, Activity Choice Index;
- Sociodemographics and general health data;
- Anthropometrics;
- Biochemical indicators of nutritional intake (in sub-sample).

Data were collected in two interviews by trained interviewers using Computer-Assisted Personal Interviewing, during 12 months (from October 2015 to September 2016), distributed over the four seasons and including all days of the week in order to incorporate seasonal effects and day-to-day variation in food intake (250). A total of 5811 participants completed two interviews and 6553 completed only the first one. From the total children (<10 years) 1329 had complete data in the two face-to-face interviews. For the present thesis, we included data from children aged 3-9 years of age (n=604). The collection of children's data is briefly explained below.

3.2.1. Sociodemographic, behavioural and health-related data (IAN-AF)

For children's participants, parents or other main caregiver answered the questionnaire. Children sociodemographic data included questions on sex and birth date (retrieved from the sample selection lists), parents' education level and professional activity, and parents and child's country of origin and nationality. The presence of any disease that currently required regular medical care was also inquired. The general questionnaire also included questions related to breastfeeding, consumption of types of milk ion and food diversification, and a brief food frequency questionnaire.

3.2.2. Children's dietary intake (IAN-AF)

For children under 10 years, dietary intake was obtained by two non-consecutive one-day food diaries followed by a face-to-face interview. This interview allowed the parent or another main caregiver to add details related to food description and quantification. The time interval between the first and second interviews was set at 8 to 15 days. The days of reporting were randomly selected, but participants were able to change them, according to their own availability for the interview.

All foods, including beverages and dietary supplements consumed by the children during a 24-hour period (from midnight to 11.59pm), were recorded per eating occasion and quantified and described as eaten. The place (at home, kindergarten, friends/family, restaurant or another) and mealtime of consumption were also recorded for each eating occasion. The food diary also included photos of household measures that caregivers could choose to quantify children's consumption. Complete description of the food or beverage was asked, including the brand, nutrition composition (e.g sugar sweetened, skimmed), preservation methods (e.g. frozen, fresh, canned), packaging (paper, plastic, glass), preparation and cooking methods (fried, microwaved, grilled) and also quantification method. The description of home-cooked meals were also requested, including name of the recipe, ingredients, complete description of the foods included in the recipe and quantity.

The 'eAT24' Module

During the face-to-face interview, the food diaries completed by the caregiver were included in the 'eAT24' module (Electronic Assessment Tool for 24-hours recall) which allowed the assessment of dietary data by an Automated Multiple-Pass Method for 24-hours (5 steps) (263). The 'eAT24' includes 12 predefined food consumption occasions to standardize the reporting of events (intake of foods and/or drinks) during the 24hours: 'before breakfast', 'breakfast', 'during morning', 'before lunch', 'lunch', 'after lunch', 'during afternoon', 'before dinner', 'dinner', 'after dinner', 'during evening/at night' and 'liquids intake'. Breakfast, lunch and dinner could only be selected once. The software allows subsequent conversion of foods into nutrients, using by default the Portuguese food composition table (264).

The quantification of foods consumed was performed using different methods:

a) direct quantification by the caregiver of food consumed in grams or in millilitres;
b) standard unit method, for foods consumed in distinguishable units with more or less standard weights (e.g. 1 apple);
c) photo method (a digital colour food picture book was developed including 186 food photo series with six portions each food/recipe item and 11 household measures photo series); a electronic picture book for the quantification of food portions was produced and included in the eAT24 module.
d) household measure method (predefined household measure list – e.g. 1 cup)

e) unknown method (a mean portion is associated with the food item, when the subject is not able to estimate the quantity consumed).

Meal definition

For IAN-AF children, the ‘participant-identified’ approach was used to define an eating occasion, main meal and snack. The parents or another main caregiver could choose any of the 12 predefined food consumption occasions (ranging from ‘before breakfast’ to ‘during evening/at night’), keeping in mind that breakfast, lunch and dinner could only be selected once. For each food consumption occasion a place and time of consumption was attributed.

All eating occasions reported in the food diaries and included in the ‘eAT24’ module were considered separate eating occasions if the time of eating was different from other eating occasion, providing at least 50 Kcal of energy. The differentiation between main meals and snacks were similar to the definition applied to study in G21: the main meal was defined as breakfast, lunch or dinner (respondents could only select one of each) and all the other eating occasions were considered snacks. Daily eating frequency describes the total number of separate eating occasions during a 24-hour period, as the result of the average of the two reported days.

3.2.3. Children’s physical activity (IAN-AF)

For children aged six years or older, physical activity was assessed by diaries including two consecutive days during the week and two days on the weekend. These diaries were adapted from a model previously proposed (265) in which children registered their activities in a logbook for each 15 minutes interval during four days, according to written instructions. However, the diaries were not used in children younger than 6 years and so additional questions on sedentary behaviours were asked for all children, including children from 3-9 years of age, and were used in this research as proxies of children’s physical exercise. These questions included usual daily screen time spent in a week and weekend day (watching TV or playing games) and practice of regular and structured physical exercise (categorized as non-practitioners vs. practitioners). The daily time spent watching TV or playing games were averaged between weekdays and weekend and converted into hours (categorized as: less than 2 hours per day watching TV or playing games, between 2 and 3 hours per day and at least 3 hours per day), and used as an indicator of a more sedentary lifestyle (259).

3.2.4. Anthropometrics (IAN-AF)

Anthropometric measurements, including length/height, weight and body circumferences, were performed in both children and adults according to standard procedures (266), by trained researchers. In addition, for children weight and height were retrieved from their health booklets, considering the last registry. Height was measured to the nearest centimetre, with participants in a stand position with light clothing and barefoot, using a portable wall stadiometer (SECA® 213, Hamburg, Germany). Body weight was measured in the same conditions, to the nearest tenth of a kilogram using a digital scale (SECA® 813, Hamburg, Germany). The child's age- and sex-specific body mass index (BMI) z-scores were calculated according to the WHO criteria (262).

RESULTS

Paper I

Evaluation of a short food frequency questionnaire for dietary intake assessment among children

Sofia Vilela, Milton Severo, Teresa Moreira, Elisabete Ramos, Carla Lopes

[Submitted]

Abstract

Background/Objectives: The objective of this study was to evaluate the performance of a short food frequency questionnaire (FFQ) to assess dietary intake at 4 and 7 years of age, against 3d food diaries (FD) and serum biomarkers, using two methods to convert the FFQ to daily intake in grams and nutrients (standard and z-score method).

Subjects/Methods: The present analysis comprises data from 2482 4-year-old children and 3511 7-year-old children, from the birth cohort Generation XXI (Porto, Portugal). To estimate daily consumption from the FFQ, the frequency response was multiplied by a standard mean portion (standard method) or adjusted with data from the FD (z-score method). The dietary intake obtained from the FFQ was compared with the FD and serum biomarkers, using Intra-Class Correlation Coefficients (ICC), de-attenuated Pearson's correlation coefficients and Bland Altman analysis.

Results: In general, the mean daily food intake estimated by the z-score method had a higher agreement with the FD, than the standard method. The highest ICC was obtained for 'vegetable soup' (ICC=0.536), using the z-score method, compared to an ICC of 0.373 using the standard method. Significant correlation coefficients were observed for all nutrients; the average of correlation coefficients was 0.39 at 4 years and 0.42 at 7 years of age. For the majority of nutrients, the correlation between mean and mean difference was lower using the z-score method, in comparison with the standard method.

Conclusions: The results suggest that the FFQ is a reasonably good instrument to estimate dietary intake in children. Moreover, adjusting the FFQ portion size, by using a z-score method, seems to increase the accuracy of dietary data in children.

Keywords: children; cohort studies; food frequency questionnaire; food diaries

Introduction

Accurately measuring dietary intake is considered one of the greatest challenges for epidemiological purposes.[1-3] In children, the collection of dietary data is particularly difficult due to the requirement of using a proxy respondent. Considering this, the evaluation of the performance of dietary intake assessment methods among children is essential to obtain high quality data on food intake in this population.

Dietary methods commonly used to assess the diets of children include respondent-based methods, such as food diaries (FD) and food frequency questionnaires (FFQ)[3]. Specifically, FFQs are widely used in large-scale prospective studies investigating links between diet and disease in both adults and children[4-7], because they are easy and simple to administer, relatively inexpensive and have a low respondent burden. Even though FFQ are broadly used, there are limited valid and reliable brief FFQ for measuring whole-of-diet intake in young children.[8]

Multiple 24-h recalls and FD are usually used for the evaluation of the validity of a FFQ.[9, 10] When available, biomarkers may be an alternative or supplementary reference method for the validation of some nutrients intakes since their measurement errors are independent of those of FFQ. [11] A combination of methods, self-reported methods and biomarkers (for specific nutrients), improve the process of validation of an FFQ, and has been used in several large prospective studies [12-14]. In a given population, one of the main components of the adaptation of an FFQ is the portion size assessment. Inappropriate portion sizes can lead to significant inaccuracy in dietary intake estimation. The classical method that uses a 'standard' portion applied equally to all responders might reduce sensitivity if portion sizes vary within the population.[15] Researchers have tested other methods to handle missing portion size in FFQ, in both adults and adolescents, such as stochastic methods[16], or a combination of FFQ with FD or 24-h recall data.[17-19] New methods to calibrate FFQ portion size data are necessary to be tested in children.

To improve children's diet it is required to accurately measure their current consumption. Moreover, the use of methods with low validity seriously attenuates the associations between nutritional intakes and outcomes in health.[20] Therefore, it is necessary to develop and validate age-specific instruments for the evaluation of usual food and nutrient consumption.

This study aimed to evaluate the performance of a short FFQ to assess diet of children at 4 and 7 years of age, against 3-non-consecutive-day FD and serum biomarker measurements, using two methods to convert the FFQ to nutrients:

a) the standard method, classical method of multiplying the frequency response option by a standard mean portion, specified for each item.

b) the z-score method, adjusted with data from 3d FD, at 4 and 7 years of age.

Subjects and Methods

The present study was based on the population-based birth cohort Generation XXI, previously described.[21] Briefly, newborns and respective mothers were recruited during 2005-2006 at the five-level III maternity units of metropolitan area of Porto, Portugal. Recruitment was conducted according to the following eligibility criteria: mothers living in one of the six municipalities of the metropolitan area of Porto; delivering at the public hospitals covering those municipalities; and giving birth to live babies with gestational age >24 weeks At enrolment, these maternity units were responsible for 91.6% of the deliveries in the whole eligible population. Mothers were invited to participate 24 to 72 hours after delivery, and of the invited mothers, 91% accepted to participate. A total of 8647 children and 8495 mothers were enrolled at baseline. Data were collected by trained interviewers using structured questionnaires that gathered information on sociodemographic, clinical and behavioural characteristics. Anthropometric measures were also performed. At 4 and 7 years of age, the entire cohort was invited to participate in the follow-up evaluation, and 86% and 81% of the children were reevaluated, respectively. Trained interviewers, in face-to-face interviews and using structured questionnaires, were responsible for data collection on demographic and social conditions, lifestyles (including dietary intake), child's health status and objective anthropometric measures, at baseline and follow-up evaluations. Children's body mass index (BMI) was classified according to age- and sex-specific BMI standard z-scores developed by WHO.[22]

The study was approved by the University of Porto Medical School/S. João Hospital Centre Ethics Committee. The signed informed consent, according to the Helsinki Declaration, was required for all participants and was taken for legal representative of the children. The present analysis includes children that had data from the FFQ and 3d FD in each follow-up, achieving a sample of 2482 children at 4 years and 3511 at 7 years of age.

FFQ

At the 2 years follow-up evaluation from G21 cohort, a 17-item-FFQ was developed to evaluate the consumption of energy-dense foods, not so often consumed. [23] At 4 and 7 years the FFQs were developed with the aim of assessing habitual dietary intake of children and queried frequency of intake for 35 food items at 4 years, and 38 food items at 7 years. At 4 years the FFQ was based on the 2-year-FFQ and on the information collected from 2-day food diaries at 2 years.

At 7 years, a few items were included in the FFQ, taking into consideration information reported in the 3-day food diaries from 4-year-evaluation and difficulties in reporting children's dietary intake in the 4-year-FFQ. The items included were milk with chocolate, breakfast cereals and fresh fruit juice; some alterations were also performed such as separation between salty pastry based on fish or meat, all meat was grouped together (red and white meat), as well as yogurts with or without sugar. We decided not to separate the yogurts due to its low consumption at 4 years and the difficulty of caregivers to distinguish the type of yoghurts. As previously described[24], parents or another caregiver were asked how many times on average the child had consumed each food item in the previous 6 months. The nine frequency response options, ranging from 'never' to '4 times or more per day', were transformed into daily frequency of consumption. At both ages, a standard mean portion was defined for each food item (Supplemental Table 1) and similar food groups were created: 'Dairy', 'Cereals, cereal products and potatoes', 'Fruit & Vegetables', 'Meat, fish and eggs', 'Drinks', 'Fat spread', 'Sweets', and 'Salty snacks'.

Three-day FD

As previously explained[25], when the child was 4 and 7 years of age, parents or another main caregiver were asked to complete a three non-consecutive days estimated FD, before the face-to-face interview. Oral and written instructions were given to parents for the correct use of FD and how to quantify food portions; they were also instructed to let the children follow their usual diet. Parents were asked to provide a detailed description of each food and drink consumed by the child, including the method of preparation, recipes and place of consumption, whenever possible. A team of trained nutritionists was responsible for reviewing and coding the FD, using an age-specific food coding manual previously developed by our research team. The proportion of reported days was similar across different days of the week and weekend, at both ages.

Nutrient conversion

Nutrient intake was estimated using the software Food Processor SQL (2004-2005 ESHA Research, Salem, Oregon), based on the Food Composition Table of the United States of America Department of Agriculture.[26] For typically Portuguese foods or culinary dishes, new codes were created with national nutritional information, as previously described.[25, 27]

Biomarkers

A fasting blood sample was collected on the morning of the evaluations, at 4 and 7 years of age. Serum samples were stored in approximately 500µ aliquots at -80°C until analysis. Using a subsample of 160 children (50% from each follow-up evaluation), measurements of vitamin A and folate were conducted by S. João Hospital Center. Folate was measured by chemiluminescence using the immunoassay analyzer Architect i2000 SR (Abbott, USA) and vitamin A were measured by an HPLC Liquid Chromatography (Gilson, USA). UV detection was performed by a detector model 116 (Gibson, USA).

Statistical analysis

All statistical analyses were performed using the SPSS statistical software package version 22.0 (SPSS inc., Chicago IL., USA) and R 3.01. A significance level of 5% was adopted.

At both ages, to estimate daily consumption from the FFQ (as grams per day), two methods were performed and tested:

a) *standard method*: each frequency response option was multiplied by a standard mean portion (Supplemental Table 1) specified for each item;

OR

b) *z-score method*: adjustment of each food item, with the overall sample mean and standard deviation (SD) of that food item from FD, applying the formula:

$$\frac{(y - \bar{y})}{Sy} = \frac{(x - \overline{x})}{Sx}$$

⇔

$$y = \frac{(x - \overline{x})}{Sx} * Sy + \bar{y}$$

Legend:

$y = grams\ from\ food\ diaries, per\ each\ food\ item$

$x = frequency\ from\ FFQ, per\ each\ food\ item$

$\bar{y} = mean\ of\ grams\ from\ food\ diaries, per\ each\ food\ item$

$x = mean\ of\ frequency\ from\ FFQ, per\ each\ food\ item$

$Sy = standard\ deviation\ of\ grams\ from\ food\ diaries, per\ each\ food\ item$

$Sx = standard\ deviation\ of\ grams\ from\ FFQ, per\ each\ food\ item$

Dietary intake overestimation from FFQ is widely described in the literature [12, 28-30] and theoretically is possible to calibrate for this bias. In the present study the food diaries data were used to calibrate the dietary information from the FFQ.

To evaluate absolute agreement at both ages, mean intake of dietary intake obtained from FFQ (standard and z-score method) was compared with those from the FD using Intra-Class Correlation Coefficients and respective 95% confidence interval [ICC (95%CI)]. In the z-score method, all negative values were transformed into zero. Guidelines for interpreting ICC statistics suggest that values between 0.81-1.00 indicate almost perfect agreement, 0.61 - 0.80 substantial agreement, 0.41 - 0.60 moderate agreement, 0.21 - 0.40 fair agreement, and values less than 0.21 indicate a poor or slight agreement.[31]

At the nutrient level, several statistical analyses were performed. To evaluate the strength of association at the individual level, Pearson's correlation coefficients were calculated to estimate the association between nutrient intake derived by the FFQ (standard and z-score method) and those obtained through FD, or serum biomarkers. Pearson's correlation coefficients were de-attenuated using the following formula:

Cr=$ro\sqrt{1} + (\lambda x/nx)$

In the formula, Cr is the corrected correlation coefficient, *ro* is the crude correlation coefficients, λx is the ratio of within-person variance and between-person variance for each nutrient, and nx is the number of reports per child.

To evaluate the presence, direction and extent of bias at the group level, Bland and Altman's statistical method[32] was applied to nutrient data, including the mean difference, limits of agreement and correlation between mean and mean difference. We also calculated the paired t-test for the difference in all nutrients, to assess agreement at the group level. However, as we have a large sample, any difference was considered statistically significant ($p<0.05$), so we decided to calculate the Cohen effect size [33] to understand how substantial the differences were. The effect size was calculated by dividing the mean change in nutrient data (between FFQ and food diaries) by the SD of the difference. Cohen classified effect sizes as small ($d = 0.2$), medium ($d = 0.5$), and large ($d >= 0.8$) Bland and Altman analysis[32] was generated for the difference between the mean obtained with 3d FD and FFQ, using the equations [mean of FD –mean of FFQ], against the average of the 2 methods ([mean of FD + mean of FFQ]/2). Based on the Bland and Altman methodology, two methods are considered comparable if 95% of data plots lay within the limits of agreement (mean difference ± 1.96 s.d. of the difference). Regarding total energy intake, Bland and Altman's plots were also generated (for standard and z-score methods)

Results

Table 1 compares the individual and socio-demographic characteristics of the children with complete data on food intake through FD and FFQ (our sample), with those without FD information, at both ages. No significant differences were found for child's sex and BMI. At both ages, our sample had mothers slightly older and higher educated. At 4 years, our sample had a higher mean daily intake of 'Fruit & Vegetables' ($p<0.001$) and a lower intake of 'Drinks' ($p=0.007$). At 7 years, the same trend was observed. Furthermore, at 7 years our sample also had a higher mean intake of 'Meat, fish and eggs' ($p=0.006$) and a lower mean intake of 'Sweets' ($p=0.001$) and 'Salty snacks' ($p<0.001$).

At 4 years, comparing with FD, the standard method seemed to overestimate the food consumption more than the z-score method. Overall, the mean daily food intake obtained using the z-score method had a higher agreement with those from the FD, than the standard method (table 2).

The lowest ICC obtained was 0.048 (95%CI: 0.002,0.094) for 'Carbonated soft drinks (except colas)' in the standard method, while the same food item using the z-score method had an ICC of 0.139 (95%CI: 0.102,0.178). The highest ICC was obtained for 'Vegetable soup' (ICC:0.536; 95%CI: 0.508,0.564), using the z-score method, compared to an ICC of 0.373 (95%CI: -0.013,0.673), using the standard method (table 2).

At 7 years, using the same methodology, the z-score method was still the best method to estimate the food consumption through the FFQ, comparing to the consumption obtained through the FD. Similar to the results obtained at 4 years, the standard method seemed to overestimate the consumption in comparison with the z-score method. The highest ICC obtained was also for 'Vegetable soup' [(ICC=0.539, 95%CI: 0.515, 0.562)], using the z-score method, comparing to an ICC of 0.430 (95%CI: 0.080,0.637) using the standard method (table 3).

Table 4 presents the de-attenuated correlation coefficients between the FFQ and FD, for daily energy and nutrient intake, at both ages. Significant correlation coefficients were observed for all nutrients and were similar using the standard or z-score method. However, the conversion of the FFQ using the z-score method presented averages of mean nutrient intake more similar to the FD, than the conversion using the standard method. At 4 years, and using the z-score method, the correlation coefficients ranged from 0.112 for vitamin B12 intake to r=0.565 for total fat intake. The average of correlation coefficients was 0.39. At 7 years of age, and using the same methodology, the average of correlation coefficients was 0.42.

For the subsample of 160 children (80 at 4 years and 80 at 7 years of age), the de-attenuated correlation coefficients between FFQ standard method and plasma concentration of vitamin A [r=0.531 (p=0.008), at 4 years and r=0.282 (p=0.120), at 7 years] and folate [r=0.176 (p=0.365), at 4 years and r=0.425 (p=0.027), at 7 years) were similar to the correlation coefficients between FFQ z-score method and plasma concentration of vitamin A [r=0.552 (p=0.005), at 4 years and r=0.269 (p=0.187), at 7 years) and folate [r=0.183 (p=0.345), at 4 years and r=0.340 (p=0.079), at 7 years]. The ICCs between plasma concentration and FFQ were similar for z-score and standard method, at both ages, although not statistically significant.

Table 5 presents ICC, mean differences, limits of agreement, correlation between mean and mean difference and Cohen effect size, for daily energy and nutrients obtained from FFQ z-score method and FD, at 4 and 7 years. The same information for the standard method is shown in Supplemental Table 2.

At 4 years, the ICC ranged from 0.036 for omega 6 to 0.350 for calcium, using the z-score method, and raging from 0.013 for vitamin E to 0.265 for sodium, using the standard method. At 7 years, the ICC ranged from 0.032 for iron to 0.328 for calcium, using the z-core method, and raging from 0.041 for iron to 0.313 for Vitamin B6, using the standard method. Overall the correlation between mean and mean difference, as well as the Cohen effect size, was lower using the z-score method (table 5), in comparison with the standard method (Supplemental Table 2), at both ages. The limits of agreement were wide for most nutrients and ranged from positive to negative values. The Bland-Altman plot for energy intake at 4 years (figure 1A and B) and 7 years (figure 1C and D) indicated that around 95% of data plots fell within the limits of agreement, at both ages and using both methods. However, the graph suggested a lower concordance using the standard method (Figure 1A and C), with a more marked trend of overestimation of energy intake.

Discussion

The present results showed that the FFQ performed reasonably well in estimating intake of a number of food items and nutrient intake, at 4 and 7 years of age, using a z-score calibration.

Considering the definition of ICC values[31], the results showed that 17 out of 30 food items had ‘fair’ to ‘moderate’ agreement with FD using the z-score method, against only 12 food items using the standard method. At 7 years, using the same z-score equations, the results were similar, the z-score method had a higher agreement with the FD. Low agreement for food items such as ‘Meat’, ‘Sweets’ and ‘Salty snacks’ was observed. Due to the nature of our method of reference, 3d FD collected over a one week period, it is expected that items eaten more often, such as ‘Fruits & Vegetables’ or ‘Dairy products’, would be more correlated compared to food items ate less often, such as candy or fast food.[34] An sensitivity analysis was perfomed in random sample at 4 years. Using 70% of the sample we calculated the z-score equations and apllied it to the others 30% of the sample. The ICC obtained to major groups were similar to those obtained using the all sample.

The differences in the two methods (standard and z-score method) regarding the food intake translated into differences in nutrient intake. For most of the nutrients, the z-score method obtained a higher correlation with FD, than the standard method. Previous studies reported similar correlation coefficients between nutrient intake estimated by FFQ and FD or 24-h recall.[12, 35] In addition, at 4 years the correlation between FFQ and plasma concentration of vitamin A and folate was also higher for the z-score method in comparison to the standard method. At both ages, in the majority of nutrients *the correlation* between mean and mean difference, as well as the Cohen effect size, was much lower using the z-score method in comparison with the standard method. This shows a decrease in the proportional bias using the z-score approach. The limits of agreement were wide for most nutrients and ranged from positive to negative values, implying that both overestimation and underestimation occurred in children's dietary intake estimation from FFQ, comparing to food diaries. Although the z-score method performed better, the limits of agreement for some nutrients fell outside the dietary reference intakes (DRI). For example, the Estimated Energy Requirement (EER) at 4 years ranged from 1113 to 1629 kcal in girls, and from 1195 to 1763 kcal in boys.[36] The mean intake obtained from the z-score method was 1741 kcal (LOA: 870-2385kcal) and from the standard method was 2470 kcal (LOA: 689-2567 kcal). Regarding the calcium intake (Recommended Dietary Allowances (RDA): 1000mg/day, 4-8 years[37]), the LOA (using the z-score method) ranged from 403 to 1764 mg/day at 4 years and from 299 to 1754 mg /day. Although the lower limit was below the Estimated Average Requirement (EAR: 800 mg/day[37]), the upper limit was below the Tolerable Upper Intake Level (UL: 2500 mg/day). On the other hand, some nutrients had the LOA within the recommendation. For example, the LOA of protein (DRI: 10-30%) ranged between 9% to 27% of total energy intake, at 4 years and between 10% to 28% at 7 years.

The validation of usual food consumption's measurements is an essential part of large-scale epidemiological studies especially in prospective studies in which it is possible to relate food habits with health outcomes. Accordingly, the impact of measurement error on measures of association is of greatest relevance. *In the present study, we observed that using a z-score calibration approach estimated food* and nutrient mean intake more similar to the mean obtained through a reference method (i.e. FD), which support a reduction on the exposure measurement error.

Our classical method of converting a FFQ, using specific standard portions, showed an overall overestimation of dietary intake, comparing to the FD. This trend of the FFQ to overestimate the dietary intake was also described in previous studies, in both adults and children.[28, 29, 38-40] Such overestimation has been explained by the large number of foods asked in the FFQ, providing a broader selection of options as compared to other methods, or an inaccurate reporting of the frequency of consumption of commonly consumed foods. However, it is possible that the portion sizes assumed in the FFQ are incorrectly high, or that increasing frequency of consumption translates in decreasing the portion size. Young children seem to self-regulate their energy intake by adjusting their portion sizes depending on the number of eating occasions per day.[41] On the other hand, the caregiver could decrease the portion size with increasing frequency.

A previous study[16] among adults also handled missing portion size in the FFQ. They reported a bias when using median imputation and described advantages in using stochastic methods to substitute missing portion size values instead of using standard portions or medians. Although the amounts consumed by individuals are considered an important component in estimating food intake, it is still controversial as to whether or not to include portion size questions in the FFQ. As the frequency of consumption, comparing to portion sizes, has been found to be a greater contributor to the variance in intake of most foods, some researchers prefer to use FFQs without the additional respondent burden of reporting portion sizes.[12]

Previous studies have shown that portion size estimation is difficult for the majority of people, varying with personal characteristics, such as appetite status, sex, age and BMI[28, 42-44] or foods' characteristics, such as energy density and number of food standard units (eg. 2 apples).[29] Furthermore, portion size may be intentionally misreported due to the social desirability effect.[45] A qualitative study among mothers of 6-7-year-old and 10-11-year-old children in the UK, showed that mothers have difficulties perceiving what is the recommended age-appropriate serving sizes for their children.[46] These results raise questions regarding the quality of parents' report on children's portion data intake.

The few number of days included in the FD is one of the limitations of this study. We only use 3 days over a period of one week to represent a period of 6 months (FFQ). It is described that to capture the day-to-day variability of some nutrients it may be necessary to include more than the 3 days; it was predicted between 2 to 6 days to estimate nutrient intake with good accuracy (*r=0.8), and even more days to estimate food intake [47].* Although in the present study, it was not possible to have more than three report days from children, 2 weekdays and 1 weekend day, as that would increase the burden of the caregiver and could result in more losses to follow-up. Since the food collection was performed every season on both weekdays and weekends, the average of 3 days might be an accurate estimate of long-time usual intake for food groups frequently consumed. However, we could not exclude a less precise estimation for food groups not expected to be consumed daily. This may have contributed to the low agreement of ICC obtained, for example, for sweets and salty snacks.

As usual in studies using FD as the method of food assessment[3], more educated individuals are more prone to participate. In our sample, in both ages, statistical differences were not found for children's sex or BMI, but mothers were slightly more educated and older. The socio-demographic characteristics of our sample seem to influence the consumption of particular food items, increasing the intake of fruit and vegetables and decreasing the intake of energy-dense foods. This might not influence the internal validity, since we expected to have this effect in both assessment methods and ages. Lastly, with the proposed method (z-score calibration), we obtained the same unconditional mean and variance as the 3d FD, estimating the population distribution of the 3d FD, and not the distribution of the true usual intake.

In conclusion, the short FFQ used to evaluate dietary intake among children performed reasonably well and seems to be a useful instrument for evaluating a wide range of food groups and key nutrient intake in children at 4 and 7 years of age. These results also support that adjusting the portion size when converting a FFQ, by using a z-score method, increase the accuracy of dietary data in young children.

Supplementary information is available at European Journal of Clinical Nutrition's website.

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Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

S.V. contributed to the design of study, performed statistical analyses and interpretation of the data and wrote the first draft of the paper. M.S. contributed the statistical analysis and to the interpretation of data. T.M. and E.R. contributed to the design of data collection instruments and contributed to the interpretation of data. CL contributed to the design of study, coordinated the design of data collection instruments and contributed to the discussion of results. All the authors critically reviewed the manuscript and approved the final version as submitted. The authors declare no conflict of interests.

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Figure legends

Figure 1. Bland-Altman plot of the difference between energy intake, estimated by the FFQ standard method at 4 years (A) and 7 years (C) or z-score method at 4 years (B) and 7 Years (D) and 3d food diary.

Table 1. Participants' characteristics, comparing the sample of children with FFQ data plus food diaries (study sample) with children with only FFQ, at 4 and 7 years of age

	FFQ+FD (n=2482)	Only FFQ (n=3505)	P value[a]	FFQ+FD (n=3511)	Only FFQ (n=2331)	P value[a]
	Mean (s.d.)			Mean (s.d.)		
Maternal characteristics						
Maternal age (years)	34.2 (5.17)	33.7 (5.44)	<0.001	37.2 (5.03)	36.1 (5.64)	<0.001
Maternal education (years)	11.5 (4.23)	10.8 (4.24)	<0.001	11.9 (4.18)	10.8 (4.27)	<0.001
Children's characteristics						
Child's food intake (frequency/day)						
Dairy	4.5 (1.48)	4.4 (1.45)	0.123	4.1 (1.66)	4.2 (1.74)	0.124
Cereals, cereal products and potatoes	5.6 (1.54)	5.5 (1.55)	0.528	5.5 (1.50)	5.6 (1.62)	0.069
Fruit & vegetables	5.2 (1.81)	5.0 (1.75)	<0.001	4.7 (1.65)	4.3 (1.63)	<0.001
Meat, fish and eggs	2.2 (0.65)	2.2 (0.67)	0.963	2.3 (0.76)	2.2 (0.80)	0.006
Drinks	1.2 (1.53)	1.3 (1.60)	0.007	1.3 (1.16)	1.6 (1.35)	<0.001
Fat spread	0.7 (0.67)	0.7 (0.66)	0.235	0.8 (0.73)	0.8 (0.76)	0.562
Sweets	1.8 (1.34)	1.8 (1.41)	0.631	1.1 (0.96)	1.2 (1.07)	0.001
Salty snacks	0.3 (0.23)	0.3 (0.23)	0.864	0.4 (0.27)	0.4 (0.33)	<0.001
	%			%		
Child's sex (boy)	51.1	50.6	0.718	52.4	50.2	0.093
Child's BMI (kg/m^2)						
Underweight/normal	69.1	67.1		62.6	62.5	
Overweight	20.6	22.2		22.1	21.5	
Obese	10.3	10.7	0.143	15.3	16.0	0.729

FD, food diaries; FFQ, food frequency questionnaire; s.d., standard deviation.
[a]Student's t-test for for continuous variables; Chi-square for categorical variables.

Table 2. Mean daily food intake and intraclass correlation coefficients (FFQ and food diaries) at 4 years of age

Groups/foods (g)	*Food diary*	*Food Frequency questionnaire*			
	Original Mean (s.d.)	*Standard method Mean (s.d.)*	*ICC (95%CI)*	*Z-score method Mean (s.d.)*	*ICC (95%CI)*
Dairy	**586 (202.2)**	**959 (304.4)**	**0.179 (-0.072, 0.403)**	**591 (208.4)**	**0.437 (0.405, 0.469)**
Milk	439 (197.3)	562 (197.4)	0.392 (0.178, 0.544)	443 (189.7)	0.462 (0.430, 0.492)
Yogurt	140 (86.5)	198 (118.0)	0.395 (0.218, 0.526)	140 (86.5)	0.479 (0.448, 0.509)
Cheese	8 (11.5)	17 (20.4)	0.266 (0.153, 0.363)	8 (11.2)	0.353 (0.318, 0.387)
Cereals, cereal products and potatoes	**186 (59.0)**	**263 (55.6)**	**0.132 (-0.044, 0.294)**	**190 (52.8)**	**0.241 (0.203, 0.277)**
Bread	42 (25.0)	76 (38.4)	0.183 (-0.009, 0.347)	42 (24.9)	0.309 (0.273, 0.345)
Semi-sweet type biscuits	14 (16.4)	22 (18.0)	0.232 (0.159, 0.300)	14 (15.7)	0.251 (0.213, 0.287)
Other cookies	4 (9.7)	13 (17.0)	0.192 (0.097, 0.277)	5 (9.2)	0.253 (0.216, 0.290)
Rice, pasta and potatoes	126 (46.1)	152 (25.9)	0.122 (0.044, 0.196)	127 (40.1)	0.164 (0.125, 0.202)
Fruit & Vegetables	**608 (209.2)**	**794 (207.6)**	**0.370 (0.016, 0.595)**	**608 (199.0)**	**0.521 (0.491, 0.549)**
Vegetable soup	370 (155.2)	512 (145.2)	0.373 (-0.013, 0.612)	372 (148.0)	0.536 (0.508, 0.564)
Vegetable on plate	69 (37.5)	56 (45.9)	0.234 (0.177, 0.288)	69 (37.5)	0.257 (0.220, 0.293)
Fruit	166 (100.5)	226 (108.6)	0.361 (0.198, 0.488)	167 (98.5)	0.419 (0.386, 0.451)
Meat, fish and eggs	**145 (45.3)**	**176 (42.8)**	**0.117 (0.044, 0.186)**	**1478 (52.1)**	**0.137 (0.098, 0.176)**
White meat	33 (27.8)	64 (28.9)	0.101 (-0.007, 0.203)	34 (27.3)	0.153 (0.114, 0.191)
Red meat	48 (33.0)	43 (19.5)	0.131 (0.092, 0.170)	50 (31.9)	0.146 (0.107, 0.184)
Ham	13 (13.5)	8 (8.8)	0.168 (0.119, 0.215)	13 (13.5)	0.196 (0.158, 0.234)
Fish	43 (27.7)	49 (20.7)	0.253 (0.209, 0.295)	43 (26.6)	0.264 (0.227, 0.301)
Eggs	8 (9.48)	11 (8.85)	0.203 (0.149, 0.254)	8 (9.03)	0.205 (0.167, 0.243)
Drinks	**71 (111.3)**	**449 (537.2)**	**0.073 (0.002, 0.141)**	**96 (138.3)**	**0.257 (0.218, 0.296)**
Coffee	2 (7.6)	2 (6.3)	0.283 (0.246, 0.319)	2 (7.3)	0.282 (0.245, 0.318)
Tea	6 (26.6)	20 (52.3)	0.323 (0.259, 0.381)	9 (25.8)	0.421 (0.388, 0.454)
Colas	2 (16.2)	26 (68.2)	0.052 (0.014, 0.090)	4 (15.4)	0.118 (0.079, 0.157)
Other carbonated soft drinks	32 (68.7)	211 (283.9)	0.048 (0.002, 0.094)	38 (65.3)	0.139 (0.102, 0.178)
Ice tea	17 (58.2)	130 (207.2)	0.125 (0.042, 0.202)	26 (53.6)	0.289 (0.252, 0.326)
Nectars (fruit juice with sugar)	12 (36.1)	59 (87.1)	0.127 (0.048, 0.201)	17 (33.2)	0.204 (0.166, 0.242)
Fat spread	**3 (2.8)**	**5 (4.7)**	**0.283 (0.203, 0.355)**	**3 (2.8)**	**0.350 (0.315, 0.384)**
Butter/margarine	3 (2.8)	5 (4.7)	0.283 (0.203, 0.355)	3 (2.8)	0.350 (0.315, 0.384)
Sweets	**26 (27.3)**	**44 (31.7)**	**0.217 (0.120, 0.304)**	**28 (27.6)**	**0.225 (0.187, 0.262)**
Ice cream	8 (16.3)	7 (10.7)	0.150 (0.112, 0.189)	8 (16.0)	0.158 (0.119, 0.196)
Cakes	12 (18.3)	13 (18.4)	0.222 (0.184, 0.259)	12 (18.2)	0.221 (0.183, 0.258)
Chocolate	3 (5.6)	17 (17.6)	0.059 (-0.007, 0.123)	4 (5.2)	0.149 (0.110, 0.187)
Added sugar	2 (3.8)	1 (3.6)	0.201 (0.066, 0.144)	2 (3.8)	0.204 (0.166, 0.241)
Candy	2 (4.6)	5 (6.4)	0.154 (0.074, 0.228)	2 (4.2)	0.178 (0.140, 0.216)
Salty snacks	**10 (22.7)**	**12 (10.0)**	**0.123 (0.084, 0.162)**	**14 (24.0)**	**0.194 (0.156, 0.232)**
Pizza & burger	6 (19.1)	5.0 (5.3)	0.105 (0.066, 0.144)	7 (19.0)	0.204 (0.166, 0.241)
Salty pastry (e.g. potato croquettes)	3 (10.7)	4 (4.5)	0.133 (0.084, 0.180)	5 (9.6)	0.130 (0.091, 0.168)
Crisps	1 (4.9)	4 (5.2)	0.115 (0.068, 0.160)	2 (4.6)	0.118 (0.079, 0.156)

95%CI, 95% confidence interval; ICC, Intraclass correlation coefficient; s.d., standard deviation.

Table 3. Mean daily food intake and intraclass correlation coefficients (FFQ and food diaries) at 7 years of age

Groups/food (g)	*Food diaries*	*Food frequency questionnaire*			
	Original Mean (s.d.)	*Standard method Mean (s.d.)*	*ICC (95%CI)*	*Z-score method Mean (s.d.)*	*ICC (95%CI)*
Dairy	518 (184.8)	742 (301.0)	0.288 (0.025, 0.482)	513 (198.6)	0.448 (0.421, 0.474)
Milk	382 (178.2)	561 (273.5)	0.348 (0.079, 0.534)	378 (173.8)	0.496 (0.471, 0.521)
Yogurt	126 (89.4)	166 (112.8)	0.445 (0.346, 0.526)	124 (87.9)	0.490 (0.465, 0.515)
Cheese	11 (13.6)	16 (19.0)	0.343 (0.289, 0.393)	11 (13.5)	0.380 (0.351, 0.408)
Cereals, cereal products and potatoes	234 (64.0)	278 (62.3)	0.134 (0.054, 0.209)	236 (64.9)	0.142 (0.109, 0.174)
Bread	54 (30.1)	87 (36.8)	0.239 (0.004, 0.425)	54 (29.2)	0.360 (0.330, 0.388)
Breakfast cereals	16 (20.0)	20 (18.6)	0.436 (0.387, 0.481)	16 (18.0)	0.452 (0.425, 0.478)
Semi-sweet type biscuits	12 (13.4)	14 (13.7)	0.346 (0.316, 0.375)	13 (13.1)	0.348 (0.318, 0.377)
Other cookies	7 (12.7)	13 (15.1)	0.270 (0.197, 0.336)	7 (11.4)	0.300 (0.269, 0.329)
Rice, pasta and potatoes	145 (48.5)	144 (36.5)	0.014 (-0.019, 0.048)	146 (47.8)	0.015 (-0.018,0.048)
Fruit & vegetables	591 (226.1)	750 (234.2)	0.443 (0.140, 0.629)	609 (191.6)	0.531 (0.506, 0.555)
Vegetable soup	331 (126.1)	458 (170.1)	0.430 (0.080, 0.637)	338 (123.3)	0.539 (0.515, 0.562)
Vegetable on plate	79 (43.4)	58 (45.5)	0.282 (0.194, 0.360)	82 (44.5)	0.313 (0.283, 0.343)
Fruit	181 (106.5)	234 (110.2)	0.409 (0.272, 0.516)	190 (104.6)	0.456 (0.429, 0.482)
Meat, fish and eggs	161 (41.1)	179 (48.7)	0.071 (0.037, 0.105)	164 (46.0)	0.072 (0.039, 0.105)
Meat	94 (40.1)	95 (36.4)	0.127 (0.095, 0.160)	95 (38.1)	0.128 (0.095, 0.160)
Ham	8 (12.4)	13 (11.5)	0.105 (0.069, 0.141)	9 (11.5)	0.113 (0.080, 0.145)
Fish	50 (17.8)	60 (25.9)	0.201 (0.158, 0.242)	52 (17.4)	0.185 (0.153, 0.217)
Eggs	8 (10.0)	11 (8.8)	0.173 (0.131, 0.214)	8 (9.6)	0.185 (0.153, 0.217)
Drinks	163 (168.9)	346 (313.6)	0.313 (0.104, 0.471)	170 (180.6)	0.467 (0.441, 0.493)
Coffee	2 (7.2)	2 (6.3)	0.312 (0.282, 0.342)	2 (7.1)	0.315 (0.285, 0.345)
Tea	9 (34.7)	23 (60.0)	0.390 (0.336, 0.439)	11 (32.6)	0.473 (0.446, 0.498)
Fresh fruit juice	8 (30.8)	27 (56.1)	0.265 (0.197, 0.328)	10.7 (28.7)	0.340 (0.310, 0.369)
Colas	8 (33.9)	27 (66.3)	0.210 (0.163, 0.255)	9 (30.0)	0.272 (0.242, 0.303)
Other carbonated soft drinks	49 (99.8)	101 (156.1)	0.285 (0.217, 0.347)	50 (90.4)	0.340 (0.310, 0.369)
Ice tea	55 (108.1)	119 (187.9)	0.387 (0.289, 0.469)	54 (103.1)	0.485 (0.459, 0.510)
Nectars (fruit juice with sugar	33 (62.6)	48 (72.5)	0.301 (0.265, 0.336)	32 (55.9)	0.310 (0.280, 0.340)
Fat spread	4 (3.3)	6 (5.1)	0.351 (0.261, 0.430)	4 (3.2)	0.421 (0.393, 0.447)
Butter/margarine	4 (3.3)	6 (5.1)	0.351 (0.261, 0.430)	4 (3.2)	0.421 (0.393, 0.447)
Sweets	29 (30.0)	29 (23.6)	0.221 (0.189, 0.252)	29 (29.6)	0.221 (0.189, 0.252)
Ice cream	8 (17.2)	7 (10.0)	0.185 (0.153, 0.217)	9 (15.6)	0.214 (0.182, 0.245)
Cakes	16 (22.2)	12 (16.5)	0.148 (0.116, 0.181)	15 (21.0)	0.157 (0.125, 0.189)
Chocolate	2 (5.5)	4 (5.5)	0.214 (0.179, 0.248)	3 (5.4)	0.220 (0.188, 0.251)
Added sugar	1 (2.4)	2 (3.4)	0.285 (0.226, 0.340)	1 (2.2)	0.321 (0.291, 0.351)
Candies	2 (5.2)	4 (5.9)	0.145 (0.093, 0.194)	2 (4.6)	0.162 (0.130, 0.194)
Salty snacks	17 (30.7)	18 (13.1)	0.079 (0.046, 0.112)	20 (29.2)	0.100 (0.067, 0.133)
Pizza & burger	11 (26.8)	6 (7.0)	0.057 (0.024, 0.089)	12 (24.5)	0.119 (0.087, 0.152)
Salty pastry (eg. potato croquettes	4 (13.9)	9 (8.0)	0.066 (0.032, 0.099)	6 (10.6)	0.081 (0.048, 0.114)
Crisps	2 (5.2)	3 (4.6)	0.085 (0.051, 0.118)	2 (4.7)	0.090 (0.057, 0.123)

95%CI, 95% confidence interval; ICC, Intraclass correlation coefficient; s.d., standard deviation.

Table 4. Mean daily intakes of nutrients and de-attenuated Pearson's correlation coefficients (FFQ and food diaries) at 4 and 7 years of age

Nutrients (units/day)	**4 years of age**					**7 years of age**				
	Food diary	*Food Frequency Questionnaire*				*Food diary*	*Food Frequency Questionnaire (3441)*			
		Standard method		*Z-score method*			*Standard method*		*Z-score method*	
	Mean (s.d.)	*Mean (s.d.)*	*De-attenuated correlation coefficient*	*Mean (s.d.)*	*De-attenuated correlation coefficient*	*Mean (s.d.)*	*Mean (s.d.)*	*De-attenuated correlation coefficient*	*Mean (s.d.)*	*De-attenuated correlation coefficient*
Energy (kcal/day)	1627 (249.2)	2470 (454.7)	0.418**	1741 (334.8)	0.437**	1772 (298.2)	2229 (417.9)	0.444**	1714 (346.3)	0.420**
Protein (g/day)	76 (15.3)	95 (17.3)	0.411**	72 (16.4)	0.392**	80 (14.2)	99 (19.6)	0.435**	80 (16.4)	0.394**
Total carbohydrates (g/day)	199 (40.8)	283 (79.5)	0.377**	180 (41.5)	0.435**	224 (44.8)	296 (64.8)	0.536**	218 (52.2)	0.500**
Total Fat (g/day)	58 (13.2)	100 (16.7)	0.560**	79 (16.1)	0.565**	62 (12.1)	68 (13.9)	0.517**	54.3 (12.1)	0.512**
Dietary fibre (g/day)	13 (3.8)	19 (5.5)	0.328**	12 (3.2)	0.484**	14 (4.0)	23 (5.3)	0.531**	17 (4.4)	0.475**
Omega 3 (g/day)	0.5 (0.26)	0.8 (0.15)	0.528**	0.6 (0.14)	0.537**	0.6 (0.33)	0.4 (0.10)	0.372**	0.6 (0.13)	0.307**
Omega 6 (g/day)	6.5 (2.27)	14 (2.2)	0.369**	12 (2.5)	0.372**	7.4 (2.39)	5.3 (1.33)	0.266**	6.2 (1.33)	0.248**
Vitamin A (µg/day)	389 (150.8)	506 (135.4)	0.565**	394 (120.6)	0.574**	410 (334.3)	520 (187.6)	0.382**	375 (133.8)	0.375**
Vitamin E (mg/day)	3.3 (1.27)	12 (1.9)	0.402**	10 (2.0)	0.372**	3.5 (1.32)	3.2 (0.80)	0.192**	2.4 (0.73)	0.129**
Vitamin B1 (mg/day)	1.0 (0.35)	1.2 (0.19)	0.321**	0.9 (0.17)	0.314**	1.1 (0.40)	1.2 (0.30)	0.559**	0.92 (0.27)	0.561**
Vitamin B6 (mg/day)	1.4 (0.53)	1.4 (0.19)	0.223**	1.1 (0.19)	0.225**	1.7 (0.66)	1.6 (0.45)	0.626**	1.3 (0.42)	0.585**
Vitamin B12 (µg/day)	4.6 (2.93)	4.4 (0.90)	0.107*	3.6 (0.91)	0.112*	5.2 (3.97)	5.0 (1.30)	0.310**	4.1 (1.15)	0.303**
Vitamin C (mg/day)	61 (27.6)	74 (23.0)	0.409**	60 (20.4)	0.420**	66 (33.0)	92 (34.5)	0.619**	60 (21.8)	0.588**
Folate (µg/day)	216 (95.4)	307 (46.6)	0.186**	244 (40.8)	0.201**	271 (120.0)	378 (88.5)	0.471**	278 (77.8)	0.464**
Calcium (mg/day)	1084 (298.0)	1457 (389.4)	0.493**	1067 (310.6)	0.497**	1021 (297.0)	1021 (297.0)	0.520**	1000 (314.5)	0.513**
Iron (mg/day)	24 (21.7)	33 (7.0)	0.179**	24 (5.4)	0.202**	23 (18.3)	21 (7.2)	0.185**	16 (5.9)	0.184**
Magnesium (mg/day)	233 (44.8)	343 (82.6)	0.222**	228 (45.6)	0.320**	239 (45.3)	338 (65.0)	0.410**	256 (51.7)	0.369**
Phosphorus (mg/day)	1209 (253.6)	1671 (345.5)	0.430**	1229 (275.9)	0.439**	1238 (251.2)	1701 (388.7)	0.517**	1281 (286.3)	0.471**
Potassium (mg/day)	2714 (557.3)	3382 (609.7)	0.415**	2514 (513.5)	0.442**	2713 (560.0)	3807 (754.3)	0.489**	2860 (559.3)	0.464**
Sodium (mg/day)	2446 (537.4)	2753 (530.2)	0.539**	2080 (503.5)	0.558**	2478 (549.6)	3251 (726.8)	0.664**	2315 (530.9)	0.643**

s.d., standard deviation. *P ≤ 0.05. **P ≤ 0.001, for the observed correlation.

Table 5. Intraclass correlation coefficients (ICC), Bland Altman analysis and Cohen effect size between daily intakes of energy and nutrients from the food diaries and FFQ (z-score method) at 4 and 7 years of age

	4 years of age						7 years of age					
	ICC	Mean difference	Limits of agreement		Correlation between mean and mean difference	Cohen Effect size	*ICC*	Mean difference	Limits of agreement		Correlation between mean and mean difference	Cohen Effect size
Energy (kcal/day)	0.232 (0.178,0.283)	-113.0	-870.9	644.9	-0.133**	-0.29	0.208 (0.175, 0.241)	66.8	-771.5	905.1	-0.037*	0.16
Protein (g/day)	0.187 (0.145,0.228)	4.2	-35.4	43.8	-0.073**	0.21	0.185 (0.152, 0.217)	-0.3	-39.4	38.8	-0.099**	-0.02
Total carbohydrates (g/day)	0.206 (0.141,0.267)	19.0	-81.4	119.4	-0.019	0.37	0.245 (0.213, 0.276)	6.7	-114.3	127.7	-0.086**	0.11
Total Fat (g/day)	0.110 (-0.043,0.256)	-20.7	-56.8	15.4	-0.205**	-1.13	0.135 (0.073, 0.194)	8.2	-28.0	44.4	0.276**	0.44
Dietary fibre (g/day)	0.291 (0.232, 0.347)	1.2	-6.9	9.3	0.170**	0.29	0.241 (0.102, 0.359)	-2.9	-12.7	6.9	-0.062**	-0.58
Omega 3 (g/day)	0.123 (0.069,0.175)	-0.1	-0.7	0.4	0.554**	-0.40	0.040 (0.006, 0.074)	0.2	-0.5	0.8	0.837**	0.47
Omega 6 (g/day)	0.036 (-0.031,0.108)	-5.1	-11.3	1.1	-0.091**	-1.61	0.036 (-0.006, 0.078)	2.1	-3.3	7.5	0.564**	0.76
Vitamin A (µg/day)	0.312 (0.275,0.348)	-5.8	-319.7	308.1	0.232**	-0.04	0.137 (0.105, 0.170)	37.3	-640.6	715.2	0.748*	0.11
Vitamin E (mg/day)	0.021 (-0.022,0.081)	-7.0	-11.2	-2.8	-0.446 **	-3.27	0.036 (-0.003, 0.074)	1.1	-1.9	4.0	0.559**	0.69
Vitamin B1 (mg/day)	0.138 (0.098,0.177)	0.1	-0.7	0.8	0.608**	0.16	0.238 (0.124, 0.337)	0.2	-0.6	1.0	0.401**	0.48
Vitamin B6 (mg/day)	0.065 (0.019,0.111)	0.3	-0.8	1.3	0.783**	0.48	0.241 (0.098, 0.362)	0.4	-0.9	1.7	0.468**	0.60
Vitamin B12 (µg/day)	0.025 (-0.013,0.062)	0.9	-5.0	6.8	0.823**	0.29	0.064 (0.031, 0.097)	1.1	-6.8	9.0	0.849**	0.27
Vitamin C (mg/day)	0.235 (0.197,0.273)	0.8	-58.2	59.7	0.303**	0.02	0.294 (0.256, 0.330)	6.8	-59.2	72.8	0.434**	0.20
Folate (µg/day)	0.079 (0.039,0.119)	-28.0	-222.6	166.6	0.693**	-0.28	0.239 (0.207, 0.270)	-6.5	-257.2	244.2	0.448**	-0.05
Calcium (mg/day)	0.350 (0.314,0.384)	16.6	-663.6	696.8	-0.044**	0.05	0.328 (0.298,0.358)	26.4	-701.2	754.0	0.032	0.07
Iron (mg/day)	0.057 (0.017,0.097)	-8.9	-52.2	34.4	0.883**	-0.40	0.032 (0.001,0.063)	7.1	-39.0	53.1	0.877**	0.30
Magnesium (mg/day)	0.212 (0.173,0.250)	5.2	-106.0	116.4	-0.019	0.09	0.194 (0.155, 0.231)	-15.2	-141.9	111.5	-0.030	-0.24

Phosphorus (mg/day)	0.295 (0.258,0.331)	-19.9	-636.4	596.6	-0.088**	-0.06	0.292 (0.260, 0.323)	-37.2	-686.6	612.2	-0.062**	-0.11
Potassium (mg/day)	0.264 (0.203,0.320)	199.2	-1059.6	1457.9	0.085**	0.31	0.279 (0.241, 0.315)	-138.4	-1469.3	1192.5	0.026	-0.20
Sodium (mg/day)	0.256 (0.098,0.386)	366.3	-824.8	1557.4	0.069*	0.60	0.081 (0.048, 0.113)	200.6	-2520.5	2921.7	0.733**	0.14

*p<0.05; **p<0.01, for the observed correlation.

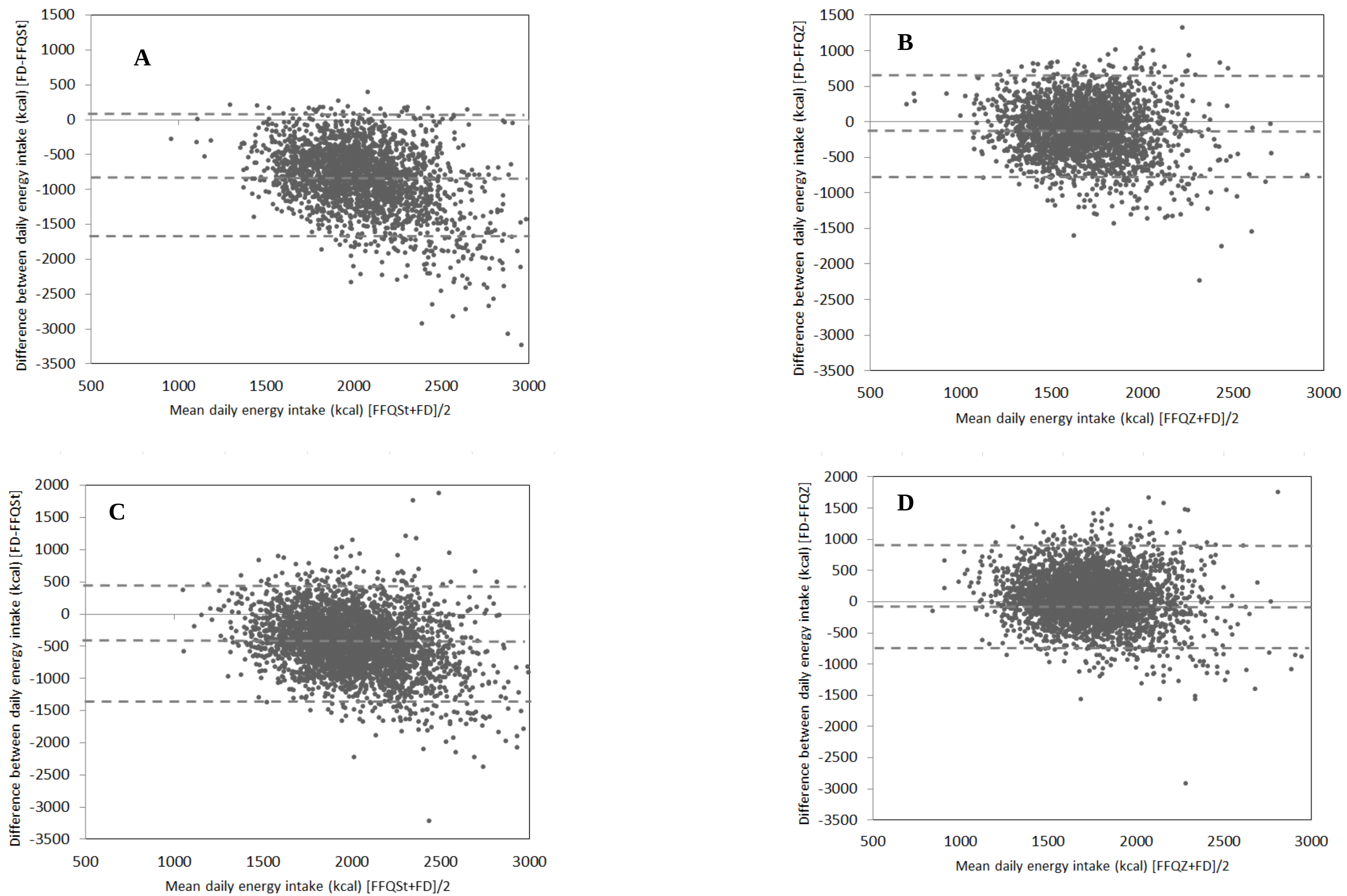

Figure 1. Bland-Altman plot of the difference between energy intake, estimated by the FFQ standard method at 4 years (A) and 7 years (C) or z-score method at 4 years (B) and 7 Years (D) and 3d food diary.

SUPPLEMENTARY MATERIAL

Table S1

Dietary intake at 4 and 7 years – FFQ (frequency of consumption)

Table S2

Nutrients intake at 4 and 7 years –FFQ (standard method) vs. food diaries

Supplemental Table 1. FFQ's mean and s.d. of food intake at 4 and 7 years of age.

	4 years		7 years	
	FFQ (frequency/day) mean (s.d.)	FFQ Portions (g)	FFQ (frequency/day) mean (s.d.)	FFQ Portions (g)
Groups/food				
Milk	2.3 (0.81)	244	2.3 (1.15)	244
Yogurt	1.6 (0.94)	125	1.4 (0.92)	125
Ice cream	0.1 (0.21)	50	0.1 (0.22)	80
Cheese	0.6 (0.68)	30	0.5 (0.64)	30
Eggs	0.2 (0.18)	50	0.2 (0.18)	50
White meat	0.6 (0.27)	105	0.9 (0.37)[a]	105
Red meat	0.4 (0.19)	105		
Ham	0.4 (0.44)	20	0.7 (0.59)	25
Fish	0.6 (0.23)	90	0.6 (0.29)	90
Bread	1.7 (0.87)	44	2.0 (0.85)	44
Semi-sweet type biscuits	1.0 (0.87)	21	0.7 (0.66)	21
Other cookies	0.4 (0.56)	30	0.4 (0.53)	30
Cakes	0.2 (0.23)	80	0.2 (0.22)	80
Chocolate	0.9 (0.88)	20	0.2 (0.27)	20
Added sugar	0.3 (0.72)	5	0.3 (0.68	5
Candies	0.3 (0.43)	15	0.3 (0.41)	15
Vegetable soup	1.7 (0.49)	295	1.5 (0.59)	295
Vegetable on plate	1.1 (0.92)	50	1.1 (0.89)	50
Fruit	1.9 (0.91)	120	1.9 (0.93)	120
Rice, pasta and potatoes	1.9 (0.33)	80	1.8 (0.46)	80
Crisps	0.1 (0.17)	30	0.1 (0.16)	30
Pizza & burger	0.1 (0.07)	80	0.1 (0.09	80
Salty pastry (eg. potato croquettes	0.1 (0.09)	50	0.2 (0.18)	50
Butter/margarine	0.7 (0.67)	7	0.8 (0.74)	7
Coffee	0.1 (0.28)	23	0.1 (0.28)	23
Tea	0.1 (0.22)	237	0.1 (0.26)	240
Colas	0.1 (0.21)	330	0.1 (0.22	330
Ice tea	0.4 (0.63)	330	0.4 (0.58	330
Nectars (fruit juice with sugar)	0.3 (0.44)	200	0.3 (0.39	200
Other carbonated soft drinks	0.6 (0.86)	330	0.3 (0.51	200
Fresh fruit juice	-	-	0.1 (0.26	220
Breakfast's cereals	-	-	0.7 (0.63	30

FFQ, food frequency questionnaire; s.d., standard deviation.
aIncludes all meats.

Supplemental Table 2. Intraclass correlation coefficients (ICC), Bland Altman analysis and Cohen effect size between daily intakes of energy and nutrients from the food diaries and FFQ (standard method) at 4 and 7 years of age

	4 years of age						7 years of age					
	ICC	Mean difference	Limits of agreement		Correlation between mean and mean difference	Cohen Effect size	*ICC*	Mean difference	Limits of agreement		Correlation between mean and mean difference	Cohen Effect size
Energy (kcal/day)	0.063 (-0.049, 0.190)	-841.9	-1781.3	97.5	-0.420**	-1.76	0.131 (-0.025, 0.272)	-448.6	-1374.5	477.3	-0.227**	-0.95
Protein (g/day)	0.117 (-0.024, 0.247)	-19.6	-60.1	20.9	-0.126**	-0.95	0.121 (-0.015, 0.246)	-19.6	-62.8	23.6	-0.279**	-0.89
Total carbohydrates (g/day)	0.085 (-0.028, 0.194)	-84.1	-244.6	76.4	-0.591**	-1.03	0.144 (-0.035, 0.306)	-70.8	-207.6	66.0	-0.301**	-1.01
Total Fat (g/day)	0.045 (-0.040, 0.150)	-41.4	-78.4	-4.4	-0.239**	-2.19	0.160 (0.121, 0.199)	-5.1	-43.0	32.8	0.143**	-0.26
Dietary fibre (g/day)	0.115 (-0.023, 0.243)	-5.6	-17.3	6.1	-0.366**	-0.94	0.117 (-0.069, 0.313)	-8.8	-19.6	2.0	-0.261**	-1.60
Omega 3 (g/day)	0.085 (-0.011, 0.176)	-0.2	-0.8	0.3	0.532**	-0.86	0.075 (0.042, 0.108)	0.0	-0.7	0.7	0.726**	0.07
Omega 6 (g/day)	0.016 (-0.019, 0.058)	-8.0	-13.8	-2.1	0.033**	-2.68	0.053 (0.016, 0.089)	1.3	-4.1	6.7	0.565**	0.46
Vitamin A (µg/day)	0.234 (0.053, 0.383)	-117.4	-446.6	211.8	0.113**	-0.70	0.164 (0.120, 0.207)	-108.1	-811.3	595.1	0.555**	-0.30
Vitamin E (mg/day)	0.013 (-0.013, 0.053)	-9.2	-13.1	-5.3	-0.384**	-4.60	0.083 (0.050, 0.116)	0.2	-2.8	3.2	0.493**	0.16
Vitamin B1 (mg/day)	0.131 (0.065, 0.193)	-0.2	-0.9	0.6	0.535**	-0.46	0.285 (0.252, 0.318)	-0.1	-0.9	0.8	0.314**	-0.15
Vitamin B6 (mg/day)	0.081 (0.041, 0.121)	0.0	-1.1	1.1	0.770**	-0.01	0.313 (0.283, 0.343)	0.1	-1.3	1.4	0.414**	0.07
Vitamin B12 (µg/day)	0.025 (-0.015, 0.065)	0.2	-5.7	6.1	0.828**	0.06	0.078 (0.045, 0.111)	0.3	-7.6	8.2	0.811**	0.07
Vitamin C (mg/day)	0.207 (0.126, 0.281)	-13.4	-74.9	48.1	0.187**	-0.43	0.275 (0.094, 0.419)	-25.0	-101.5	51.5	-0.021	-0.64
Folate (µg/day)	0.050 (-0.013, 0.112)	-90.4	-289.4	108.6	0.618**	-0.89	0.172 (0.007, 0.315)	-105.5	-363.8	152.8	0.336**	-0.80
Calcium (mg/day)	0.212 (-0.021, 0.403)	-373.1	-1156.5	410.3	-0.277**	-0.93	0.216 (0.006, 0.387)	-381.0	-1276.8	514.8	-0.326**	-0.83
Iron (mg/day)	0.055 (0.014, 0.095)	-8.9	-52.2	34.4	0.813**	-0.40	0.041 (0.008, 0.074)	2.1	-44.4	48.6	0.826**	0.09
Magnesium (mg/day)	0.052 (-0.031, 0.137)	-110.3	-282.8	62.2	-0.550**	-1.25	0.091 (-0.052, 0.235)	-97.7	-240.4	45.0	-0.259**	-1.34
Phosphorus (mg/day)	0.129 (-0.059, 0.308)	-461.3	-1175.3	252.7	-0.312**	-1.27	0.149 (-0.052, 0.331)	-457.2	-1242.8	328.4	-0.366**	-1.14
Potassium (mg/day)	0.160 (-0.025, 0.322)	-668.6	-2057.1	719.9	-0.093**	-0.94	0.126 (-0.064, 0.312)	-1085.1	-2655.1	484.9	-0.279**	-1.35
Sodium (mg/day)	0.265 (0.148, 0.366)	-306.9	-1536.7	922.9	0.014	-0.49	0.085 (0.033, 0.136)	-735.8	-3577.4	2105.8	0.551**	-0.51

*p<0.05; **p<0.01

Paper II

Association between energy-dense food consumption at 2 years of age and diet quality at 4 years of age.

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Association between energy-dense food consumption at 2 years of age and diet quality at 4 years of age

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Abstract
The present study aimed to evaluate the association between the consumption of energy-dense foods at 2 years of age and the consumption of foods and diet quality at 4 years of age. The sample included 705 children evaluated at 2 and 4 years of age, as part of the population-based birth cohort Generation XXI (Porto, Portugal). Data on sociodemographic and lifestyle factors of both children and mothers were collected by face-to-face interviews. The weight and height of children were measured by trained professionals. Based on FFQ, four energy-dense food groups were defined: soft drinks; sweets; cakes; salty snacks. A healthy eating index was developed using the WHO dietary recommendations for children (2006) aged 4 years. The associations were evaluated through Poisson regression models. After adjustment for maternal age and education, child's carer, child's siblings and child's BMI, higher consumption of energy-dense foods at 2 years of age was found to be associated with higher consumption of the same foods 2 years later. An inverse association was found between the intake (≥median) of soft drinks (incidence rate ratio (IRR) = 0·74, 95 % CI 0·58, 0·95), salty snacks (IRR = 0·80, 95 % CI 0·65, 1·00) and sweets (IRR = 0·73, 95 % CI 0·58, 0·91) at 2 years of age and the consumption of fruit and vegetables at 4 years of age (≥5 times/d). Weekly and daily consumption of energy-dense foods at 2 years of age was associated with a lower healthy eating score at 4 years of age (IRR = 0·75, 95 % CI 0·58, 0·96; IRR = 0·56, 95 % CI 0·41, 0·77, respectively). The consumption of energy-dense foods at young ages is negatively associated with the diet quality of children a few years later.

Key words: **Energy-dense foods: Diet quality: Preschool children**

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Energy-dense foods are foods generally high in energy but with a low nutrient content[1]. The consequences of the consumption of this type of foods on health have been studied through different components. Some studies have focused more on foods high in sugar such as sugar-sweetened beverages[2–4], candies[5,6], confectionery[7] and chocolates[8–10]. Others have been more interested in foods with a high fat content such as snacks[11] and fast foods[12,13]. The per capita daily energy contribution from sugar-sweetened beverages in US children aged 2–5 years increased from 448 kJ (107 kcal) in 1988–94 to 519 kJ (124 kcal) in 1999–2004[14]. Snacking has also increased in US children[15]. Compared with children of other age groups, children aged 2–6 years consumed the highest amount of daily snacks and showed the largest increase in intake from 1977 to 2006 (approximately an increase of 1·41 events)[15]. In Europe, the daily consumption of soft drinks ranges from 10 % (Finland) to 40 % (Bulgaria) in school-aged children. Almost one-third of children eat sweets or chocolates on a daily basis[16]. In a national survey carried out in 1997/1998 among British children aged 4–6 years, 80 % of children were found to consume foods such as savoury snacks, potato chips and confectionery at least once a week[17]. In Portugal, among school-aged children, the daily consumption of soft drinks was observed in 21 % of girls and 30 % of boys and sweets were consumed daily by 20 % overall[16,18].

Previous studies have highlighted the adverse health effects of the consumption of energy-dense food in both children and adults, particularly on body weight[12,19–22]. The consumption of energy-dense foods also seems to have an adverse effect on the overall diet quality in children. A study[13] conducted among children and adolescents in a national household survey in the USA has found that children who ate fast

Abbreviation: IRR, incidence rate ratio.

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foods, compared with those who did not, had a significantly higher intake of total energy, total fat, saturated fat, added sugars and sugar-sweetened beverages and a significantly lower intake of dietary fibre, milk, fruit and starchy vegetables. Other studies[23,24] have found an association between increasing intake of added sugars and decreasing intake of some nutrients, such as protein, fat, vitamins A and E and folate. Moreover, higher consumption of sugar-sweetened beverages has been shown to be negatively associated with diet quality in children and adolescents[25,26]. Lower diet quality scores have been reported to be associated with increased disease risk and all-cause mortality rates in adulthood[27]. Most of these studies had used a cross-sectional approach to study the association between the consumption of presumed less-healthy foods and the diet quality of children[13,23,24,26,28,29]. The use of a longitudinal analysis among young preschool children has been less explored[25,30,31].

The aim of the present study was to evaluate the prospective association between the consumption of less-healthy foods at 2 years of age and the consumption of foods at 4 years of age. The relationship between the consumption of energy-dense foods at 2 years of age and the consumption of similar food groups and diet quality at 4 years of age was explored.

Methods

Subjects

The present study is based on the prospective population-based birth cohort Generation XXI, which has been described elsewhere[32–34]. Generation XXI recruited newborns and their mothers during 2005–6 at five level III maternity units of Porto. Of the invited mothers, 91·4 % agreed to participate. A total of 8647 children and 8495 mothers were enrolled at baseline. At 2 years of age, a subsample of 855 children was re-evaluated (April–August 2007 and January 2008). In 2009/2011, all the children (at 4–5 years of age) and their mothers were invited to attend the first follow-up session of the entire cohort. During this evaluation period, the participants were invited to participate in an interview and a physical examination, with 86 % of the children being re-evaluated at 4–5 years of age.

Data obtained from 708 singleton children and their mothers evaluated at both 2 years of age (25 (SD 3·5) months) and 4 years of age (49 (SD 7·2) months) were analysed.

Data collection

Data, at both 2 and 4–5 years of age, were collected by trained interviewers in face-to-face interviews, through structured questionnaires including information on parents' sociodemographic characteristics and children's health status and behavioural characteristics.

Dietary intake

Information on dietary intake at 2 and 4–5 years of age was collected using a FFQ, which the main carer (usually the mother) answered in a face-to-face interview. At 2 years of age, the FFQ queried about the current frequency of consumption of seventeen food items not usually consumed on a daily basis (e.g. crisps, cakes and burgers). A total of six response options were available: 'every day'; '3–6 times per week'; '1–2 times per week'; '1–3 times per month'; 'less than once a month'; 'never'. At 4–5 years of age, the FFQ queried about the frequency of intake of thirty-five food items. For each food item, the parents or carers were asked as to how many times, on average, their children had consumed these food items during the previous 6 months. The nine frequency responses were as follows: '4 or more times per d'; '2–3 times per d'; '1 time per d'; '5–6 times per week'; '2–4 times per week'; '1 time per week'; '1–3 times per month'; 'less than once a month'; 'never'. Daily frequencies of consumption were calculated using both the questionnaires (e.g. 3–6 times per week was converted into a mean of 4·5 times per week, meaning $4{\cdot}5/7\,d = 0{\cdot}6423$ times per d).

At 2 and 4 years of age, four similar energy-dense food groups were created, including only foods comparable at both ages: soft drinks (sweetened carbonated drinks and other sweetened drinks, including diet drinks); salty snacks (crisps, pizzas and burgers); cakes (creamy cakes, not creamy cakes and sweet pastries); sweets (chocolates and candies). At both ages, the sample median consumption of energy-dense food groups was used to create dichotomic variables: consumption lower than the median *v.* consumption higher or equal to the median. At 2 years of age, in addition to the median consumption, tertiles of consumption of energy-dense foods were obtained.

At 2 and 4 years, the carers were asked to complete 2 and 3 d food records, respectively. Pearson's correlation coefficients were calculated for key groups comparing the responses from the FFQ and those from the food records at both ages, to assess the validation of the FFQ. A weak-to-moderate correlation was found for most of the food groups evaluated (data not shown). With the exception of sweets (r 0·531), a weak correlation was found at 2 years of age.

Healthy eating index

Based on dietary recommendations for children proposed by the World Health Organization[35], a healthy eating index was developed at 4 years of age, including only data of foods and not those on nutrient content. This index comprises seven food groups: fruit and vegetables (vegetable soup, raw and cooked vegetables and fruit); dairy foods (semi-skimmed milk, skimmed milk, cheese and yogurts); red meat and meat products (pork, beef, veal, goat, processed meats and savoury pastries); white meat and fish (rabbit, poultry, eggs and fish); soft drinks (sweetened carbonated drinks and other sweetened drinks, including diet drinks); salty snacks (crisps, pizzas and burgers); sweet snacks (cakes, sweet pastries, chocolates and candies). For each food group, quartiles of consumption were calculated, and a score ranging from 1 to 4 was assigned. For 'healthy foods' such as fruit, vegetables, white meat and fish, and dairy foods, the lowest quartile of consumption was assigned a score of 1, intermediate quartiles were given the scores 2 and 3, and the highest quartile was given a score of 4. The food groups that are not recommended for a healthy diet such as soft drinks and salty and sweet snacks were scored in the reverse direction with the highest quartile of consumption receiving the lowest score. The possible range score of the final index is 7–28. Overall, a higher score represents a better diet at 4 years of age. The final score was stratified by the median score of 17.

Covariables

Variables collected at the 4–5-year follow-up evaluation and used for the present analysis include the following: maternal age and education (as continuous variables); child's siblings (none, younger or older); child's current carer (family/babysitter or kindergarten/school); child's sex. The weight and height of children were measured by a team of experienced investigators. Weight was measured in light clothing and without shoes using a digital scale 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 to the nearest 0·1 cm. The BMI of children was defined as weight in kg divided by height in m^2. This continuous variable was then categorised using specific cut-offs for sex and age specified by the WHO[36] and re-categorised into underweight/normal (BMI <2 SD) and overweight/obese (BMI ≥2 SD).

Ethical approval

The project Generation XXI was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethical Committee of São João Hospital/University of Porto Medical School. The project was approved 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 baseline and follow-up evaluations.

Statistical analyses

Mean values with standard deviations and frequency differences were compared using Student's *t* test and χ^2 test, respectively.

Associations between consumption at 2 years of age and that at 4 years of age were estimated by crude and adjusted incidence rate ratios (IRR) and respective 95 % CI, using Poisson regression. The models were adjusted for maternal age and education in years (as continuous variables), child's siblings (none, younger or older), child's current carer (family/babysitter or kindergarten/school) and child's BMI (underweight/normal or overweight/obese). The total person-time at risk was calculated, and the log of person-months (mean = 24 months) was included as the offset variable. A potential interaction effect of maternal education levels on the association between the consumption of energy-dense foods at 2 years of age and the healthy eating index at 4 years of age was also assessed, by including an interaction term in the final models. The analyses were conducted using the SPSS 20.0 software (SPSS Inc., 2011).

Results

In Table 1, the characteristics of eligible participants are compared with those of the remaining cohort evaluated at baseline. In the present study, mothers were slightly more educated (11·2 (SD 4·33) *v.* 10·4 (SD 4·24) years, $P<0·001$) and older (30·4 (SD 5·02) *v.* 29·4 (SD 5·64) years, $P<0·001$) than the remaining mothers evaluated at baseline. In the study sample, more children were being taken care of by family or babysitter (18·4 *v.* 10·7 %, $P<0·001$). No significant differences were found concerning child's sex and BMI and child's siblings.

Fig. 1 shows the proportion of children consuming each energy-dense food group at least once a week at 2 and 4 years of age. Most children were consuming sweets (92·0 %) and soft drinks (63·2 %) at least once a week at 4 years of age. Cakes and salty snacks were least consumed at both ages. Among those who had already been consuming these foods at least once a week at 2 years of age, the percentage of consumers at 4 years of age was 97·4, 87·8, 63·8 and 72 % for sweets, soft drinks, cakes and salty snacks, respectively.

Table 2 summarises the crude and adjusted associations between the consumption of energy-dense foods at 2 years of age and that at 4 years of age. Overall, higher consumption at 2 years of age was positively associated with higher consumption of the same foods at 4 years of age. The strongest

Table 1. Comparison between characteristics of eligible participants and those of the remaining cohort evaluated at baseline*

(Number of participants and percentages; mean values and standard deviations)

	Sample† (*n* 705)		Remaining cohort‡ (*n* 7942)		
	n	%	*n*	%	*P*
Child's sex (boy)	362	51·3	4042	50·9	0·822
Child's siblings					
None	320	45·5	3008	44·7	
Younger	13	1·8	196	2·9	
Older	370	52·6	3523	52·4	0·264
Child's carers§					
Family/babysitter	127	18·4	670	10·7	
Kindergarten/school	562	81·6	5611	89·3	<0·001
Child's BMI§ (kg/m²)					
Underweight/normal	618	90·1	4469	89·5	
Overweight/obese	68	9·9	524	10·5	0·640
Maternal age (years)					<0·001
Mean	30·4		29·4		
SD	5·02		5·64		
Maternal education (years)					<0·001
Mean	11·2		10·4		
SD	4·33		4·24		

* For each variable, the total may not add to 705/7942 due to missing data.
† Children evaluated at 2 and 4 years of age.
‡ Cohort evaluated at baseline.
§ Characteristics evaluated in the follow-up evaluation at 4–5 years of age (*n* 6753).

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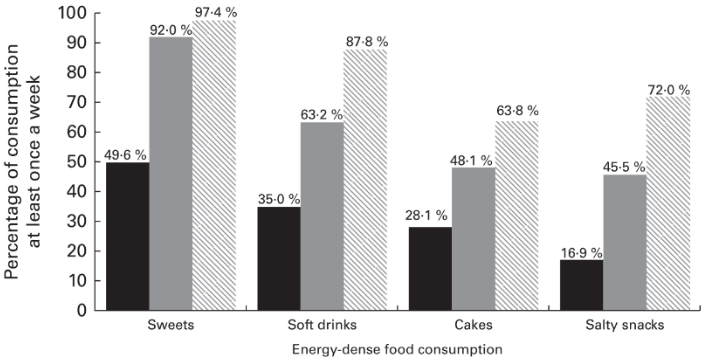

Fig. 1. Proportion of children consuming energy-dense foods at least once a week at 2 and 4 years of age. ■, 2 years (all sample); ■, 4 years (all sample); ▧, 4 years (among those who consumed ≥1/week at 2 years).

effect was found for soft drinks (third *v.* first tertile: IRR = 3·33, 95% CI 2·36, 4·70).

Table 3 presents the associations between the consumption of energy-dense foods at 2 years of age and the consumption of the remaining food groups included in the healthy eating index at 4 years of age. The final index of the study sample ranged from 7 to 25. After adjustment, a higher intake of soft drinks (IRR = 0·74, 95% CI 0·58, 0·95), salty snacks (IRR = 0·80, 95% CI 0·65, 1·00) and sweets (IRR = 0·73, 95% CI 0·58, 0·91) at 2 years of age was inversely associated with the intake of fruit and vegetables at 4 years of age. No significant associations with dairy foods, red meat and meat products, and white meat and fish were found. An increasing frequency of consumption of any energy-dense food was significantly associated with a lower intake of fruit and vegetables (weekly *v.* monthly: IRR = 0·77, 95% CI 0·59, 0·99; daily *v.* monthly: IRR = 0·61, 95% CI 0·44, 0·83). A significant crude association was found between the consumption of energy-dense foods and the intake of red meat and meat products (IRR = 1·50, 95% CI 1·09, 2·06); however, after adjustment, the association did not remain statistically significant.

In the multivariate analysis, higher consumption of each of the energy-dense foods, except cakes, at 2 years of age was significantly associated with a lower score in the healthy eating index at 4 years of age. Weekly and daily consumption of any energy-dense food was associated with a worse diet quality, compared with less frequent consumption (IRR = 0·75, 95% CI 0·58, 0·96; IRR = 0·56, 95% CI 0·41, 0·77, respectively) (Table 4). The potential modifying effect of maternal education was tested using an interaction term in the final model, and no significant interaction effect was observed (P>0·05).

Table 2. Associations between the consumption of energy-dense foods at 2 years of age and the consumption of similar foods at 4 years of age (Incidence rate ratios (IRR) and 95% confidence intervals)

	Consumption at 4 years (≥median *v.* <median)*			
	Crude		Adjusted†	
	IRR	95% CI	IRR	95% CI
Soft drinks at 2 years‡				
First tertile	1§		1§	
Second tertile	2·37	1·73, 3·25	2·22	1·59, 3·09
Third tertile	3·64	2·65, 4·98	3·33	2·36, 4·70
Salty snacks at 2 years‡				
First tertile	1§		1§	
Second tertile	1·55	1·21, 1·98	1·53	1·18, 1·97
Third tertile	1·66	1·34, 2·07	1·63	1·30, 2·04
Cakes at 2 years‡				
First tertile	1§		1§	
Second tertile	1·23	0·91, 1·66	1·26	0·93, 1·71
Third tertile	1·61	1·24, 2·08	1·60	1·23, 2·10
Sweets at 2 years‡				
First tertile	1§		1§	
Second tertile	1·45	1·13, 1·87	1·44	1·11, 1·88
Third tertile	1·69	1·32, 2·18	1·65	1·27, 2·16

* Median of consumption at 4 years of age: soft drinks – 1·5 times per week; salty snacks – 0·9 times per week; cakes – 1 time per week; sweets – 6 times per week.
† Adjusted for maternal age and education, child's siblings, child's carer and child's BMI.
‡ Tertiles of consumption at 2 years of age: soft drinks – first tertile (0 times per week), second tertile (>0–1·5 times per week), and third tertile (>1·5 times per week); salty snacks – first tertile (≤0·1 times per week), second tertile (>0·1–0·5 times per week), and third tertile (>0·5 times per week); cakes – first tertile (≤0·1 times per week), second tertile (>0·1–0·6 times per week), and third tertile (>0·6 times per week); sweets – first tertile (≤0·5 times per week), second tertile (>0·5–2·0 times per week), and third tertile (>2·0 per week).
§ Reference class.

Discussion

The present study shows that the consumption of energy-dense foods at 2 years of age is independently associated with higher consumption of these foods later and is related to a poorer diet quality at 4 years of age.

Children consuming soft drinks more often at 2 years of age were approximately three times more likely to consume soft drinks at 4 years of age, and this was the strongest association found. This finding raises concern, as sugar-sweetened beverages have been reported to be significantly associated with childhood obesity due to both a high glycaemic index and a weak compensatory response to beverages[37]. On the other hand, salty snacks, cakes and sweets are high-energy-dense

Table 3. Associations between the consumption of energy-dense foods at 2 years of age and the consumption of different food groups at 4 years of age (Incidence rate ratios (IRR) and 95 % confidence intervals)

	Consumption at 4 years (≥median *v.* <median)*															
	Fruit and vegetables				Dairy foods				Red meat and meat products				Lean meat and fish			
	Crude		Adjusted†		Crude		Adjusted†		Crude		Adjusted†		Crude		Adjusted†	
	IRR	95 % CI	IRR	95 % CI	IRR	95 % CI	IRR	95 % CI	IRR	95 % CI	IRR	95 % CI	IRR	95 % CI	IRR	95 % CI
Soft drinks at 2 years‡																
< Median	1§		1§		1§		1§		1§		1§		1§		1§	
≥ Median	0·68	0·54, 0·85	0·74	0·58, 0·95	1·10	0·92, 1·34	1·07	0·87, 1·30	1·29	1·04, 1·60	1·18	0·94, 1·49	0·92	0·76, 1·13	1·00	0·81, 1·25
Salty snacks at 2 years‡																
< Median	1§		1§		1§		1§		1§		1§		1§		1§	
≥ Median	0·77	0·63, 0·95	0·80	0·65, 1·00	1·03	0·85, 1·25	1·00	0·83, 1·21	1·20	0·97, 1·49	1·14	0·91, 1·43	0·94	0·78, 1·15	0·97	0·80, 1·19
Cakes at 2 years‡																
< Median	1§		1§		1§		1§		1§		1§		1§		1§	
≥ Median	0·84	0·68, 1·05	0·89	0·70, 1·12	1·06	0·88, 1·29	1·05	0·86, 1·28	1·16	0·93, 1·44	1·08	0·86, 1·36	0·84	0·68, 1·04	0·87	0·70, 1·08
Sweets at 2 years‡																
< Median	1§		1§		1§		1§		1§		1§		1§		1§	
≥ Median	0·67	0·55, 0·83	0·73	0·58, 0·91	1·06	0·88, 1·27	1·05	0·86, 1·27	1·13	0·90, 1·41	1·14	0·91, 1·43	1·00	0·83, 1·22	1·09	0·89, 1·34
Energy-dense foods at 2 years‖																
< 1 time per week	1§		1§		1§		1§		1§		1§		1§		1§	
Weekly	0·73	0·57, 0·93	0·77	0·59, 0·99	1·11	0·86, 1·41	1·07	0·82, 1·38	1·17	0·86, 1·60	1·09	0·80, 1·50	0·85	0·66, 1·08	0·90	0·70, 1·17
Daily	0·53	0·40, 0·71	0·61	0·44, 0·83	1·13	0·87, 1·48	1·09	0·82, 1·45	1·50	1·09, 2·06	1·31	0·94, 1·84	0·86	0·66, 1·12	0·97	0·72, 1·30

* Median of consumption: fruit and vegetables – 5 times per d; dairy foods – 3·5 times per d; fatty meat and meat products – 11·5 times per week; lean meat and fish – 1·3 times per d.
† Adjusted for maternal age and education, child's siblings, child's carer and child's BMI.
‡ Median of consumption: soft drinks 0·5 times per week; salty snacks 0·2 times per week; cakes 0·5 times per week; sweets 1·0 time per week.
§ Reference class.
‖ Includes soft drinks, salty snacks, cakes and sweets. Sample size by categories of consumption: <1 time per week (*n* 147); weekly (*n* 339); daily (*n* 214).

Table 4. Associations between the consumption of energy-dense foods at 2 years of age and the healthy eating index at 4 years of age

(Incidence rate ratios (IRR) and 95 % confidence intervals)

	Consumption at 4 years					
	Healthy eating index (≥median *v.* <median)*					
	Crude				Adjusted†	
	Mean	SD	IRR	95 % CI	IRR	95 % CI
Soft drinks at 2 years‡						
< Median	18·1	3·52	1§		1§	
≥ Median	16·3	4·92	0·55	0·44, 0·69	0·64	0·50, 0·83
Salty snacks at 2 years‡						
< Median	18·1	3·77	1§		1§	
≥ Median	15·9	3·36	0·65	0·53, 0·80	0·70	0·57, 0·87
Cakes at 2 years‡						
< Median	20·3	4·50	1§		1§	
≥ Median	17·4	3·67	0·78	0·62, 0·97	0·84	0·66, 1·06
Sweets at 2 years‡						
< Median	19·3	5·62	1§		1§	
≥ Median	18·1	3·66	0·62	0·50, 0·76	0·73	0·58, 0·91
Energy-dense foods at 2 years‖						
< 1 time per week	19·4	3·32	1§		1§	
Weekly	17·0	3·46	0·67	0·53, 0·85	0·75	0·58, 0·96
Daily	15·2	3·37	0·44	0·33, 0·59	0·56	0·41, 0·77

* Median = 17, ranging from 7 to 25.
† Adjusted for maternal age and education, child's siblings, child's carer and child's BMI.
‡ Median of consumption: soft drinks 0·5 times per week; salty snacks 0·2 times per week; cakes 0·5 times per week; sweets 1·0 time per week.
§ Reference class.
‖ Includes soft drinks, salty snacks, cakes and sweets. Sample size by categories of consumption: <1 time per week (*n* 147); weekly (*n* 339); daily (*n* 214).

foods with a high glycaemic index and a low fibre content[38]. These dietary factors may also increase energy intake, hence promoting a positive energy balance and increasing obesity risk. Decreasing energy density of foods along with other strategies may lead to sustained decreases in energy intake[39].

The consumption of energy-dense foods might have a diluting effect on nutrient intake and total diet quality if they displace nutrient-dense foods such as milk and fruit[40,41]. In the present study, increased consumption of soft drinks, salty snacks (including fast foods) and sweets at an early stage was found to be associated with lower consumption of fruit and vegetables later in life. Fruit and vegetables have a low glycaemic index[42] and a high fibre content, which *per se* may protect against excessive weight gain[43]. Consistent scientific evidence from epidemiological studies, such as case–control and prospective studies, support an inverse relationship between dietary consumption of fruit and vegetables and CVD[44–46]. Moreover, the WHO has highlighted the potential for the consumption of fruit and vegetables to reduce the risk of type 2 diabetes mellitus and to help to achieve or maintain a healthy body weight[47]. Previous studies[13,31,40], mostly conducted among school-aged children and adolescents, have found an inverse association between the consumption of energy-dense foods, mainly soft drinks, and the consumption of dairy foods. This relationship could be partially explained by the replacement of milk and milk products by soft drinks. However, in the present study, the consumption of energy-dense foods at 2 years of age was found to be not significantly associated with the consumption of dairy foods at 4 years of age. The reason for this may be that dairy foods are mainly consumed at breakfast (data not shown) and the prevalence of skipping breakfast is low among these children (4 %). On the other hand, this replacement of dairy foods by soft drinks may be more likely later in life and not at such young ages[34].

The main finding of the present study was the association of high consumption of energy-dense foods at young ages with lower scores in healthy eating index 2 years later, particularly through lower consumption of fruit and vegetables. Decreased diet quality scores were found to be consistently associated with higher rates of all-cause mortality and rates and mortality of select diseases (e.g. CVD and cancer) in adulthood[27]. A pattern of high consumption of energy-dense food could also contribute, *per se*, to worst future health outcomes, namely obesity, as has been suggested in previous studies[21,22]. Claims that energy-dense nutrient-poor foods can be part of a healthful diet[48] should be discouraged.

Moreover, special attention should be given to the role of a carer in the establishment of a child's dietary patterns. At the age range focused upon in the present study, children do not shop for foods themselves; therefore; their eating patterns are reflective of the food shopping and nutrition knowledge of the carers. Thus, there is a great need for intervention from the carers, as they have the main responsibility for the eating habits of children.

A major strength of the present study is the use of a prospective approach to evaluate the association between poor food habits early in life and dietary habits a few years

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later. It used a sample of children from a population-based cohort, with characteristics similar to those of the remaining cohort. Only a few not relevant differences were found, such as maternal age and education, and these were controlled for in the adjusted analysis.

The accurate assessment of dietary intake in children is a challenge in epidemiological studies, and the use of parents as proxy reporters of their children's food consumption patterns is consensual. Dietary information reported by parents or carers at both ages could be biased, since they might not be always aware of all the foods eaten by the children when they are being taken care of by others. However, at this early stage of life, this is less likely to occur. A social desirability bias regarding food intake could be present, as dietary intake data were collected through face-to-face interviews. Data on consumption recorded could reflect attitudes about what should be consumed as opposed to what was really consumed. If lower intakes of 'unhealthy' foods and/ or higher intakes of 'healthy' foods have been reported, our associations could be underestimated.

The weak correlation found between 2 d food record and FFQ at 2 years of age could be the result of a low intake of these types of foods at this age, and a 2 d food record might not be a good method to validate extremely low intakes. Compared with food records, the FFQ seems to have overestimated the consumption at both ages, and as we did not expect to have a differential error, this would not compromise the conclusions of the present study regarding the associations found.

The application of a healthy eating index to summarise the overall diet quality represents a complementary approach to the study of single foods and accounts for cumulative and interactive effects of nutrients and foods and thus may provide a more comprehensive approach.

In conclusion, the consumption of energy-dense foods at young ages is associated with the diet quality of children, particularly in relation to the adequate consumption of fruit and vegetables.

As dietary habits of childhood might persist into adulthood, the present results suggest that the consumption of energy-dense nutrient-poor foods should be limited in childhood in order to prevent lifelong adverse effects on the diet quality of children.

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The authors' contributions are as follows: S. V. was responsible for the analysis and interpretation of the data and wrote the first draft of the paper; A. O. and C. L. were also responsible for the analysis and interpretation of the data. All authors contributed to the concept and design of the study and paper review.

None of the authors has any conflicts of interest to declare.

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Paper III

Adherence to a healthy eating pattern from pre-school to school age and its associations with socioeconomic and early life factors.

Marta Costa, Catarina Durão, Carla Lopes, Sofia Vilela

Abstract

Introduction: Childhood has been described as an important period for the development of healthy eating behaviours. The objective of this study was to evaluate the tracking of food consumption, including the adherence to a healthy eating index from pre-school to school age and its association with early life factors and socioeconomic characteristics.

Methods: The sample includes 5013 children evaluated at 4 and 7 years old from the Portuguese birth cohort Generation XXI with complete information on food frequency questionnaire. A healthy eating index was developed at both ages to assess adherence to the WHO's dietary recommendations, including eight food groups. Consumption quartiles were obtained for each group and assigned a score between 1 and 4. A higher score represents a better diet. The associations between early life factors and socioeconomic characteristics and the adherence to a healthy eating index at 7 years were evaluated through linear regression models.

Results: The healthy eating index had an average score of 21.4 ± 3.53 (range:12-32) at 4 years and 19.6 ± 3.65 (range: 9-32) at 7 years. After adjustment for confounders, a positive association was found between the index at 4 and 7 years (β=0.329, 95%CI=0.295; 0.363). Maternal years of education (β=0.044, 95%CI=0.016; 0.071) and maternal dietary score (β=0.175, 95%CI=0.143; 0.207) were positively associated with increasing dietary quality from 4 to 7 years.

Conclusion: A healthier diet at preschool age, higher maternal education and a healthier maternal diet, increase the likelihood to maintain a healthy eating pattern in school age.

Keywords: tracking, cohort studies, children, dietary habits, maternal behaviours.

Introduction

Childhood is a key period in life for the development of healthy eating habits that can persist for a long time[1-5]. The transition from pre-school to school can influence changes in children's food consumption[6]. Previous literature has been shown that eating habits are defined early and can be maintained throughout childhood, being more difficult to modify behaviours in older children[2, 3]. However, eating habits have mainly been tracked in adults or between adolescence and adulthood, while few studies have tracked diet during childhood[5, 7]. Understanding how children's eating patterns are acquired is essential in public health planning, supporting strategies to improve quality of children's diet.

Maternal behaviours during pregnancy and after birth influence the development of the child's eating habits. In fact, children whose mothers smoked during pregnancy are more likely to be overweight throughout their lives(8). Breastfeeding is also one of the factors that plays a significant role in establishing long-term child's eating habits[9], and its duration is inversely associated with the child's risk of being overweight later[10].

Childhood is also an important period for the development of positive attitudes towards eating behaviours[6] and several environmental factors can influence food choices in this period[11]. The diet of pre-school children can be influenced by parental behaviours (e.g. parental food habits) and family characteristics[12-15]. In particular, parental socioeconomic status seems to have a strong influence in children's food intake, since low levels of maternal education have been associated with lower compliance with food recommendations[2, 14, 16], and with a higher consumption of energy-dense foods (EDF)[12], compared to children of mothers with high levels of education. This negative effect of low levels of education on food intake might be, in part, the result of parents' poor health literacy (e.g., ability to obtain, process and apply basic health information)[17, 18].

On the other hand, children with older and more educated mothers tend to have a healthier diet[19, 20]. The maternal age is another important determinant, as children of younger mothers seem to have a more similar diet to those of children with less educated mothers, presenting, for instance, a lower consumption of whole-wheat bread and fruit[19, 21].

Understanding whether children's food consumption changes during childhood, when food consumption remains stable and what factors influence them is important since it allows to formulate interventions that promote a healthy diet at an early childhood stage[3, 22, 23]. Thus, the present study aimed to evaluate the tracking of food consumption, as well as the adherence to a healthy eating index from 4 to 7 years of age, and its association with early life factors and maternal socioeconomic characteristics.
socio-economic characteristics.

Methods

Participants

The present study was based on the population-based birth cohort Generation XXI, described elsewhere[24]. For this cohort, all the newborns in the five public maternity hospitals in the metropolitan area of Porto were invited to participate, between 2005 and 2006. Of the invited mothers, 91.4% agreed to participate, with a total of 8647 children enrolled at baseline. At 4 years of age, the first follow-up of the entire cohort was performed, where 86% of the children were evaluated. The second follow-up was performed at 7 years of age, and 80% of the children were re-evaluated. During the follow-up periods, participants were invited to participate in a face-to-face interview. For those families that were not able to be present during the face-to-face interview, data were collected by telephone with a shorter version of the questionnaire.

For the final sample, we considered all children with complete information on the food frequency questionnaire (FFQ) at both ages (n=5046). After exclusion of children with diseases who might influence the analysis of food intake (celiac disease, allergy and food intolerance, n=15) and children with malformations that might affect feeding (n=18), 5013 children were included in the final sample.

Data collection

Data were collected by trained interviewers using structured questionnaires. The following variables collected at baseline were included in the study: maternal socioeconomic characteristics at delivery (maternal working status, age and complete years of education), maternal and child characteristics during pregnancy and delivery (gestational diabetes, hypertension status, smoking status during pregnancy and weight gain during pregnancy; child's sex, birth weight and birthweight for gestational age). From the 4-year follow-up evaluation, we have included maternal and child's body mass index (BMI) and dietary intake, child's physical activity and if the child was breastfeeding and the duration. From the 7-year follow-up, the children's dietary intake was used.

Child's dietary intake

Data on children's food consumption was collected through an FFQ, applied by trained interviewers to the main caregiver of the child (usually the mother), referring to the 6 months prior the interview, at both ages. At 4 years, the FFQ asked the frequency of intake of 35 food items, while at 7 years the FFQ included 38 food items, as previously described[25, 26]. The nine possible frequency options were converted into daily consumption frequencies. Similar food groups were created at both ages, based on the food recommendations of the Portuguese food wheel guide[27]: 'Dairy' (semi-skimmed milk, skimmed milk, cheese and yogurts); 'Meat and meat products' (pork, beef, rabbit, poultry and processed meat); 'Fish and eggs'; 'Cereal products and potatoes' (rice, pasta, potatoes, bread and semi-sweet type biscuits); 'Fruit and vegetables' (vegetable soup, raw and cooked vegetables, and fruit); 'Sweets' (cakes, sweet pastry, chocolate, chocolate powder, chocolate milk and candies); 'Salty snacks' (crisps, pizzas and burgers) and 'Soft drinks' (sweetened carbonated drinks and other sweetened drinks, including diet drinks).

In a subsample of children, dietary intake was also collected using 3-day food diaries at 4 and 7 years of age (n=2993), completed by the main caregiver, regarding two weekdays and one weekend day[(30)]. Parents were asked to record all the foods and drinks that children had consumed during those days, describing the amount, brand, recipe and place of consumption, through food diaries. The codification process was conducted *a posteriori* by a team of trained nutritionists, and nutrient intake was estimated using the software Food Processor SQL[(28)].

Healthy eating index

A healthy eating index, based on WHO's dietary recommendations, previously developed[(29)] for the 4-year-old children, was adapted to this study. The index was developed to evaluate the adherence to a healthy eating pattern at 4 and 7 years of age and includes the eight previously defined food groups. For each food group, quartiles of the frequency of consumption were obtained and a score was assigned ranging from 1 to 4 points. For foods considered healthier, such as fruit, vegetables, dairy, fish and eggs, the lowest consumption quartile was scored with 1 point, intermediate quartiles with 2 to 3 points, and the highest consumption quartile was scored with 4 points. In the food groups with less healthy foods, such as meat, meat products, salty snacks, sweets and soft drinks, the score was assigned inversely, that is, with the highest consumption quartile receiving the lowest score. In the end, a higher score represented a higher adherence to a healthy eating pattern and the possible range score of the final index was between 8 and 32 points.

To validate the index, we used dietary information collected through food diaries. The information used in the validation was the children's total energy intake (kcal/day), the percentage of energy from carbohydrates, proteins and fat, and the fiber intake (g/day), at both ages.

Other child's characteristics

A detailed description of newborns' anthropometrics is described elsewhere [(31)]. Briefly, birth weight was abstracted from medical records by trained interviewers at baseline evaluation. During the hospital stay, trained examiners weighed babies to the nearest 1 g using infant scales. The birth weight adjusted for gestational age was computed as a continuous variable according to the Canadian reference (32). Accordingly, children were categorized as small for gestational age (children with birth weight below the 10th percentile for their gestational age and sex), appropriate for gestational age or large for gestational age (children weighing above the 90th percentile for their gestational age and sex).

Objective measurements of children's height and weight at 4 years were performed by a team of trained examiners, according to standard procedures. The children's BMI was defined by the weight in kg divided by the height in m^2. This variable was then categorized using specific cut-offs for sex and age as defined by the WHO[33] [Underweight/Normal weight (≤1SD); Overweight (>1SD to ≤2SD); Obesity (>2SD)].

Regular practice of structured physical exercise was used as qualitative variable and children were classified as non-practitioners or practitioners. The variable breastfeeding, collected as any breastfeeding duration in weeks, was categorized as never or less than 16 weeks, between 16 weeks and 20 weeks, and more than 20 weeks.

Maternal characteristics

Maternal working status at baseline was evaluated as a nominal variable, converted into working (part or full-time and working student) and not working (unemployed, student, seeking the first job, incapacitated and retired). As described before[34] mothers were considered as having gestational diabetes or hypertension during pregnancy when recorded on obstetrical records as a diagnosis during the current pregnancy. The maternal smoking status was asked at baseline for each of the pregnancy trimesters and categorized as not smoking during that period or smoking (less than 1 cigarette/day or at least 1 cigarette/day). The weight gain during pregnancy was obtained by subtracting the self-reported final pre-delivery weight by the preconceptional weight. The results were categorized as ≤10kg, >10kg and ≤20kg, >20kg.

Objective measures of maternal height and weight were performed when the child was 4 years of age by a team of trained examiners, according to standard procedures. The maternal BMI was defined by the weight in kg divided by the height in m^2. This variable was then categorized as normal or underweight (<25kg/m^2) and overweight/obese (≥25kg/m^2), according to the WHO cut-off points[35].

Information on maternal dietary intake at the 4-year-evaluation was collected through an adapted FFQ from a previous questionnaire validated for the general adult population[36] and for pregnant women in a subsample of this cohort[37]. This FFQ collected information on food intake of the previous year and included 18 food items with 9 response options ranging from 'never' to '4 or more times per day'. Frequencies of consumption were converted into daily frequencies. A maternal food index previously developed was used in this study to evaluate the influence of maternal food intake on the child's food intake[38]. Briefly, a score was obtained considering eight food items/groups: milk, fish, red meat, bread, fruit, vegetables, EDF and sugar-sweetened beverages (SSB). For each item/food group, quartiles of frequency of consumption were obtained and a score was assigned with a range from 1 to 4 points, according to the increase in the quartile of consumption (milk, bread, fruit and vegetables) or decrease in quartiles of consumption (red meat, EDF and SSB). The food score ranged from 8 to 32 points. In the end, a higher score represents a better diet.

Ethical approval
The Generation XXI project was conducted in accordance with the guidelines defined in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Hospital de São João/ University of Porto Medical school. The legal representatives of each participant were informed about the benefits and potential discomforts, through written informed consent.

Statistical analysis
Categorical variables were described as frequency and compared by the McNemar test. Continuous variables were summarized by mean and standard deviation (SD) or median and interquartile range (IQR). Comparison between groups was performed using the t-student test for paired samples or the Wilcoxon test, respectively. The adherence to the healthy eating index at both ages was compared using the t-student test for paired samples. The correlation between nutrient intake and the score of the healthy eating index, at both age, was obtained using the Pearson correlation coefficient.

The associations between adherence to the healthy eating index at 4 years, early life factors and socioeconomic characteristics, and adherence to the index at 7 years were evaluated through linear regression models - regression coefficients and respective 95% confidence intervals [β, 95%CI]. A step-by-step approach was used: predefined blocks of variables were fitted separately, with each block mutually adjusted, and introduced cumulatively into the analysis, with a fixed order. The model 1 was adjusted for maternal socioeconomic characteristics (maternal age, education and work status at delivery); the model 2 was furthermore adjusted for maternal and children characteristics during pregnancy and at delivery (gestational diabetes, hypertension, weight gain during pregnancy, smoking status, child's weight for the gestational age and breastfeeding); the model 3 included variables from model 2 plus maternal and child's characteristics at 4-year evaluation (maternal BMI, maternal dietary score and child's physical exercise); and model 4 included all the previous variables plus the healthy eating index score at 4 years. All the models were adjusted for child's sex and BMI. The models' goodness-of-fit was evaluated through adjusted R squared.

The software used was the Statistical Package for the Social Sciences (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). We considered a significance level lower than 0.05.

Results
Table 1 shows the sociodemographic and early life characteristics. At baseline, the mothers presented a mean age of 30 (standard deviation (s.d) = 5.2) years, had in average 11 (s.d= 4.3) years of education and 77% were working. Around 6% of the mothers had gestational diabetes and less than 1% had hypertension during that pregnancy. The mean weight gain during pregnancy was 13.6 kg (s.d=5.8), 20% were smoking during the first trimester of pregnancy, 13% in the second and 12% in the last trimester of pregnancy. At

birth, the majority of children (82%) had a weight considered suitable for their gestational age. Less than half of the children (46%) were breastfed beyond 20 weeks. Among the children included in the analysis (49% girls) the majority was underweight or had a normal weight, at both ages. However, the percentage of obese children increased from 4 to 7 years of age (9.8% vs. 15.0%).

Table 2 presents the comparison of daily dietary intake and scores in the healthy eating index, between 4 and 7 years of age. The daily frequency of 'Meat and meat products', 'Fish and eggs', 'Sweet' and 'Soft drinks' increased significantly from 4 to 7 years. On the other hand, the frequency of consumption of 'Cereal products and potatoes' and 'Dairy' have decreased in the same period of time. The proportion of children that followed the Portuguese dietary recommendations for healthy foods and the proportion that consumed daily EDF is also described in table 2. At age 4, almost 90% of the children consumed dairy at least 3 times per day, while at 7 years there was a significant decrease in the daily frequency ($p<0.001$). From 4 to 7 years there was an increased in the proportion of children consuming daily 'Meat and meat products' (72% vs. 80%, $p<0.001$), 'cereal products and potatoes' (≥5/d: 50% vs. 64%, $p<0.001$) and weekly 'fish and eggs' (≥5-6/week: 53% vs. 64%, $p<0.001$), 'At both ages, less than half of the children were consuming five daily servings of 'Fruit and vegetables'. Around 1% of children consumed salty snacks daily at both ages. The daily consumption of 'Sweets' increased from 4 to 7 years (52% vs. 66%, $p<0.001$), and during the same period the proportion of children consuming soft drinks daily, increased significantly to (23% vs. 34%, $p<0.001$).The healthy eating index score decreased slightly from 4 to 7 years [21.4 (s.d.=3.53) vs.19.6 (s.d.=3.85), respectively, $p<0.001$]. The scores ranged from 12 to 32 points at 4 years, and from 9 to 32 points at 7 years (table 2).

The correlation between the index's score at 4 and 7 years with nutrient intake was obtained through 3-day food diaries. At both ages, the index was positively and significantly correlated with protein ($r= 0.111$, $p<0.001$ and $r= 0.167$, $p<0.001$) and fiber intake ($r= 0.205$, $p <0.001$ and $r= 0.246$, $p<0.001$), but inversely with energy intake ($r= −0.060$, $p = 0.006$ and $r= −0.069$, $p<0.001$) and total fat intake ($r= −0.106$, $p <0.001$ and $r= −0.057$, $p=0.002$). The intake of carbohydrates, saturated fat, monounsaturated and polyunsaturated fat did not present a statistically significant association with the index scores, at both ages (Table Supplemental 1).

Figure 1 shows the proportion of children by tertiles of the healthy eating index score at both ages. Almost half of the children (46.6%) that were in the lowest tertile of score at 4 years of age continued in the lowest tertile at the age of 7, while the remaining moved to a higher tertile. Of the children who were in the 2nd tertile of the healthy index score at 4 years of age, one-third remained in the same at 7 years, while other one-third moved to the 1st tertile and the remaining (37.0%) moved to a higher tertile. More than half of the children that were in the highest tertile of score at 4 years remained in the highest tertile at 7 years (59.9%). One-fourth moved to the 2nd tertile and 15% to the 1st tertile.

In the analysis of table 3, the associations were considered by consecutive addition of blocks in the model. Regarding the association between maternal socioeconomic factors at delivery and the healthy eating index score at 7 years (model 1), a positive association was described between maternal age (β=0.042, 95%CI: 0.022;0.063) and years of education (β=0.162, 95% CI: 0.136; 0.187) and the child's dietary intake. After adjusting for maternal characteristics during pregnancy (model 2), the maternal age (β=0.035, 95%CI: 0.013; 0.057) and years of education (β=0.159, 95%CI: 0.133; 0.186) were still positively associated with the index score at age 7. When maternal behaviours at 4-year evaluation were added (model 3), maternal age ceases to be significantly associated with the index score. However, maternal education (β=0.107, 95%CI: 0.079; 0.134) and the maternal dietary score (β=0.302, 95%CI: 0.272; 0.333) were positively associated with the index score at 7 years. When the score of the healthy index at 4 years was added in model 4, a significant positive association was found (β=0.329, 95%CI: 0.295; 0.363). Also, in this model maternal education (β=0.044, 95%CI: 0.016; 0.071) and maternal dietary score (β=0.175, 95%CI: 0.143; 0.207) remained significantly associated with an increased score of the healthy eating indexfrom 4 to 7 years of age. No association was found between early life characteristics (intrauterine and infancy environment) and diet at 7 years of age.

The comparison between models was performed through the adjusted square R, which allows us to know the proportion of y variation explained by all the x's variables taken together. Model 4 was the one that best explains this variation.

Discussion

The present study supports the idea that food consumption suffers changes with the transition from pre-school to school years. These dietary changes from 4 to 7 years consisted mainly on an increase in the consumption of animal protein (meat, fish and eggs), cereals and potatoes, and soft drinks and sweets, and also in a decrease in consumption of dairy, which contributes to a lower adherence to a healthy eating index at 7 years.

The increase in consumption of animal protein from 4 to 7 years is in accordance with previous studies that show that European adolescents have a much higher consumption than the recommendations, mainly due to meat consumption(39). The increase in consumption of cereal products and potatoes may be the result of children at 7 years are already consuming these foods in the main meals and not just vegetable soup, and including them more in the morning and afternoon snacks. The decrease in daily consumption of dairy may be related to the substitution of these foods by SSB, since in the same period, and as reported in other studies, there was an increase in their consumption(40, 41). While the consumption of fruit and vegetable remain the same between 4 and 7 years of age, less than half of the children were consuming at least 5 portions a day. The consumption of foods high in sugar and energy (SSB and sweets) increased significantly; attention should be given to this trend in childhood, as previously reported from 2 to 4 years of age(29), since these food groups are associated with adverse health outcomes, namely a higher risk of obesity in children [42].

Although the adherence to a healthy eating index decreased from preschool to school age, it was verified that the better the index scores at age 4, the better the index score at age 7. One explanation for this association may be that the dietary pattern formed in the first years of life continues to have a strong influence on food intake, even if the child spends more time away from home. Another possibility is that although children are eating out more often, most of the food they consume is still consumed or prepared at home[43]. The comparison between the index score and child's nutrients intake obtained through food diaries showed that the index was indeed evaluating a better diet, at both ages. Moreover, the comparison of the healthy eating index score at 4 and 7 years of age, by tertiles, showed that the higher the tertile score at age 4, higher the probability of remaining at the highest tertile of scoring at age 7. The inverse was also observed.

Schooling and maternal age were independently associated with the child's adherence to a healthy eating index score at 7 years. These results are in concordance with previous studies, that show that healthier eating habits among pre-school and school children have been associated with higher maternal education[2, 16, 21, 44-46]. Low maternal schooling increases the probability of a poorer food quality diet in children, as this socio-demographic condition probably limits the purchasing power and the ability to obtain adequate nutritional information[2]. This association is due to the fact that parents are important role models for children's dietary intake. There is considerable evidence that children's eating behaviours, including food preferences, are very similar to that of their parents[47, 48]. In the present study, a better maternal dietary intake influenced positively the children's diet. These findings were consistent with other reports[38, 49] , with maternal diet being one of the most important factors associated with the healthy eating index score at 7, in our study. This suggests that particular efforts should be made toward food education for less educated mothers, encouraging them to adopt healthy eating habits[19, 20, 48]. Maternal work status was significantly associated with the dietary pattern of children in a study in the UK; however, the same did not occur in our sample[2].

Excessive gestational weight gain during pregnancy and gestational diabetes were previously recognized as prenatal determinants of childhood obesity[50, 51], which might be explained in part by the adoption of less healthy eating habits. However, we did not find any statistical significant association between these factors and the adherence to the index at 7 years. Moreover, maternal smoking status was associated with dietary intake at 7 years. The present results are not in line with previous studies, which show an association between low scores in a healthy pattern and smoking during pregnancy[23]. A recent study combining information from 4 European cohorts, named Habeat Project, conclude that never or short duration of breastfeeding (but not the timing of complementary feeding) were associated with a less healthy diet in early childhood, including a lower consumption of fruit and vegetable[52]. However, in the present study, we did not find a significant association between breastfeeding and a better score in the healthy eating index.

These results should be viewed in light of some limitations. The QFA was answered by parents, in a face-to-face interview, who might not be aware of everything is offered to the children to eat, when they are with other caregivers. However, in in a subsample of children from the Generation XXI cohort, dietary information collected from 3-day food diaries were compared with dietary data from the FFQ and the results support the validity of the results, particularly for the foods more frequently consumed[38]. Another possible limitation is the fact that some parents might report what, for them, the child is supposed to consume instead of what was actually consumed or report more healthy food and less unhealthy food. If this has occurred, our associations could be underestimated and be even stronger. The use of two methods to collect dietary intake information, food diaries and QFA, can be considered a strength of this study, as it allows the reduction of report errors. The main strengths of the study are the longitudinal design, based on a relatively large sample size from participants of a population-based birth cohort regularly evaluated, with a high participation rate. This allows us to ensure a temporal sequence between several exposures and the tracking of children's dietary intake. Finally, by performing the statistical analysis by models, we were able to identify easily the possible confounders of these associations.

Conclusion

This study suggests that promoting healthy eating habits at early ages is essential in order to prevent unfavourable eating habits later. The quality of dietary intake seems to decrease from preschool to school age and healthy eating habits stablished at 4 years influence healthy eating habits at 7 years. We could also verify that maternal socioeconomic and behavioural factors have a higher influence on the maintenance of healthy eating habits from 4 to 7 years, compared to metabolic or genetic factors. A higher maternal education and a healthier maternal diet, increase the likelihood of maintaining a healthy eating pattern at school age. In that way, Interventions to promote healthy eating habits among preschool children should involve mothers, especially those with lower education, with a greater focus on their diet and its consequences on children's healthy eating habits.

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Table 1. Socio-economic characteristics, pregnancy and early-life characteristics

	Evaluation		
	Baseline	4 years	7 years
Maternal age (years), mean (s.d.)	29.8 (5.2)	-	-
Maternal level of education (years), mean (s.d.)	11.1 (4.3)	-	-
Maternal working status (working), n (%)	3940 (77.1)	-	-
Gestational Diabetes, n (%)	274 (5.7)	-	-
Hypertension during pregnancy, n (%)	43 (0.8)	-	-
Weight gain during pregnancy (kg), mean (s.d)	13.6 (5.8)	-	-
Smoking during pregnancy, n (%)			
1st Trimester	1028 (20.0)	-	-
2nd Trimester	672 (13.2)	-	-
3rd Trimester	626 (12.3)	-	-
Maternal BMI, mean (s.d.)	-	26.4 (5.1)	-
Maternal Dietary Score, mean (s.d.)	-	18.8 (3.7)	-
Girls, n (%)		2462 (49.1)	
Child's birthweight for gestational age, n (%)			
Suitable for gestational age	4226 (82.3)	-	-
Small for gestational age	728 (14.1)	-	-
Large for gestational age	186 (3.6)	-	-
Breastfeeding, n (%)			
<16 weeks		2058 (39.8)	-
≥16 weeks and ≤20 weeks		601 (11.7)	-
>20 weeks		2487 (48.3)	-
Child's Structured Physical Exercise, n(%)	-	4393 (85.3)	-
Child's BMI, n (%)			
Underweight/ normal weight	-	3433 (69.2)	3176 (63.4)
Overweight	-	1041 (21.1)	1076 (21.5)
Obesity	-	487 (9.8)	751 (15.0)

BMI, body mass index; s.d., standard deviation

Table 2 - Children's dietary intake at 4 and 7 years of age.

	4 years	7 years	p-value
Dairy (frequency/d), mean (s.d.)	4.43 (1.45)	4.18 (1.70)	<0.001*
Dairy (≥3/day), n (%)	4004 (87.4)	3825 (74.9)	<0.001*
Meat and meat products (frequency/d), mean (s.d.)	1.43 (0.56)	1.55 (0.71)	<0.001*
Meat and meat products (≥1/day) n (%)	3287 (71.8)	4088 (80.1)	<0.001*
Fish and eggs (frequency/d), mean (s.d.)	0.77 (0.30)	0.87 (0.35)	<0.001*
Fish and eggs (≥5-6/week) n (%)	2404 (52.5)	3260 (64.0)	<0.001*
Cereal products and potatoes (frequency/d) mean (s.d.)	4.61 (1.33)	4.42 (1.25)	<0.001*
Cereal products and potatoes (≥5/day) n (%)	2296 (50.1)	3269 (64.0)	<0.001*
Fruit and vegetables (frequency/d), mean (s.d.)	4.64 (1.54)	4.50 (1.65)	<0.001*
Fruit and vegetables (≥5/day) n (%)	1921 (41.9)	2022 (39.1)	0.008*
Sweets, median (IQR)	1.20 (1.44)	1.27 (1.37)	<0.001#
Sweets (≥1/day) n (%)	2705 (52.3)	3393 (65.6)	<0.001
Salty snacks, median (IQR)	0.13 (0.14)	0.13 (0.13)	<0.001#
Salty snacks (≥1/day) n (%)	51 (1.1)	46 (0.9)	0.816
Soft drinks, median (IQR)	0.57 (1.00)	0.92 (1.15)	<0.001#
Soft drinks (≥1/day) n (%)	1089 (23.8)	1771 (34.3)	<0.001
Healthy eating index score, mean (s.d.)	21.4 (3.53)	19.6 (3.65)	<0.001*

BMI, body mass index; s.d., standard deviation
IQR interquartile range
*-t-student teste for paired samples
Wilcoxon test

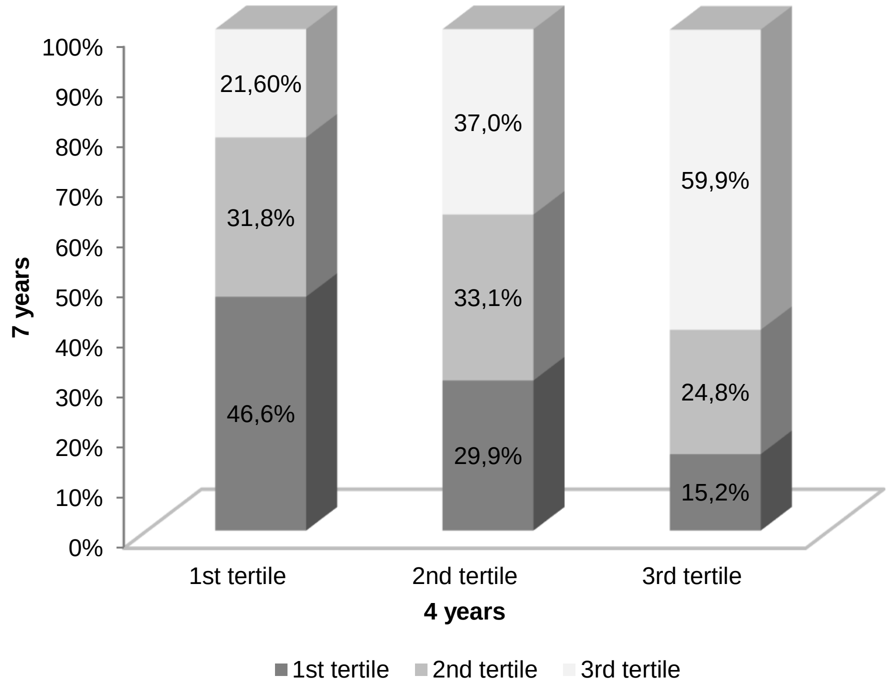

Fig. 1 Proportion of children by tertiles of healthy eating index score at 4 and 7 years of age

Table 3. Associations between maternal and child characteristics and adherence to a healthy eating index scores at 7 years of age

	Model 1 β (95%CI)	**Model 2** β (95%CI)	**Model 3** β (95%CI)	**Model 4** β (95%CI)
Sociodemographic factors at child's delivery				
Maternal age (years)	**0.042 (0.022; 0.063)**	**0.035 (0.013; 0.057)**	-0.012 (-0.034; 0.010)	-0.017 (-0.038; 0.004)
Maternal education (years)	**0.162 (0.136; 0.187)**	**0.159 (0.133; 0.186)**	**0.107 (0.079; 0.134)**	**0.044 (0.016; 0.071)**
Maternal work status				
Working	Ref.	Ref.	Ref.	Ref.
Not Working	-0.156 (-0.419; 0.108)	-0.124 (-0.401; 0.152)	-0.048 (-0.317; 0.221)	-0.001 (-0.259; 0.258)
Early life factors – At pregnancy and delivery				
Gestational diabetes				
No		Ref.	Ref.	Ref.
Yes		0.244 (-0.237; 0.726)	0.151 (-0.316; 0.617)	0.042 (-0.408; 0.492)
Maternal hypertension during pregnancy				
No		Ref.	Ref.	Ref.
Yes		0.427 (-0.709; 1.563)	0.603 (-0.487; 1.692)	0.482 (-0.576; 1.540)
Weight gain during pregnancy (kg)				
≤10		Ref.	Ref.	Ref.
>10 and ≤20		-0.134 (-0.383; 0.114)	-0.075 (-0.317; 0.166)	-0.085 (-0.317; 0.148)
>20		-0.286 (-0.696; 0.123)	-0.225 (-0.371; 0.166)	-0.295 (-0.679; 0.089)
Child's birthweight for gestational age				
Suitable for gestational age		Ref.	Ref.	Ref.
Small for gestational age		0.079 (-0.237; 0.394)	0.169 (-0.138; 0.476)	0.108 (-0.188; 0.404)
Large for gestational age		-0.074 (-0.670; 0.521)	-0.167 (-0.745; 0.412)	-0.216 (-0.774; 0.341)
Maternal smoking status during pregnancy				
1st trimester				
No		Ref.	Ref.	Ref.
Yes		-0.101 (-0.518; 0.316)	0.093 (-0.314; 0.499)	0.048 (-0.342; 0.438)
2nd trimester				
No		Ref.	Ref.	Ref.
Yes		-0.314 (-1.309; 0.682)	-0.369 (-1.340; 0.603)	-0.330 (-1.261; 0.601)

3rd trimester				
No		Ref.	Ref.	Ref.
Yes		-0.101 (-1.076; 0.859)	0.071 (-0.873; 1.015)	0.099(-0.805; 1.003)
Breastfeeding				
<16 weeks		Ref.	Ref.	Ref.
≥16 weeks and ≤20 weeks		0.235 (-0.112; 0.582)	0.139 (-0.196; 0.474)	0.163 (-0.159; 0.486)
>20 weeks		0.235 (-0.001; 0.471)	0.021 (-0.209; 0.251)	0.003 (-0.218; 0.225)
Maternal and child's behaviours at 4 years of age				
Maternal dietary score			**0.302 (0.272; 0.333)**	**0.175 (0.143; 0.207)**
Maternal BMI				
Normal or underweight (<25 kg/m²)			Ref.	Ref.
Overweight/obese (≥25 kg/m²)			0.014 (-0.235; 0.208)	0.029 (-0.184; 0.243)
Child's Structured Physical exercise				
Non-practicioners			Ref.	Ref.
Practicioners			0.034 (-0.272; 0.340)	0.055 (-0.242; 0.351)
Child's behaviours				
Dietary score at 4 years of age				**0.329 (0.295; 0.363)**
Adjusted R square	0.044	0.047	0.125	0.196

BMI, body mass index; Statistically significant associations are highlighted in bold; Blocks of models (maternal socioeconomic characteristics; maternal and children characteristics during pregnancy and at delivery; maternal characteristics at 4-year evaluation; and children characteristics) were added sequentially into the analysis; Models are adjusted for sex and BMI of children.

SUPPLEMENTARY MATERIAL

Supplemental Table 1. Correlation between the healthy eating index score at 4 and 7 years and the nutrients obtained by food diaries (n=2993).

Nutrient	4 years		7 years	
	Pearson Correlation	p-value	Pearson Correlation	p-value
Energy intake (Kcal)	-0.060	0.006	-0.069	<0.001
Protein (%TEI)	0.111	<0.001	0.167	<0.001
Carbohydrates (%TEI)	0.040	0.064	-0.022	0.238
Fat (%TEI)	-0.106	<0.001	-0.057	0.002
Saturated fat (%TEI)	-0.141	<0.001	-0.096	<0.001
Monounsaturated fat (%TEI)	-0.039	0.071	0.010	0.603
Polyunsaturated fat (%TEI)	0.003	0.894	-0.025	0.177
Fiber (g)	0.205	<0.001	0.246	<0.001

TEI, total energy intake

Paper IV

Tracking diet variety in childhood and its association with eating behaviours related to appetite: Generation XXI study

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Tracking diet variety in childhood and its association with eating behaviours related to appetite: The generation XXI birth cohort

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ABSTRACT

Research on the influence of early eating habits on eating behaviours related to appetite using a prospective approach is scarce, especially in children. The aim of this study was to explore the relationship between changes in diet variety from 4 to 7 years of age and appetitive traits measured at 7 years of age. Participants are from the population-based birth cohort Generation XXI (2005–2006). The present analysis included 4537 children with complete data on a food frequency questionnaire (FFQ) at both ages, and on the Children's Eating Behaviour Questionnaire at 7y. A healthy diet variety index (HDVI) was calculated at both ages using data from the FFQ. To assess tracking of diet variety, tertiles of HDVI scores were calculated and then re-categorized as 'maintain: low', 'maintain: high', 'increase' and 'decrease'. Although the HDVI score decreased from 4 to 7y ($p < .001$), it showed a high stability, a positive predictive value, and a fair agreement. Increasing diet variety, compared to maintaining a low variety, was inversely associated with the 'Desire to Drink' ($\beta = -0.090$, 95%CI: 0.174; −0.006) and 'Satiety Responsiveness' ($\beta = -0.119$, 95%CI: 0.184; −0.054) subdimensions and positively with 'Enjoyment of Food' ($\beta = 0.098$, 95%CI: 0.023; 0.172) and 'Emotional Overeating' ($\beta = 0.073$, 95%CI: 0.006; 0.139). Those classified as either increase or maintain a high diet variety, in comparison with maintaining a low variety, had lower scores of 'Food Fussiness'. In conclusion, diet variety decreased from 4 to 7y with a fair tracking. Children with a higher diet variety were less fussy, had a lower desire to drink and a higher general interest in food.

1. Introduction

Eating behaviour patterns acquired during childhood and adolescence are likely to track into adulthood (Mikkila, Rasanen, Raitakari, Pietinen, & Viikari, 2004, 2005). Diet tracking might be represented by the maintenance of eating habits, nutrient intake or food intake over time. Poor eating habits established in early life are likely to remain stable, therefore establishing healthy eating habits early on is essential. Dietary tracking has been demonstrated in adults and between adolescence and adulthood period, while few studies have tracked diet during childhood (Northstone & Emmett, 2008). It has been shown that the introduction of a variety of foods as early as the beginning of the complementary feeding, positively influences the variety of the diet later on (Maier, Chabanet, Schaal, Leathwood, & Issanchou, 2008; Skinner, Carruth, Bounds, Ziegler, & Reidy, 2002). Variety of the free food choices between 2 and 3 years has been associated with the variety of food consumed up to the age of 22 years (Nicklaus, Boggio, Chabanet, & Issanchou, 2005). Several paediatric diet quality indices, including assessment of the food variety, have been administered in both developed and developing countries and studied in relation to health-related outcomes (Marshall, Burrows, & Collins, 2014). However, the tracking of the overall food variety throughout childhood, using the same validated instrument, is lacking in the literature.

An earlier study has proposed that other aspects of eating behaviour, such those related to appetite, also track throughout childhood (Ashcroft, Semmler, Carnell, van Jaarsveld, & Wardle, 2008). The authors highlighted that some changes in the magnitude of appetitive traits can, however, occur as the result of

Abbreviations: HDVI, Healthy Diet Variety Index.

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interaction with child's food environment (Mallan, Fildes, Magarey, & Daniels, 2016; de Barse et al., 2017). One aspect previously explored was the decrease of food fussiness behaviour with an increase of diet variety offered to children (Mallan et al., 2016; de Barse et al., 2017).

Recommendations (WHO, 1996) to increase the diet variety in order to achieve a higher quality diet are included in most European national dietary guidelines, including Portugal (WHO European Region, 2003). The term 'diet variety' indicates the number of different foods or food groups consumed over a given reference period. Increasing the variety of nutrient-dense foods, within and across food groups, is thought to ensure adequate intake of essential nutrients and to improve health outcomes (Conklin, Monsivais, Khaw, & Wareham, 2016; Foote, Murphy, Wilkens, Basiotis, & Carlson, 2004; Murphy et al., 2006; Steyn, Nel, Nantel, Kennedy, & Labadarios, 2006; Vadiveloo, Parkeh, & Mattei, 2015). The World Health Organization (WHO) recommends the consumption of a varied diet originated mainly from plants, rather than animals (WHO, 1996). Eating a variety of foods is crucial to achieving optimal nutritional status and complete coverage of essential nutrients. It is also a key factor to tackle chronic micronutrient deficiencies, also known as hidden hunger (Burchi, Fanzo, & Frison, 2011).

A greater variety of healthy foods has been also associated with a lower prevalence of overweight or obesity (Vadiveloo, Dixon, & Parekh, 2013). However, intake of a variety of less healthy foods, such as energy-dense foods, has been positively associated with adiposity (Vadiveloo et al., 2013). It is not known whether exposure to a higher food variety environment might stimulate appetite and increase food consumption, leading to excess energy intake and an unhealthy body weight gain. Short-term controlled feeding studies consistently show that the variety of food offered during a meal increases energy intake, and limiting variety across days tends to reduce food consumption (Brondel et al., 2009; Raynor & Epstein, 2001). The stimulating effect of a high diet variety on appetite might be attributable to lower sensory-specific satiation for multiple components of a meal, to delayed satiation or by making the eating occasion more enjoyable (Brondel et al., 2009). These short-term studies are limited by the tendency to offer highly palatable, energy-dense foods. When low energy-dense foods are offered, for example, fruits and vegetables (FV), the variety effect seems to stimulate intake in both children (Roe, Meengs, Birch, & Rolls, 2013) and adults (Meengs, Roe, & Rolls, 2012a).

The majority of previous studies have investigated the effects of food variety on appetite using short-term controlled feeding studies, however, to date no published research has investigated the association in children between tracking of diet variety and eating behaviours related to appetite, using a prospective approach. Therefore, we aimed to prospectively assess the effect of changes in diet variety from 4 to 7 years on eating behaviours related to appetite at 7 years of age.

2. Material and methods

2.1. Study design and participants

Participants were from the population-based birth cohort Generation XXI, assembled in the five-level III public maternity units in the Porto Metropolitan Area (Northern Portugal), during 2005/2006 (Larsen et al., 2013). At enrolment, these maternity units were responsible for 91.6% of the deliveries in the whole catchment population. Of the invited mothers, 91.4% accepted to participate at baseline (n = 8647 children). Data on demographic and social conditions, lifestyles, medical history and anthropometrics were collected by trained interviewers within 72 h after delivery. When the children were 4 and 7 years of age, an evaluation of the entire cohort occurred, achieving a participation rate of 86% and 81%, respectively. In baseline and follow-up evaluations, information was collected in face-to-face interviews, and for those families that were not able to participate in-person, the evaluation was performed by telephone using a shorter version of the questionnaire (20% and 15% at 4 and 7 years of age, respectively).

The present analysis included 4748 children with complete data using a food frequency questionnaire (FFQ) (Durao et al., 2015) at 4 and 7 years of age, and complete information on the Children's Eating Behaviour Questionnaire (CEBQ) (Wardle, Guthrie, Sanderson, & Rapoport, 2001), at 7 years of age. We excluded twins (n = 183) and children with congenital anomalies or diseases that might influence dietary intake (cerebral palsy, celiac disease, food allergy, food intolerance, and phenylketonuria; n = 28), resulting in a sample of 4537 children. Comparing the children included in the analysis with the remaining cohort, we found no statistical differences regarding sex children. However, mothers of children included in the study at baseline were slightly older (mean (SD): 29.8 (5.25) vs. 28.1 (5.82) years, p < .001) and more educated (mean (SD): 11.2 (4.27) vs. 9.6 (4.10) years, p < .001). The prevalence of obesity in our sample was also higher (68% vs. 67%, p = .009).

2.2. Data collection

2.2.1. Dietary intake

The children's dietary intake was evaluated through an FFQ that queried frequency of intake for 35 and 38 food items at 4 and 7 years of age, respectively, that was previously tested. As previously described (Durao et al., 2015), for each food item, parents or another caregiver were asked how many times on average his/her child had consumed that food during the previous 6 months.

For each food item, the selected frequency response option (4 times or more per day, 2–3 times per day, 1 time per day, 5–6 times per week, 2–4 times per week, 1 time per week, 1–3 times per month\once a month, or never) was converted into daily frequency (e.g. 5–6 times per week was converted into a mean of 5.5 times per week, meaning 5.5/7d = 0.78 times per day). Five food groups were defined: starchy foods (3 original food items: rice, pasta, potatoes, bread and semi-sweet type biscuits); fruits (only one food item); vegetables (3 original food items: vegetable soup and vegetable on plate); meat, fish and alternatives (5 original food items: meat, sausage, ham, fish and eggs); and dairy products (5 original food items at 4y and 4 food items at 7y: yoghurt, cheese and milk).

In a subsample of 2482 children at 4 years and 3511 at 7 years, FFQ data was compared with 3-day food diaries. For food groups eaten more often, fair-to-moderate agreement was obtained. Significant positive intraclass correlation coefficients (ICC) were found for vegetable soup [(ICC = 0.54, 95%CI: 0.51; 0.56) at 4 years and (ICC = 0.54, 95%CI: 0.52; 0.56) at 7 years], fruit [(ICC = 0.42, 95%CI: 0.39; 0.45) at 4 years and (ICC = 0.46, 95%CI: 0.43; 0.48) at 7 years], milk [(ICC = 0.46, 95%CI: 0.43; 0.49) at 4 years and (ICC = 0.50, 95% CI: 0.47; 0.52) at 7 years], yoghurt [(ICC = 0.48, 95%CI: 0.45; 0.51) at 4 years and (ICC = 0.49, 95%CI: 0.47; 0.52) at 7 years], sweets [(ICC = 0.23, 95%CI: 0.19; 0.26) at 4 years and (ICC = 0.22, 95%CI: 0.19; 0.25) at 7 years] and salty snacks [(ICC = 0.19, 95%CI: 0.16; 0.23) at 4 years and (ICC = 0.10, 95%CI: 0.07; 0.13) at 7 years].

2.2.2. Diet variety index

A healthy diet variety index (HDVI) (Jones et al., 2015), based on the Food Variety Index for Toddlers by Cox et al. (Cox, Skinner, Carruth, Moran, & Houck, 1997), was calculated at 4 and 7 years using data from the FFQ. This was done by considering variety within and among the five food groups previously described, and considering the number of servings recommended in the food plate model healthy eating guidelines promoted by the U.S. Department of Agriculture (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010). As indorsed by Cox et al. (Cox et al., 1997), truncations were applied in the next steps to ensure variety in intake both within and between food groups. As recommended in the original index (Cox et al., 1997), a higher variety of a particular food item or food groups could not compensate a low intake of other food item or food groups. First, within each food groups (except FV) the contribution of a particular food item was truncated at 33%. Foods within a food group, which were similar (e.g. yoghurt without sugar and sweetened yoghurt at 4 years) were grouped together and counted as a single food, so they did not contribute more than 33% of the total. Due to the relatively limited number of questions of the FFQ, it was not possible to assess variety of FV. For these items, the index instead reflects whether or not the children ate the recommended number of servings. The number of servings for each food group was completed after the groupings and truncations were applied. Food group scores were calculated by dividing the total number of servings by the recommended number of servings per day, for each food group. The following recommended number of servings was used: starchy foods = 7, fruit = 2, vegetables = 3, meat, fish and alternatives = 2 and dairy foods = 3. Then, a second truncation was applied to ensure variety between the food groups. Each food group score was truncated at 1 point (e.g. if a child ate 3 different types of meat, fish and alternatives daily when this was divided by 2 it gave a potential score of 1·5 points which was then truncated to 1 point). This meant that a high intake of one food group could not compensate for a low intake of another food group. The final HDVI was the sum of the five food group scores and the maximum score was 5, representing a higher diet variety and adequacy.

2.2.3. Children's eating behaviours

Appetitive traits were assessed using a Portuguese version of the Children's Eating Behaviour Questionnaire – CEBQ, originally developed by Wardle et al. (Wardle et al., 2001) which had been previously translated and tested, and shown to have good psychometric properties in 7-year-old Portuguese children (Albuquerque, Severo, & Oliveira, 2017). This questionnaire is a parental report (94% of the questionnaires were answered by mothers) constructed to assess eight subdimensions of eating behaviours in children: 'Food Responsiveness' (FR), 'Enjoyment of Food' (EF), Emotional Overeating' (EOE) and 'Desire to Drink' (DD) as 'food-approach' appetitive traits, and 'Satiety Responsiveness' (SR), 'Slowness in Eating' (SE), 'Emotional Undereating' (EUE) and 'Food Fussiness' (FF) as 'food-avoidant' appetitive traits. This 35-item instrument is rated on a 5-point Likert scale ("never", "seldom", "sometimes", "often" and "always"), scored 1 to 5. Each of the eight subscales contained 3 to 6 items. The items 3 (SR), 4 (SE), 10 (FF), 16 (FF) and 32 (FF) were reverse-scored items. In individuals with missing data for less than 50% of the items, missing data (around 3%) were handled by imputation, replacing the average of the remaining questions within each subdimension.

2.2.4. Covariates

Problematic eating behaviours were also assessed at 4 years of age by caregiver report. Caregivers were asked about any perceived eating problem observed in their child. The following questions were included in the final model as potential confounders: "my child does not eat enough", "my child eats very slowly" and "my child eats too much". Caregivers reported if any of these behaviours happened in the last year and their level of concern (very concerned, somewhat concerned, no concern). These variables were used as proxies of the subdimensions of the CEBQ at 4 years of age. Significant differences were found between different levels of parental concern with children's eating behaviours and scores of the CEBQ subdimensions at 7 years of age (Supplemental Table 1). For example, children with a higher score in 'Enjoyment of Food' and 'Food Responsiveness' were the ones with a higher level of parental concern in "my child eats too much" (9%). Parents that were more concerned with "not eating enough" (35%) had children with higher scores in 'Satiety Responsiveness', 'Slowness in Eating' and 'Food Fussiness,' but lower scores in 'Food Responsiveness' and 'Enjoyment of food' ($p < .001$, Supplemental Table 1).

Other child and maternal characteristics that may confound the association between diet variety and appetitive traits were also considered. The maternal education was evaluated, at 4-year-old evaluation, as the number of completed school years; the maternal age was obtained as the difference between the date of birth and the date of the 4-year interview. Breastfeeding duration (expressed or directly from the breast) was recorded in weeks, and a variable was defined as any breastfeeding duration (recorded as never or less than 16 weeks, between 16 and 20 weeks and more than 20 weeks). The practice of physical exercise (defined as regular physical exercise) was collected at 4 years of age as a qualitative variable (non-practitioners vs. practitioners). Daily screen time was calculated as the daily minutes spent in front of a screen (television, computer or game devices) during both week and weekend days, and categorized into less than 120 min and 120 min or more per day. At 7 years of age, timing of complementary feeding and first food eaten by the infant (cereals porridge (56.9%), fruit (5.2%), vegetable soup (36.6%) or other (1.2%)), was recorded and re-categorized as 'vegetable soup' vs. 'cereals porridge/fruit/other'. The vegetable soup was separated from the other categories due to the beneficial effect previously described in children having vegetables as the first food, in appetite and acceptance of new foods (Cox et al., 1997; de Barse et al., 2017).

In both evaluations, children's anthropometric measurements were performed by a team of experienced examiners, according to standard procedures. Weight was measured in underwear and without shoes using a digital scale 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 to the nearest 0.1 cm. Children's body mass index (BMI) was classified according to age- and sex-specific BMI standard z-scores developed by WHO (WHO Multicentre Growth Reference Study Group, 2006).

2.3. Statistical analysis

Mean (standard deviation) and frequency differences were compared through Student's t-test and chi-square test, respectively. The tracking of behaviours was estimated in different ways, each assessing a specific aspect of tracking. First, tertiles for the score of the HDVI were calculated at 4 and 7 years of age. The proportion of stability was assessed as the proportion of children who remained in the same tertile at both ages divided by the total number of children. If chance alone determined the tertile at age 7, one third would be expected to be placed in the same tertile; a value higher than 0.33 suggests that children do not stay in the same tertile completely by chance. A predictive value for remaining in the highest tertile was calculated as the proportion of children in the highest tertile at 4 years of age who remained in the highest tertile at 7 years, divided by the children in the highest tertile at 4 who had moved to other tertiles at 7 years. A value higher than 1 suggests more children remained than changed. Confidence intervals were calculated for the predictive value. For testing the overall tracking, the ICC was applied. Guidelines for interpreting ICC statistics suggest that values between 0.81 and 1.00 indicate almost perfect agreement, 0.61–0.80 substantial agreement, 0.41–0.60 moderate agreement, 0.21–0.40 fair agreement, and values less than 0.21 indicate a poor or slight agreement (Landis & Koch, 1977).

The effect size for the HDVI was calculated by dividing the mean change in the score (between 4 and 7 years of age) by the standard deviation (SD) of the initial mean score (at 4 years). Cohen classified effect sizes as small ($d = 0.2$), medium ($d = 0.5$), and large ($d \geq 0.8$) (Cohen, 1992). Furthermore, tertiles of HDVI score were transformed as following: 'maintain: high', for children who were at 2nd or 3rd tertile in both ages, 'maintain: low', for children who were at 1st tertile at both ages, 'increase', for children whose scores changed to a higher tertile (e.g. 1st tertile to 2nd or 2nd to 3rd) and 'decrease', for children whose scores fell to a lower tertile (e.g. 3rd tertile to 1st or 3rd to 2nd).

Associations between tracking behaviour (maintain: high; maintain: low; increase; and decrease - exposure) and appetitive subdimensions of the CEBQ (outcome) were evaluated through linear regression models – regression coefficients and respective 95% confidence intervals [$\hat{\beta}$, 95%CI]. Two regression models were presented, one crude model and the other model adjusted for maternal age, maternal education (in years) and child's BMI at 4 years (adjusted model). Other potential confounders, such as parental concerns of problematic eating behaviours at 4 years, breastfeeding duration, the practice of physical exercise, daily screen time and the first food eaten, were tested and included in the adjusted model if statistically significant for the model. An interaction of the child's sex in these associations was studied by including an interaction term in the final models, but no significant interaction was found; thus, results are reported for all children.

The software used was the Statistical Package for the Social Sciences (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Significance was established a priori at 0.05.

2.4. Ethical consideration

The project Generation XXI was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Ethical Committee of the São João Hospital/the University of Porto Medical School. The project was approved by the Portuguese Authority of Data Protection. Parents or legal guardians of each participant received an explanation on the purposes and design of the study and gave written informed consent at baseline and follow-up evaluations.

3. Results

3.1. Participants characteristics

Table 1 presents characteristics of children and their mothers by age and sex. The sample in this study had 49% of girls. No significant differences were found for the maternal level of education and age, between girls and boys. At both ages, there were no significant differences between the groups, regarding HDVI score. At 4 years, a higher prevalence of overweight and obesity was observed among girls, in comparison with boys ($p = .027$). At 7 years, there were no statistical differences for BMI between girls and boys ($p = .167$). However, when comparing between the two periods of time, the HDVI score decreased from 4 to 7 years (mean (SD): 4.01 (0.52) and 3.60 (0.35), respectively, $p < .001$), with a large effect size (0.8). The HDVI score at 4 years ranged from 1.45 to 4.99 and at 7 years from 1.27 to 4.95 (from a possible maximum of 5). From 4 to 7 years of age, for each food group that contributes to the HDVI, a statistically significant ($p < .001$) decrease was observed in the frequency of consumption per day. Compared to girls, boys had a higher frequency of intake of starch in both ages. At 7 years, boys had a higher intake of foods from the dairy category than girls [mean (SD): 2.08 (0.61) vs. 2.03 (0.63), $p = .010$].

3.2. Diet variety tracking

Fig. 1 shows the proportion of children by tertiles of HDVI score at 4 and 7 years of age. More than half of the children that were in the lowest tertile of HDVI score at 4 years remained in the lowest tertile at 7 years. The same trend was observed for the highest tertile. In a subsample with dietary intake evaluated by 3-day food diaries at 4 ($n = 1984$) and 7 years of age ($n = 2779$), energy intake, macronutrients and fiber intake were compared across HDVI tertiles. The energy intake increased across tertiles of diet variety in both ages [1st tertile vs. 3rd tertile: at 4 years, mean (SD) = 1567 (297.4) kcal vs. 1682 (291.4) kcal, $p < .001$; at 7 years, 1792 (364.3) kcal vs. 1842 (314.7) kcal, $p = .06$]. Children with a high diet variety, compared to children in the lowest tertile of variety (3rd vs 1st tertile of intake) had a significantly higher intake of fiber at 4 years [mean (SD) = 14 (3.6) g vs. mean (SD) = 12 (3.7) g, $p < .001$] and at 7 years [mean (SD) = 16 (4.0) g, vs. mean (SD) = 13 (4.0) g, $p < .001$]. On the other hand, in both ages, children with the lowest diet variety had a higher intake of total fat, including saturated fat.

Table 2 presents the tracking values for the HDVI score and for each food group included. Overall, the HDVI showed a stability of 48% and a positive predictive value (1.13, 95%CI: 1.02; 1.25). Regarding the food groups, the 'fruit and vegetables' group had the highest stability value (0.52) and the highest predictive value (1.30; 95%CI: 1.18, 1.44). The agreement was moderate for this food group (ICC = 0.464, 95%CI: 0.388; 0.529). All the other food groups had stability values higher than 0.33, but negative predictive values. They all had a fair agreement with the exception of meat, fish and alternatives that showed only slight tracking.

3.3. Diet variety and eating behaviours related to appetite

The mean scores in the CEBQ subdimensions at 7 years ranged from 1.8 (SD = 0.64) for ' Emotional Overeating' to 3.0 (SD = 0.80) for 'Enjoyment of Food' (Supplemental Table 1).

Associations between levels of tracking of the HDVI (exposure) and eating behaviours related to appetite at 7 years (outcome) are shown in Table 3. Parental concerns for problematic behaviours were used at 4 years as a proxy of appetitive traits. and were tested in the final model as potential confounders.

In the adjusted models, increasing diet variety from 4 to 7 years, in comparison with maintaining a low variety, was inversely associated with the 'Desire to Drink' ($\beta = -0.090$, 95% CI: −0.174; −0.006) and 'Satiety Responsiveness' ($\beta = -0.119$, 95% CI: −0.184; −0.054) subdimensions, and positively with the 'Enjoyment of Food' ($\beta = 0.098$, 95%CI: 0.023; 0.172) and 'Emotional Overeating' ($\beta = 0.073$, 95%CI: 0.006; 0.139). Increase or maintain a high diet variety, in comparison with maintaining a low variety, were associated with lower scores in the 'Food Fussiness'. No effect of changes in HDVI score from 4 to 7 years was observed on 'Food Responsiveness', 'Slowness in Eating', and 'Emotional Undereating'.

4. Discussion

To our knowledge, this is the first study assessing the tracking of diet variety from preschool to school age and its association with individual differences in appetitive traits. It is important to know which children will benefit most from practical and targeted interventions to promote healthy diets in early life. The results of this

Table 1
Sample characteristics by age and sex.

	all	4 years			all	7 years		
		Girls	Boys	p-value		Girls	Boys	p-value
Maternal level of education at 4y (years), mean (SD)	11.4 (4.22)	11.4 (4.24)	11.4 (4.21)	.320[a]	–	–	–	–
Maternal age at 4y (years), mean (SD)	34.2 (5.22)	34.2 (5.32)	34.1 (5.15)	.065[a]	–	–	–	–
Body Mass Index, n (%)								
Normal/Thinness	3029 (68.3)	1454 (66.6)	1575 (70.0)	.027[b]	2754 (63.1)	1375 (61.9)	1481 (64.2)	.167[b]
Overweight	966 (21.8)	493 (22.6)	473 (21.0)		940 (21.5)	504 (22.7)	472 (20.5)	
Obese	438 (9.9)	237 (10.9)	201 (8.9)		669 (15.3)	344 (15.5)	353 (15.3)	
Fruit & Vegetables, (frequency/day), mean (SD)	5.09 (1.739)	5.12 (1.739)	5.08 (1.740)	.355[a]	4.54 (1.648)**	4.54 (1.641)	4.53 (1.656)	.732[a]
Starch, (frequency/day), mean (SD)	5.04 (1.114)	5.00 (1.145)	5.07 (1.141)	.037[a]	4.62 (1.100)**	4.54 (1.127)	4.69 (1.068)	<.001[a]
Dairy, (frequency/day), mean (SD)	2.31 (0.480)	2.31 (0.485)	2.31 (0.475)	.706[a]	2.05 (0.618)**	2.03 (0.628)	2.08 (0.607)	.010[a]
Meat, fish and alternatives, (frequency/day), mean (SD)	1.80 (0.320)	1.81 (0.324)	1.80 (0.316)	.317[a]	1.42 (0.241)**	1.42 (0.241)	1.41 (0.242)	.396[a]
HDVI score, mean (SD)	4.01 (0.521)	4.02 (0.519)	4.01 (0.522)	.745[a]	3.60 (0.352)**	3.58 (0.601)	3.61 (0.58)	.122[a]
HDVI stability								
Maintain: low (n = 781)	3.34 (0.375)	3.34 (0.378)	3.34 (0.373)	.881[a]	2.83 (0.396)	2.81 (0.400)	2.86 (0.391)	.117[a]
Maintain: high (n = 1397)	4.36 (0.291)	4.36 (0.294)	4.36 (0.288)	.805[a]	3.99 (0.348)	4.00 (0.344)	4.00 (0.352)	.531[a]
Increase (n = 1202)	3.73 (0.396)	3.72 (0.400)	3.74 (0.400)	.593[a]	3.97 (0.319)	3.97 (0.317)	3.97 (0.323)	.792[a]
Decrease (n = 1157)	4.34 (0.267)	4.33 (0.265)	4.34 (0.269)	.580[a]	3.26 (0.428)	3.23 (0.439)	3.29 (0.415)	.014[a]

[a] Student's t-test: comparisons between the sexes, for continuous variables. **Paired *t*-test: comparisons between ages ($p < .001$).
[b] Chi-square: comparisons between the sexes, for categorical variables.

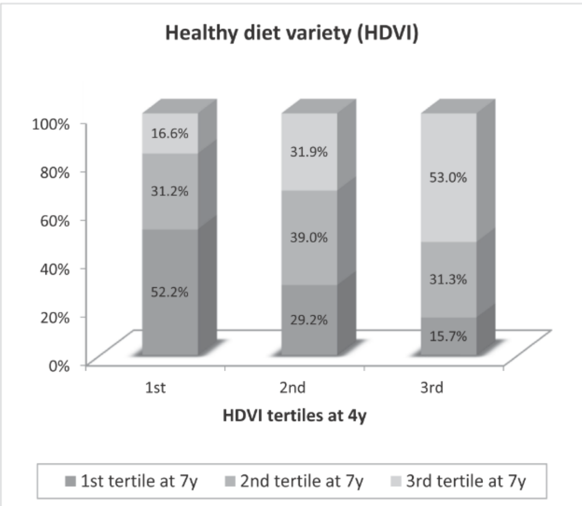

Fig. 1. Proportion of children by tertiles of healthy diet variety index (HDVI) at 4 and 7 years (y) of age.

study show that diet variety is somewhat stable throughout childhood. A higher varied diet, namely through a higher consumption of fruit and vegetables, predicted a fair to moderate tracking during childhood. There is also room for improvement of eating habits since a low tracking was found for the ‘starch’ and ‘meat, fish and alternatives’ food groups.

A previous review regarding dietary patterns from childhood to adolescence described a weak to moderate tracking throughout childhood, and between childhood and adolescence periods (Madruga, Araujo, Bertoldi, & Neutzling, 2012). In a similar birth cohort study (ALSPAC) in the UK, dietary patterns were obtained through early to mid-childhood and stability was assessed. Similar dietary patterns were obtained at 4 and 7 years of age with correlations around 0.60 (Northstone & Emmett, 2008). A previous study among Brazilian children, and using approximately the same age span, described some level of tracking for diet quality, including diet variety (Rauber, Hoffman, & Vitolo, 2014). In the present study, although a certain level of tracking was observed from 4 to 7 years, there was a general trend for a decrease in diet variety over time. Further research is warranted to test whether this trend for a decrease in diet variety extends into late childhood. This will be possible to answer with data from Generation XXI, from subsequent follow-up evaluations. Our results are in agreement with previous research, thus early food variety is a predictor of later food variety in childhood but also there is an observed decrease in overall food variety, including fruit and vegetable variety (Nicklaus, 2009). At both ages, almost none of the children reached the recommended score of 5 for the variety of healthy foods. In our sample, although a decrease of consumption of the ‘fruit and vegetables’ group was described, a higher tracking was observed for this group. However, taking into consideration that we only evaluated recommended eating per day and not fruit and vegetables variety, the tracking might be easier to achieve that using the other food group's variety. Recently, a greater variety of fruit and vegetables was associated with a better overall diet quality in a

Table 2
Tracking values from 4 to 7 years of age of the healthy diet variety index (HDVI) and food groups.

	Stability[a]	Predictive value[b] (95% CI)	ICC (95% CI)
HDVI	0.48	1.13 (1.02,1.25)	0.337 (0.104,0.506)
Food groups			
Fruit & Vegetables	0.52	1.30 (1.18,1.44)	0.464 (0.388,0.529)
Starchy	0.40	0.95 (0.85,1.06)	0.209 (0.162,0.254)
Dairy	0.43	0.48 (0.42,0.55)	0.307 (0.214,0.387)
Meat, fish and alternatives	0.39	0.85 (0.77,0.94)	0.146 (−0.051,0.325)

CI, confidence interval.
[a] Proportion of children who remained in the same tertile 3 years later.
[b] Predictive value for remaining in the highest tertile.

Table 3
Associations between levels of tracking of the healthy diet variety index (HDVI) and eating behaviours related to appetite at 7 years of age.

	Desire to drink (DD)		Enjoyment of Food (EF)		Satiety Responsiveness (SR)		Slowness in Eating (SE)	
	Crude model β^ (95% CI)	Adjusted model[a] β^ (95% CI)	Crude model β^ (95% CI)	Adjusted model[b] β^ (95% CI)	Crude model β^ (95% CI)	Adjusted model[b] β^ (95% CI)	Crude model β^ (95% CI)	Adjusted model[b] β^ (95% CI)
HDVI stability (4 to 7y)								
Maintain: low	ref	ref	ref	ref	ref	ref	ref	ref
Maintain: high	**−0.108**	−0.105	**0.188**	0.064	**−0.185**	−0.074	−0.076	−0.017
	(-0.180,-0.036)	(-0.230,0.020)	**(0.117,0.258)**	(-0.047,0.174)	**(-0.245,-0.125)**	(-0.170,0.022)	(-0.153,0.000)	(-0.139,0.104)
Increase	**−0.108**	**−0.090**	**0.174**	**0.098**	**−0.186**	**−0.119**	**−0.095**	−0.031
	(-0.182,-0.034)	**(-0.174,-0.006)**	**(0.102,0.246)**	**(0.023,0.172)**	**(-0.248,-0.124)**	**(-0.184,-0.054)**	**(-0.173,-0.016)**	(-0.113,0.051)
Decrease	−0.019	−0.058	**0.173**	0.039	**−0.148**	−0.040	−0.058	−0.007
	(-0.094,0.055)	(-0.186,0.069)	**(0.101,0.246)**	(-0.074,0.152)	**(-0.210,-0.085)**	(-0.238,0.058)	(-0.137,0.021)	(-0.131,0.118)

	Food responsiveness (FR)		Food fussiness (FF)		Emotional Overeating (EOE)		Emotional Undereating (EUE)	
	Crude model β^ (95% CI)	Adjusted model[b] β^ (95% CI)	Crude model β^ (95% CI)	Adjusted model[c] β^ (95% CI)	Crude model β^ (95% CI)	Adjusted model[d] β^ (95% CI)	Crude model β^ (95% CI)	Adjusted model[b] β^ (95% CI)
HDVI stability (4 to 7y)								
Maintain: low	ref	ref	ref	ref	ref	ref	ref	ref
Maintain: high	**0.074**	−0.020	**−0.424**	**−0.138**	0.048	0.072	−0.016	0.006
	(0.005,0.144)	(-0.129,0.089)	**(-0.490,-0.358)**	**(-0.253,-0.022)**	(-0.008,0.105)	(-0.028,0.172)	(-0.083,0.050)	(-0.110,0.122)
Increase	**0.115**	0.057	**−0.325**	**−0.219**	**0.076**	**0.073**	−0.015	0.007
	(0.044,0.187)	(-0.016,0.131)	**(-0.392,-0.257)**	**(-0.297,-0.141)**	**(0.019,0.134)**	**(0.006,0.139)**	(-0.083,0.053)	(-0.071,0.085)
Decrease	**0.130**	0.028	−**0.252**	0.055	**0.073**	0.094	−0.020	0.018
	(0.058,0.202)	(-0.083,0.140)	**(-0.321,-0.184)**	(-0.063,0.173)	**(0.015,0.132)**	(-0.007,0.196)	(-0.088,0.049)	(-0.101,0.136)

Bold entries denote statistical significance ($p<.05$).
[a] Adjusted for HDVI, maternal age and years of education, children's BMI, caloric intake and parental concern "eating too much" at 4 years.
[b] Adjusted for HDVI, maternal age and years of education, children's BMI, caloric intake and parental concerns regarding problematic behaviours at 4 years.
[c] Adjusted for HDVI, maternal age and years of education, children's BMI, caloric intake, weaning first food, and parental concerns "not eating enough" and "eating too slow" at 4 years.
[d] Adjusted for HDVI, maternal age and years of education, children's BMI, caloric intake, TV per day and parental concern "eating too much" at 4 years.

nationally representative sample of US preschool children (Ramsay, Shriver, & Taylor, 2017). In another European prospective population-based cohort study (Bjelland et al., 2013), analyzing tracking of food intake from 1 to 7 years of age, fair to moderate tracking was also described for intake of fruit and vegetables. Young children seems to benefit from being exposed consistently to a variety of healthy foods, including fruit and vegetables. In particular, the use of different strategies such as repeated exposure to unfamiliar foods, offering tangible rewards or modifying recipes to improve visual appeal are known to improve intake of healthy foods (Anzman-Frasca, Savage, Marini, Fisher, & Birch, 2012; Cooke, 2007; Remington, Anez, Croker, Wardle, & Cooke, 2012).

Diet variety can be evaluated in different ways and there is a lack of consistency in a validated method (Marshall et al., 2014). We used an index based on FFQ, previously tested in children from European birth cohorts, including the Generation XXI (Jones et al., 2015; Oliveira et al., 2015). In association with health outcomes, such as obesity, the use of the FFQ is recommended as the most appropriate tool to measure long-term diet variety (Salehi-Abargouei, Akbari, Bellissimo, & Azadbakht, 2016). The comparison between nutrient intake evaluated by food diaries and HDVI tertiles showed a better macronutrient intake profile in children with higher diet variety in both ages.

The effect of diet variety on appetite, mainly related to satiety, has been explored in previous studies. Several authors described a positive effect of offering different types of foods on food intake (Brondel et al., 2009; Bucher, Siegrist, & van der Horst, 2014; McCrory, Burke, & Roberts, 2012; Meengs et al., 2012b; Raynor & Epstein, 2001). For instance, previous studies described that both adults and children ate significantly more vegetables when presented with two or more vegetables, than just one (Bucher et al., 2014; Meengs et al., 2012b). Increasing variety within a meal has been effective in increasing energy intake in older adults with a poor appetite, in a cross-over trial study (Wijnhoven, van der Meij, & Visser, 2015). Exposure to different types of food might prevent the onset of sensory-specific satiety, that is defined as the decline in pleasure derived from consuming a known type of food in comparison to exposure to a new flavour or food (Rolls, Rolls, Rowe, & Sweeney, 1981). This effect might be also attributed to habituation, as exposure to different types of foods might retard the habituation process (Temple, Giacomelli, Roemmich, & Epstein, 2008). The variety effect in appetite has been demonstrated in both humans and animals (Bouton, Todd, Miles, León, & Epstein, 2013; Temple et al., 2006). For example, in a previous work, researchers showed that food variety could change the rate of habituation in children involved in a food-seeking task (Temple et al., 2008). In a previous randomized 8-week behavioural weight loss intervention, limiting energy-dense food group variety across several days produced long-term sensory-specific satiety and monotony (Raynor, Niemeier, & Wing, 2006).

Our study hypothesised a long-term influence of diet variety on appetitive traits of children. Overall, different levels of tracking of diet variety were associated with eating behaviours related to appetite at 7 years of age. Maintaining a high diet variety in childhood was inversely associated with problematic behaviours such as being a fussy eater. Children with a higher dietary variety also had a higher general interest in food although a lower desire to have drinks, and lower satiety responsiveness, than those with a low dietary variety. A lack of interest in food, including fussy eating, is being described as a common problem among children (Taylor, Wernimont, Northstone, & Emmett, 2015). Increasing variety of healthy food could be a strategy to decrease eating problems among children. Previous research suggests that following a less healthy dietary pattern early in life increases the risk of disordered eating behaviours later in childhood (Albuquerque et al., 2016).

Strengths of this study are the longitudinal design based on a large population-based birth cohort, and the use of the same method to describe tracking of diet variety across childhood. We also analyzed the prospective association between tracking of diet variety and appetitive traits, overcoming the limitations of cross-sectional studies. However, we evaluated children's eating behaviours only at 7 years and we could not exclude a bidirectional association between diet variety and eating behaviours. It was previously described a bidirectional relationship between children's fussy behaviour and parents feeding practices (Jansen et al., 2017). The authors also suggested an increase of variety of foods offered to increase food acceptance, decreasing fussiness behaviour (Jansen et al., 2017). Parental concerns for problematic behaviours were used at 4 years as a proxy of previously eating behaviours.

Additional limitations of this study will be discussed. Our diet variety index was based on data of a short FFQ, which did not allow measuring variety of fruit and vegetable. A previous systematic review (Marshall et al., 2014) studied different diet indices in paediatric age, including those used to evaluate variety in diet related to health outcomes. The majority of FFQ used to create the several diet variety indices had less than 60 food items, with some studies using FFQ with less than 20 food items (Marshall et al., 2014). Adding more questions to the FFQ would help increase the accuracy of our diet variety index; however, that would increase the burden of response among caregivers, which could result in more losses to follow-up or more errors in the responses. Even with the short FFQ, we were capable to find tracking in the diet variety and its associations with eating behaviours related to appetite. Eating behaviours were assessed subjectively through the CEBQ, based on caregivers' report; however, the CEBQ subdimensions have shown a good internal reliability in this population (Albuquerque et al., 2017) and good correspondence with objective measures in previous studies (Carnell & Wardle, 2007). Children's dietary intake was also based on reported by parents or caregiver who might not be aware of all the foods eaten by the child. Comparing FFQ with 3-day food diaries, a fair to moderate agreement was obtained for those food items eaten more often. The trend for an overall decrease in diet variety could be explained by the effect of the regression to the mean, which is a statistical phenomenon that can happen when repeated measurements are made on the same participant over time (Barnett, van der Pols, & Dobson, 2005). We quantified the regression to the mean effect in our sample, assuming the 3rd tertile of diet variety as the cut-off point. We found an estimated regression to the mean effect for the diet variety (0.19) much lower than the mean difference found in our sample (−0.41, 95% CI: 0.39; 0.43). These data indicate that a decrease in diet variety from 4 to 7 years of age cannot be completely explained by this statistical phenomenon.

5. Conclusions

Children with a higher diet variety were less fussy, had a lower desire to drink and had a higher general interest in food. Although these eating behaviours related to appetite track throughout childhood, they can nevertheless be managed using strategies, such as having a variety of healthy foods consistently available at home and persisting with the provision of variety over time. Diet variety seems to decrease with age in childhood; these findings enhance the need of implementing effective strategies to increase the consumption of a variety of healthy foods, including fruit and vegetables, from early life.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.appet.2017.12.030.

Potential conflict of interest and source of funding

The authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Table S1

CEBQ and parental level of concern of children's problematic eating behaviours

Supplemental Table 1. Mean differences of subdimensions of the Children Eating Behaviour Questionnaire (CEBQ) by the parental level of concern of problematic eating behaviours at 4 years.

	Eating behaviour related to appetite at 7 years (CEBQ)							
	Desire to Drink [2.2 (0.82)]*	**Enjoyment of Food** [3.0 (0.80)]*	**Satiety Responsiveness** [2.7 (0.69)]*	**Slowness in Eating** [2.9 (0.87)]*	**Food Responsiveness** [2.1 (0.80)]*	**Food Fussiness** [2.9 (0.77)]*	**Emotional Overeating** [1.8 (0.64)]*	**Emotional Undereating** [2.5 (0.76)]*
Parental concerns at 4 years								
"My child does not eat enough"								
Very concerned (15.1%)	2.3 (0.86)	2.6 (0.74)	3.2 (0.69)	3.4 (0.86)	1.8 (0.65)	3.3 (0.80)	1.7 (0.59)	2.6 (0.76)
Somewhat concerned (20.3%)	2.2 (0.80)	2.8 (0.72)	2.9 (0.65)	3.1 (0.85)	1.9 (0.64)	3.1 (0.72)	1.8 (0.59)	2.6 (0.74)
No concern (7.8%)	2.2 (0.85)	3.0 (0.75)	2.7 (0.67)	2.9 (0.80)	2.0 (0.78)	2.9 (0.75)	1.8 (0.62)	2.5 (0.77)
Never happen (56.9%)	2.2 (0.81)	3.2 (0.79)	2.5 (0.62)	2.8 (0.83)	2.2 (0.86)	2.8 (0.74)	1.9 (0.67)	2.4 (0.74)
p-value†	0.036	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
"My child eats very slow"								
Very concerned (8.3%)	2.3 (0.84)	2.6 (0.75)	3.2 (0.69)	3.6 (0.81)	1.8 (0.69)	3.3 (0.79)	1.7 (0.62)	2.7 (0.78)
Somewhat concerned (18.3%)	2.2 (0.82)	2.8 (0.75)	2.9 (0.66)	3.2 (0.83)	1.9 (0.66)	3.1 (0.70)	1.8 (0.59)	2.6 (0.75)
No concern (25.4%)	2.2 (0.83)	2.9 (0.76)	2.8 (0.70)	3.1 (0.81)	2.0 (0.74)	3.0 (0.78)	1.8 (0.61)	2.5 (0.78)
Never happen (48.0%)	2.2 (0.82)	3.2 (0.79)	2.5 (0.63)	2.6 (0.78)	2.2 (0.86)	2.8 (0.75)	1.9 (0.68)	2.4 (0.73)
p-value†	0.061	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
"My child eats too much"								
Very concerned (3.7%)	2.6 (0.79)	3.9 (0.79)	2.2 (0.62)	2.3 (0.88)	3.2 (1.07)	2.7 (0.74)	2.4 (0.91)	2.5 (0.75)
Somewhat concerned (5.1%)	2.4 (0.84)	3.6 (0.75)	2.3 (0.59)	2.4 (0.80)	2.7 (0.93)	2.8 (0.70)	2.1 (0.70)	2.5 (0.72)
No concern (3.3%)	2.3 (0.86)	3.5 (0.77)	2.4 (0.62)	2.5 (0.79)	2.4 (0.81)	2.8 (0.73)	1.9 (0.72)	2.4 (0.80)
Never happen (87.9%)	2.2 (0.82)	3.0 (0.77)	2.8 (0.68)	3.0 (0.86)	2.0 (0.71)	3.0 (0.77)	1.8 (0.60)	2.5 (0.76)
p-value†	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.268

*mean (Standard deviation)
†One-way ANOVA

Paper V

Association between eating frequency and behaviours related to appetite from 4 to 7 years of age: findings from the population-based birth cohort Generation XXI

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[Submitted]

Abstract

This study aimed to assess the association between eating frequency (EF) (main meals vs. snacks) at 4 and 7y and eating behaviours related to appetite at 7y. The participants are from the population-based birth cohort, Generation XXI (Porto, Portugal). The analysis included 1359 children evaluated at both 4 and 7y, who provided 3day food diaries, at both ages, and complete information on the Children's Eating Behaviour Questionnaire, at 7y. A time-of-day approach was used to distinguish main meals from snacks. A cross-lagged analysis was performed to check the direction of the association between EF and children's eating behaviours. Associations between EF and eating behaviours related to appetite were evaluated through linear regression models. We found a bidirectional relationship between children's EF and appetitive behaviours. The tracking of EF between 4 and 7y was moderate and statistically significant. Prospectively, a higher number of main meals at 4y was inversely associated with scores of 'Satiety Responsiveness' at 7y (β=-0.15, 95% CI: -0.28; -0.02). Moreover, a higher frequency of snacks at 4y was independently associated with higher scores in 'Desire to Drink' at 7y (β=0.05, 95% CI: 0.00; 0.09). At 7y, a higher number of main meals had a positive association with 'Enjoyment of Food' and 'Food Responsiveness'. A higher number of snacks at 7y had a positive association with 'Satiety Responsiveness' and 'Slowness in Eating', but an inverse association with 'Enjoyment of Food' and 'Food Responsiveness'. In conclusion, children who had more snacking events seem to have more food-avoidance behaviours, traits that might protect these children from gaining excessive weight.

Introduction

Specific eating habits are developed early in life and, in particular, the family's background can have a significant impact on the child's diet [1, 2]. Parents have a central role in shaping the family's food environment and eating experiences, influencing food intake self-regulation of the child [3].

Individual differences in eating behaviours, and in particular behaviours related to appetite, have been associated with variations in children's weight. In previous research, 'food-avoidance' appetitive traits such as low satiety responsiveness showed a graded negative association with weight, while high responsiveness to external food cues, was positively associated [4-6]. Certain dietary components have been shown to influence mechanisms of appetite, namely those related to satiety [7, 8]. For example, dietary proteins induce satiety, increasing secretion of gastrointestinal hormones and diet-induced thermogenesis [9]. Previous evidence has suggested that characteristics of the meals, such as nutrient composition [10-12] and frequency of meals [13-16], could influence outcomes in health, such as obesity, insulin resistance, and metabolic syndrome, independently of other risk factors. The examination of meals' characteristics provides an interesting approach by expressing cumulative and interaction effects of foods and nutrients, overcoming the limitations of studying specific foods or nutrients [17].

It has been hypothesized that regular eating frequency could modify metabolism, increasing satiety and improving glucose and insulin metabolism [14, 18]. Controlled feeding studies in adults [18] showed that reduced eating frequency negatively influence appetite control, increasing the perceived appetite and reducing the perceived satiety. Long-term studies are needed to disentangle the true role of eating frequency in the regulation of appetite.

Although there is a lack of universal definition differentiating main meals and snacks [19], snacking events have been described as increasing in recent years, particularly among children [20-23]. The literature describes a variety of approaches to define a snack, using, for example, the time of consumption, food groups or nutritional profile [24, 25].

Most associations found between eating habits and appetive-traits have focused in short-term intervention studies. Research on the influence of early eating habits on appetite-related eating behaviours in a prospective approach is scarce and usually focuses only on breastfeeding or complementary feeding [26, 27]. The majority of previous studies focused on a unidirectional relationship between eating behaviours related to appetite and dietary habits, but a bidirectional relationship cannot be discarded. The maintenance or change of a specific meal pattern, such as meal frequency, throughout childhood and its association with the children's appetite, has not been studied to date. Therefore we aimed to assess the association between main meals vs. snacks frequency from pre-school to school-age and eating behaviours related to appetite at 7 years of age.

Methods

Participants

This study was conducted within Generation XXI, a population-based birth cohort that has been previously described [28]. Briefly, a total of 8647 children and respective mothers were recruited between April 2005 and August 2006, from the public maternity units in the Porto Metropolitan Area. Of the invited mothers, 91.4% accepted to participate, corresponding to 8647 children. The follow-ups of the entire cohort occurred between April 2009 and July 2011 and again between April 2012 and April 2014, when the children were 4 and 7 years of age and 86% and 81% of the children was re-evaluated, respectively. During these evaluation waves, information was collected in face-to-face interviews and for those families that were not able to participate in-person, the evaluation was performed by telephone using a shorter version of the questionnaire (20% and 15% at 4 and 7 years, respectively).

The present study included a subsample of children evaluated at face-to-face follow-up evaluations, providing data from age 4 and again at age 7 years, using completed 3-day food diaries and information on the Children's Eating Behaviour Questionnaire (CEBQ) (n=1403). We excluded twins (n=39) and children with congenital anomalies or diseases that might influence dietary intake (cerebral palsy, celiac disease, food allergy, food intolerance and phenylketonuria; n=5), achieving a final sample of 1359 children. Comparing the children included in the analysis with the remaining cohort no statistical differences were found regarding children's sex and body mass index (BMI) and parental concerns regarding children's eating behaviours. However, mothers of children included in the study at baseline were slightly older (mean (SD): 30.3 (4.93) vs. 28.7 (5.68), p<0.001) and more educated (mean (SD): 11.7 (4.27) vs. 10.2 (4.21), p<0.001).

In both evaluations, children's anthropometric measurements were performed by a team of experienced examiners, according to standard procedures. Weight was measured in underwear and without shoes using a digital scale and was recorded to the nearest 0.1kg. Height was measured as the distance from the top of the head to the bottom of the feet without shoes, using a fixed stadiometer to the nearest 0.1cm. Children's BMI was defined as weight in kg divided by height in meters squared and age- and sex-specific z-scores were established according to the WHO criteria [29].

Dietary intake

As previously described [30], when children were 4 and 7 years of age, parents or other main caregivers were asked to complete a 3-day food diary, 2 weekdays and 1 weekend day, previously to the face-to-face interview. Oral and written instructions were given for the correct completion of food diaries and for the quantification of food portions. Parents were taught how to use household measures and standard units to quantify the food portions. They were also asked to provide detailed descriptions of each food and drink consumed by the children, including the method of preparation, recipes and place of consumption, whenever possible. It was advised to let children follow their usual diet and to ask for the help of other caregivers in the case the child was out of the home during the day.

The codification process was conducted *a posteriori* by a team of trained nutritionists, using an age-specific food coding manual previously developed by our research team. Nutrient intake was estimated using the software Food Processor SQL (2004-2005 ESHA Research, Salem, Oregon), based on the Food Composition Table of the United States of America Department of Agriculture [(31)]. For typically Portuguese foods or culinary dishes, new codes were created with national nutritional information, as previously described [(30, 32)].

Meal definitions

Based on themeal patterns reviewed by Leech [(19)], several approaches were combined to define a meal in the present study. First, a neutral approach was used: an 'eating occasion' was any occasion when food or drink was consumed 30 min apart and provided a minimum of 210kj (50Kcal) of energy.

Then to distinguish main meals from snacks, a time-of-day approach was used, using the variable previously defined as eating occasion. As parents did not distinguish the meals in the food diary, the team of trained nutritionist classified and distinguished meals (e.g. breakfast, lunch, mid-day) according to the type of foods, the period of time and place of consumption. Using this variable, a decision tree [(33)] was applied to define the best period of time for breakfast, mid-morning, lunch, mid-afternoon, dinner and supper. After that, for the period of time defined as breakfast, lunch and dinner, the eating occasion with higher energy content (>=210kj) was defined as the main meal and all other eating occasion separated by 30 minutes, was considered as snacks.

Breakfast was defined as the eating occasions with higher energy content between 6am and 9.30am. Lunch and dinner were considered as the eating occasion with higher energy content between 11 am and 2.30 pm and between 7 pm and 9.30 pm, respectively. The other time periods were considered for mid-morning, mid-afternoon and supper; and all meals within these periods of time were considered as snacks. The mean contribution of eating occasion providing <210kj to total energy was 3.4 % at 4 years and 1.8% at 7 years of age.

Eating behaviours

At 4 and 7 years of age, problematic eating behaviours were assessed by caregiver report. Caregivers, usually the mothers, were asked about specific perceived eating problem observed in their child. The following questions were included in the analyses: "my child does not eat enough", "my child eats very slowly" and "my child eats too much". They reported if any of these behaviours happened during the previous year and what was their level of concern (very concerned, somewhat concerned, no concern).

A number of psychometric instruments were developed to evaluate eating behaviour in children, including the CEBQ [34]. In the present study, 7-year-old children's eating behaviours related to appetite were assessed using a Portuguese version of the CEBQ, previously tested with good psychometric characteristics among these children [35]. This instrument is a parent-report questionnaire that measures appetitive traits in children. The sub-dimensions 'Food Responsiveness' (FR), 'Enjoyment of Food' (EF), 'Satiety Responsiveness' (SR), 'Slowness in Eating' (SE) and 'Desire to Drink' (DD) were analysed independently. All items were rated on a 5-point Likert scale ("never", "seldom", "sometimes", "often" and "always"), scored 1 to 5. Items 3 (SR) and 4 (SE) were reverse-scored items. In individuals with missing data in less than 50% of the items, missing data (around 3%) were handled by imputation, replacing the average of the remaining questions within each sub-dimensions.

Ethical considerations

The project Generation XXI was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Ethical Committee of the São João Hospital/ the University of Porto Medical School. The project was approved by the Portuguese Authority of Data Protection. Parents or legal tutors of each participant received an explanation on the purposes and design of the study and gave written informed consent at baseline and follow-up evaluations.

Statistical analysis

Continuous variables were summarized by mean and standard deviation (SD) and categorical variables by proportions. Mean (SD) and frequency differences were compared through the paired sample t-test and McNemar test (or the marginal homogeneity test), respectively.

Parental concerns regarding children's eating behaviours comprised the questions "my child does not eat enough", "my child eats very slowly" and "my child eats too much", and was used as a proxy of the sub-dimensions of the CEBQ at both ages. A higher score in the variable parental concerns represents a higher concern for the child not eating enough or eating slowly, and lower concern for the child is eating too much. A cross-lagged panel design analysis [36] was performed using the parental concerns regarding children's appetite (as a proxy of CEBQ) and main meals and snacks frequency at both ages. With cross-lagged models, it is possible to determine the reciprocal relationship, or directional influences, between variables over time, as well as the strength of this relation. The model included two components: a longitudinal analysis of main meals and snacks frequency, and parental concerns across the two periods of time; and the cross-lagged analysis of eating frequency at 4 years on parental concerns at 7 years, as well as of parental concerns at 4 years on eating frequency at 7 years. Mean eating frequency and parental concerns at both ages were standardized to enable effect size comparisons. Determination of model fit included the chi-square test, comparative fit index (CFI), the root mean square error of approximation (RMSEA) and the Tucker-Lewis index (TLI). CFI and TLI >0.93 and RMSEA <0.06 indicate good model fit.

Cross-sectional and prospective associations between eating frequency and eating behaviours related to appetite were evaluated through linear regression models – regression coefficients and respective 95% confidence intervals [B, 95%CI]. Model 1 was adjusted for the same meal at 4 years (cross-sectional analysis) or at 7 years of age (prospective analysis). Model 2 included the covariates from model 1 plus maternal age and education, children's sex and BMI at 4 years, and parental concerns regarding problematic behaviours at 4 years. Model 3 was adjusted for covariates from model 2 and in addition for energy intake (kcal/day) at 7 years. We intended to test the confounding effect of energy intake on the association between eating frequency and eating behaviours (model 3), but as the energy intake might be an intermediate step on this association, we considered the model 2 as the final model. An interaction effect between eating frequency at 4 years and child's sex, and between eating frequency and diet variety was tested in models 2 and 3, by including an interaction term in the final models. However, no interaction effect was found for these variables and results are presented for the all children.

The software Statistical Package for the Social Sciences (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.), and the R language and software environment for statistical computation (version 2.12.1, R Foundation for Statistic Computing, Austria, 2010) were used. The significance level was fixed at 0.05.

Results

At 4 years of age, the number of daily eating occasions varied between 4 and 11 (median=6), main meals between 1 and 3 (median=3) and snacks between 1 and 8 (median=4). At 7 years of age, the number of daily eating occasions ranged from 4 to 9 (median=6), main meals between 2 and 3 (median=3) and snacks between 1 and 7 (median=3).

Table 1 presents the characteristics of children at 4 and 7 years of age, and mothers. Almost half of the sample (47%) was girls; at 4 years, less than one-third of the children were overweight or obese, comparing to 36% at 7 years of age ($p<0.001$). Total energy intake increased significantly from 4 to 7 years. The overall mean daily frequency of eating occasion has significantly decreased from 6.5 eating occasions per day at 4 years to 5.8 eating occasions per day at 7 years of age, due to a decrease on the daily frequency of snacks. Regarding parental concerns with children's eating behaviours no statistical differences were found between the two periods of time, with exception of an increase of concern regarding the child being eating too much, from 4 to 7 years (somewhat concern/very concern: 8.3% vs 14.9%, $p<0.001$, respectively).

The mean scores in the CEBQ sub-dimensions at 7 years ranged from 2.0 (SD = 0.74) for 'Food Responsiveness' to 3.0 (SD = 0.78) for 'Enjoyment of Food' (Table 2). Significant differences were found between different levels of parental concern with children's eating behaviours and scores of the CEBQ sub-dimensions at 7 years (Table 2). For example, children with the higher score in 'Slowness in Eating' were the ones with a higher level of parental concern of 'eating too slow'. Parents that were more concerned with 'eating too much' had children with higher scores of 'Enjoyment of Food' and 'Food Responsiveness' but lower scores in ''Satiety Responsiveness' and 'Slowness in Eating' (Table 2).

In order to explore the direction of the associations between eating behaviours related to appetite (using 'parental concerns' as proxies of the CEBQ's scores) and eating frequency, a cross-lagged analysis was performed (Figure 1). The model presents a CFI and TLI higher than 0.90 and a RMSEA close to 0, supporting the goodness of fit.

The tracking of frequency of main meals ($\beta_{standardized}$=0.237; p<0.001) and snacks ($\beta_{standardized}$=0.419; p<0.001) was moderate and statistical significant (Figure 1). Parental concerns at 4 years had a strong association with parental concerns at 7 years ($\beta_{standardized}$=0.779; p<0.001). The cross-lagged association shows a positive bidirectional relationship between eating frequency and children's appetitive behaviours (using 'parental concerns' as proxies). A higher level of concern of not eating enough or eating too slow at 4 years was positively associated with snacks frequency at 7 years ($\beta_{standardized}$=0.081; p=0.026), but not with main meals frequency ($\beta_{standardized}$=0.039; p=0.321). The strongest direction found was from snacks frequency to the parental concern ($\beta_{standardized}$=0.108; p=0.002) (Figure 1).

In the prospective analysis, a higher frequency of daily main meals at 4 years was inversely associated with 'Satiety Responsiveness' (Table 3, model 0). After adjustment for the same eating occasion at 7 years, the strength of the association was attenuated, meaning that the association between the eating occasion at 4 years on 'Satiety Responsiveness' was through the same eating occasion at 7 years (Table 3, model 1). Eating frequency at 4 years was not significantly associated with 'Enjoyment of Food', 'Food Responsiveness' or 'Slowness in Eating' (Table 3). A higher frequency of daily snacks at 4 years was positively associated with 'Desire to Drink', independently of the number of snacks at 7 years (Model 1, β=0.08, 95% confidence interval: 0.04; 0.12) and independently of other potential confounders (Model 2, β=0.05, 95%CI: 0.00; 0.09) (Table 3).

In the cross-sectional analysis at 7 years, and after multivariate adjustment, a higher frequency of daily main meals had a statistical significant positive relationship with 'Enjoyment of Food' (β=0.22, 95%CI: 0.09; 0.35) and 'Food Responsiveness' (β=0.15, 95%CI: 0.02; 0.27) [Table 3, model 2]. A higher number of daily snacks at 7 years was independently and positively associated with 'Satiety Responsiveness' and 'Slowness in Eating', but inversely associated with 'Enjoyment of Food' (Table 3).

The additional adjustment for total energy intake (model 3) did not change significantly the association described between eating frequency and children's eating behaviours.

Discussion

This is the first study prospectively assessing the association between main meal and snack frequency, and eating behaviours related to appetite in childhood. Given that eating habits established early in life track into the later years, it is important to understand how dietary intake might influence appetite-related behaviours and vice versa. A prospective approach is the best way to clarify these relationships. This is also the first study investigating the bidirectional association between eating frequency and eating behaviours related to appetite, as the majority of previous studies assume that the relationship is unidirectional, eating behaviours influencing eating frequency. A moderate correlation was found between the number of eating occasions at 4 and 7 years of age. This suggests that eating frequency is somewhat stable during childhood.

A higher number of daily main meals at 4 years, was associated with lower scores of 'Satiety Responsiveness', mainly due to the influence of eating frequency at 7 years. On the other hand, an increasing number of daily snacks at 4 years was associated with higher scores of 'Desire to Drink' at 7 years, independently of the number of snacks eaten at 7 years. Previously, higher scores in 'Desire to Drink' was associated with a higher consumption of sugar-sweetened beverages in 11-year-old children [(37)] but not in younger children [(38)]. In these 6-8-year-old Finnish children, 'Desire to Drink' was associated with a higher consumption of fat-containing milk and lower consumption of skimmed milk [(38)]. In the present study, the 4-year-old children might have more energy-dense beverages at snack times, than at main meals, a pattern imposed by their caregivers, and this might have influenced their desire for drinks at 7 years of age, independently of the number of snacks at 7 years. The quality of snacks offered to young children, and in particular the quality of beverages, should be taken into careful consideration as this might influence the way and how much children consume beverages habitually over time.

Overall, at 7 years, a higher number of daily snacks had a positive association with scores of 'Satiety Responsiveness' and 'Slowness in Eating', and an inverse association with 'Enjoyment of Food' and 'Food Responsiveness'. On the other hand, a higher number of daily main meals had a positive association with 'Enjoyment of Food' and 'Food Responsiveness'. Originally the CEBQ was broadly categorised into two dimensions: 'food approach' eating behaviours which include 'Enjoyment of Food', 'Food Responsiveness' and 'Desire to Drink', among others, and 'food avoidance' eating behaviours which include, among others, 'Slowness in Eating' and 'Satiety Responsiveness' [(34)]. In our analyses at 7 years, a higher frequency of snack intake is linked to food avoidance traits, while a higher frequency of main meals was linked to food approach traits at 7 years. In a previous study [(5)], higher scores in food approach traits have been positively associated with children's weight, whereas more food avoidance traits have been negatively associated with children's weight.

As a higher frequency of daily snacks was linked to food avoidance traits, this might protect these children from developing overweight or obesity. Trying to understand this beneficial effect of higher snack frequency, we compared the macronutrient composition of main meals and snacks. Snacks were proportionally richer in carbohydrates and contained fewer fats and proteins than main meals [data not shown]. Compared to the dietary recommendation for macronutrients in children (4-18y) [(39)] it seems that the snacks events are closer to the recommendations. Also, young children seem to self-regulate the energy intake by adjusting their portion sizes, depending on the number of eating occasions per day [(40)], meaning that a higher frequency of snacks in early life does not necessarily translate into an excess energy intake.

While higher scores in 'Satiety Responsiveness' and 'Slowness in Eating' have been linked with a lower risk of overweight [(5)], previous studies have found an association between higher scores in these eating behaviours and higher consumption of sweets [(38)] and lower preferences for vegetables and fruit [(41)]. Children with these appetitive traits may not only be consuming less in general, but also eating disproportionally fewer nutrient-dense foods.

A recent study [(42)] has analysed the associations between eating behaviour related to appetite, such as 'Food Responsiveness' and 'Satiety Responsiveness', when the children were 16 months old, and eating frequency and meal size at 21 months. Higher 'Food Responsiveness' was associated with more frequency of meals, while lower 'Satiety Responsiveness' was associated with a larger meal size but not with eating frequency. These results cannot be generalized across childhood, as these relations could change as children get older. More recently, a published study explored the cross-sectional association between eating behaviour and eating frequency and food consumption in 6-8-year-old children [(38)]. The authors described a positive association between 'Enjoyment of Food' and the daily number of main meals in these children. These results are in accordance with ours, where 7-year-old children, who had a higher frequency of main meals, had higher scores in 'Enjoyment of Food'. Moreover, higher scores in 'Enjoyment of Food' have been associated with higher intake of fruit and vegetables whereas higher scores in 'Food Responsiveness' have been associated with higher consumption of fruit but also with a higher liking for noncore foods [(38, 41)]. These children seem to have a diet closer to recommended amounts of fruit and vegetables, foods that are usually consumed during a main meal. However, as these eating behavioural traits (a general interest in food or eating in response to environmental food cues) were previously related to a higher weight in children [(5)], these children might benefit from interventions to avoid excessive food consumption, especially from larger meals and more energy dense foods.

An earlier study has proposed that aspects of eating behaviour track throughout childhood [(43)]. However, the authors highlighted that a small-to-moderate change in the magnitude of appetitive traits occurs. One justification for possible changes in eating behaviours throughout childhood could be the result of interactions with the child's food environment. For instance, the food fussiness behaviour in a child could decrease as the variety of food in the environment increases [(26, 27)]. Particularly, researchers have reported a beneficial effect of an early introduction of vegetables with fussy eating behaviours around 4 years of age [(26, 27)]. These results support our hypothesis of a potential influence of frequency of meals, usually imposed by the caregivers in pre-school aged children, and behaviours related to appetite, a few years after.

Strengths and limitations of the present study deserve further discussion. We define the exposure, children's eating frequency, based on previously defined approaches [(19)]. Firstly, a neutral approach was used to define an eating occasion (as any occasion when food or drink was consumed 30 min apart, and providing a minimum of 50kcal (210kj)). One of the advantages of using this definition is that it can be standardized and comparable over time and across different studies. Then, a second definition was used to distinguished different types of meals, minimizing the error associated with the definition of main meal and snacks by the participant. The differentiation between a 'meal' and 'snack' is important as people tend to give a different perception to an eating occasion, which could have a significant impact on food and nutrient intake. Experimental studies showed that adults who define a particular eating occasion as a main meal, based on some eating cues (i.e. use of dishes/utensils and being seated at a table) [(44)], may reduce caloric intake later in that day [(45)]. The definition of eating occasions using data from food diaries is also an advantage, rather than simply inviting parents to specify the daily number of eating occasions of their child.

Food diaries were completed by parents or other main caregivers, who might not be with the child all day. However, parents were advised to ask information from other caregivers, during the report day. It is recognised that a social desirability bias may have occurred, resulting in a lower report of unhealthy foods (e.g. high energy-dense food) and/or over report of more healthy foods (e.g. fruit and vegetables). However, this bias might have been minimized as our main dependent variable namely eating frequency, was calculated independently of the quality of foods. The present results regarding main meals should be interpreted with caution due to the low variability of main meals frequency in our sample, as the majority of the children had the three main meals.

In the present study, we analysed cross-sectional and prospectively the association between eating frequency and eating behaviours related to appetite, overcoming the limitations of previous only cross-sectional studies. We also assessed the cross-sectional association of eating frequency and appetite at 7 years, adjusted for the previous eating frequency. A bidirectional association between eating frequency and eating behaviours was described for the first time in this study. This supports that parents are aware of the children eating behaviours as they grow and change the concerns (and consequently behaviours) accordingly.

Although the CEBQ was only assessed at 7 years of age, the use of parental concerns for children's problematic eating behaviours (used as a proxy for items of the CEBQ) allowed us to uncover a bidirectional association between eating frequency and children's eating behaviour related to appetite. Moreover, these 'parental concern' variables were included in the final models to adjust for previous eating behaviours related to appetite.

Another strength of this study is the use of a large sample of young children, well characterized since birth within the framework of a prospective cohort. This has permitted detailed measures of eating behaviours over time and to control for a wide set of potential confounders.

Conclusions

A moderate association between the number of eating occasions at 4 and 7 years of age was found. A bidirectional association was described between EF and eating behaviours between the two periods of time, with a stronger link between snack frequency at 4 years and eating behaviours related to appetite at 7 years. A higher frequency of main meals was associated with higher scores in food-approach traits a few years later, while a higher frequency of snacks was associated with food-avoidance traits. The food-avoidance behaviours identified, including a higher sensitivity to satiety signals and a healthy eating pace could be encouraged in interventions to prevent problematic eating behaviours without resulting in unnecessary weight gain in childhood. The number but also the quality of snacks should be taken into consideration in early life as it might have a lasting effect on children's behaviours related to appetite.

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The authors' contributions to the study were as follows: S.V. contributed to the design of study, performed statistical analyses and interpretation of the data and wrote the first draft of the paper. M.S. contributed with the statistical analysis and with the interpretation of data. CL contributed to the design of study, coordinated the design of data collection instruments and contributed to the discussion of results. All authors contributed with the interpretation of data and discussion of results. All the authors critically reviewed the manuscript and approved the final version as submitted. The authors declare no conflict of interests.

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Table 1. General characteristics of the participants included in the study, at 4 and 7 years of age.

	4 years	7 years	p-value
Children's characteristics			
Sex (girl), n (%)	643 (47.3)		
Body mass index, n (%)			
Normal weight/Thinness	923 (68.8)	842 (63.2)	
Overweight/Obese	419 (31.2)	491 (36.8)	<0.001
Eating occasions (number per day), mean (SD)	6.5 (1.12)	5.8 (0.87)	<0.001
Main meals (number per day), mean (SD)	2.8 (0.28)	2.8 (0.29)	0.048
Snacks (number per day), mean (SD)	3.6 (1.14)	3.0 (0.85)	<0.001
Total energy intake (kcal/d), mean (SD)	1631 (286.4)	1788 (305.5)	<0.001
Parental concerns, n (%)			
"My child does not eat enough"			
Never happen	770 (57.9)	764 (57.3)	
No concern	105 (7.9)	96 (7.2)	
Somewhat concern	288 (21.6)	301 (22.1)	
Very concern	168 (12.6)	172 (12.9)	0.515
"My child eats very slow"			
Never happen	651 (48.9)	646 (48.4)	
No concern	340 (25.5)	271 (20.3)	
Somewhat concern	242 (18.2)	336 (25.2)	
Very concern	98 (7.4)	81 (6.1)	0.126
"My child eats too much"			
Never happen	1173 (88.1)	1086 (81.4)	
No concern	47 (3.5)	49 (3.7)	
Somewhat concern	71 (5.3)	115 (8.6)	
Very concern	40 (3.0)	84 (6.3)	<0.001
Mother's characteristics, mean (SD)			
Education at 4-year-follow up (years)	11.9 (4.18)		
Age at 4-year-follow up (years)	34.7 (4.92)		

SD, standard deviation.
P value are calculated by paired sample t-test in continuous variables and McNemar test or the marginal homogeneity test in categorical variables

Table 2. Mean differences in sub-dimensions of the Children's Eating Behaviour Questionnaire (CEBQ) by parental level of concern with problematic eating behaviours at 7 years.

	Eating behaviours related to appetite at 7 years (CEBQ)				
	Satiety Responsiveness [2.7 (0.68)]*	**Enjoyment of Food** [3.0 (0.78)]*	**Food Responsiveness** [2.0 (0.74)]*	**Slowness in Eating** [2.9 (0.86)]*	**Desire to Drink** [2.2 (0.78)]*
Parental concerns at 7 years					
"My child does not eat enough"					
Very concerned	3.3 (0.64)	2.3 (0.66)	1.7 (0.56)	3.6 (0.83)	2.3 (0.94)
Somewhat concerned	3.1 (0.59)	2.6 (0.56)	1.8 (0.49)	3.3 (0.80)	2.1 (0.76)
No concern	2.8 (0.60)	2.8 (0.60)	1.8 (0.52)	3.2 (0.78)	2.2 (0.79)
Never happen	2.4 (0.52)	3.3 (0.73)	2.2 (0.82	2.6 (0.74)	2.1 (0.75)
p-value†	<0.001	<0.001	<0.001	<0.001	0.027
"My child eats very slowly"					
Very concerned	3.3 (0.64)	2.3 (0.64)	1.6 (0.50)	3.9 (0.66)	2.4 (0.86)
Somewhat concerned	3.1 (0.66)	2.6 (0.66)	1.8 (0.56)	3.5 (0.71)	2.2 (0.81)
No concern	2.9 (0.64)	2.8 (0.68)	1.8 (0.57)	3.3 (0.66)	2.2 (0.82)
Never happen	2.4 (0.53)	3.4 (0.73)	2.3 (0.82)	2.4 (0.60)	2.2 (0.78)
p-value†	<0.001	<0.001	<0.001	<0.001	0.215
"My child eats too much"					
Very concerned	2.2 (0.53)	3.7 (0.81)	3.0 (0.95)	2.2 (0.74)	2.5 (0.82)
Somewhat concerned	2.3 (0.58)	3.7 (0.71)	2.8 (0.80)	2.3 (0.75)	2.3 (0.81)
No concern	2.4 (0.46)	3.5 (0.65)	2.4 (0.70)	2.5 (0.82)	2.2 (0.64)
Never happen	2.8 (0.67)	2.8 (0.71)	1.8 (0.57)	3.1 (0.82)	2.1 (0.78)
p-value†	<0.001	<0.001	<0.001	<0.001	<0.001

*mean (Standard deviation)

†One-way ANOVA

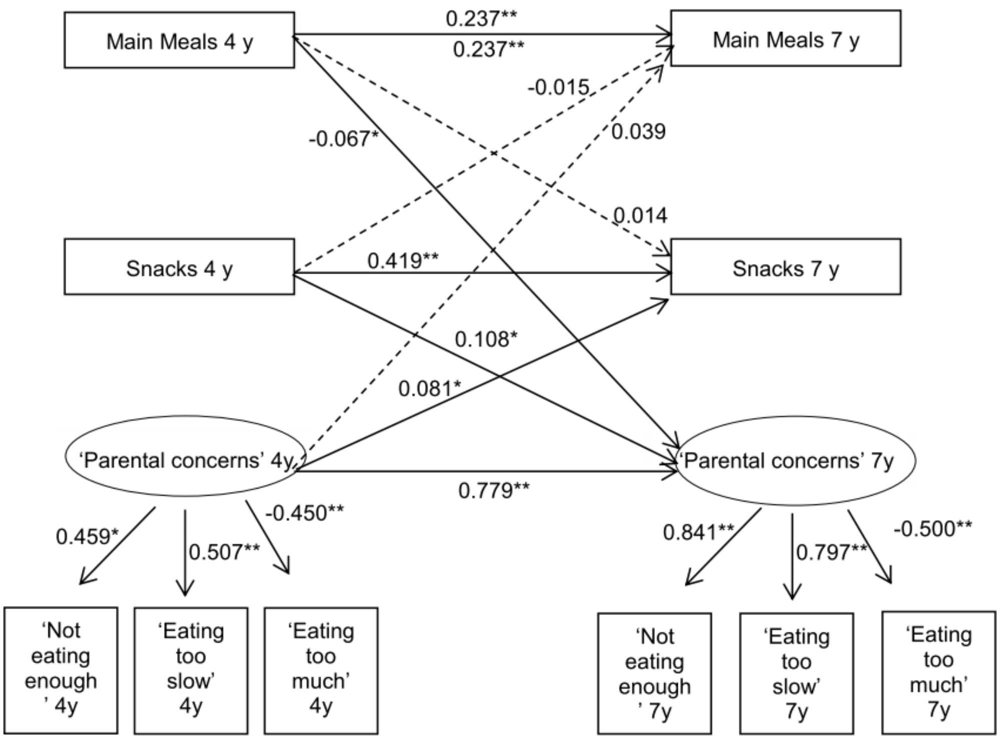
Table 3. Associations of eating frequency at 4 and 7 years and eating behaviours related to appetite at 7 years of age.

	Eating behaviours at 7 years (CEBQ)				
	Satiety Responsiveness	**Enjoyment of Food**	**Food Responsiveness**	**Slowness in Eating**	**Desire to Drink**
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Eating frequency at 4y (prospective analysis)					
Main meals (frequency/day)					
Model 0	**-0.15 (-0.28;-0.02)**	0.11(-0.03;0.26)	-0.05 (-0.19;0.10)	-0.13 (-0.29;0.03)	-0.15 (-0.30;0.00)
Model 1	-0.13 (-0.26;0.00)	0.07(-0.08;0.22)	-0.08 (-0.22;0.07)	-0.13 (-0.30;0.04)	-0.13 (-0.29;0.02)
Model 2	-0.06 (-0.18;0.06)	0.01 (-0.13;0.15)	-0.06 (-0.20;0.07)	-0.06 (-0.21;0.09)	-0.09 (-0.25;0.06)
Model 3	-0.06 (-0.18;0.06)	0.01(-0.12;0.15)	-0.06 (-0.20;0.07)	-0.06 (-0.21;0.09)	-0.09 (-0.24;0.07)
Snacks (frequency/day)					
Model 0	0.03 (-0.00;0.06)	-0.01(-0.05;0.03)	-0.00 (-0.04;0.03)	0.04 (-0.00;0.08)	**0.08 (0.04;0.12)**
Model 1	0.00 (-0.03;0.04)	0.02 (-0.02;0.06)	0.01 (-0.02;0.05)	0.02 (-0.03;0.06)	**0.08 (0.04;0.12)**
Model 2	0.02 (-0.01;0.05)	-0.00(-0.04;0.04)	0.00 (-0.03;0.04)	0.03 (-0.01;0.07)	**0.05 0.00;0.09)**
Model 3	0.02 (0.01, 0.06)	-0.01(-0.04;0.03)	0.00 (-0.03;0.04)	0.04 (-0.01;0.098)	**0.04 (0.00;0.08)**
Eating frequency at 7y (cross-sectional analysis)					
Main meals (frequency/day)					
Model 1	-0.08 (-0.20;0.05)	**0.18 (0.03;0.33)**	0.12 (-0.02;0.26)	-0.01 (-0.18;0.15)	-0.06 (-0.21;0.09)
Model 2	-0.10 (-0.21;0.02)	**0.22 (0.09;0.35)**	**0.15 (0.02;0.27)**	-0.05 (-0.20;0.09)	-0.03 (-0.18;0.12)
Model 3	-0.09 (-0.20;0.03)	**0.23 (0.10;0.36)**	**0.14 (0.01;0.27)**	-0.06 (-0.20;0.09)	-0.05(-0.20;0.10)
Snacks (frequency/day)					
Model 1	**0.08 (0.04;0.13)**	**-0.10(-0.15;-0.05)**	**-0.06(-0.11;-0.01)**	**0.07(0.10;0.13)**	-0.00 (-0.06;0.05)
Model 2	**0.06 (0.02;0.10)**	**-0.07(-0.12;-0.02)**	-0.02 (-0.07;0.02)	**0.05 (-0.00;0.10)**	-0.00 (-0.06;0.05)
Model 3	**0.09 (0.04;0.13)**	**-0.09(-0.14;-0.04)**	-0.04 (-0.08;0.01)	**0.06 (0.01;0.12)**	-0.02 (-0.08;0.04)

95%CI, 95% confidence interval

Model 0: unadjusted; model 1: adjusted for the same eating occasion at 4 (cross-section analysis) or 7 years of age (prospective analysis); and model 2: adjusted for covariates from model 1 and in addition for maternal age and education at 4 years, children's sex and BMI, and parental concerns regarding problematic behaviours at 4 years; model 3 adjusted for covariates from model 2 and in addition for energy intake (kcal/day) at 7 years.

Significant associations are highlighted in bold-type



$X^2(29) = 123.08$; p<0.001; CFI=0.960; TLI=0.939; RMSEA=0.050

Figure Cross-lagged models for the associations between parental concerns regarding children's eating behaviours and children's eating frequency at 4 and 7 y of age.
All values represent standardized β-regression coefficients.
**P<0.05; **P<0.001.* CFI, comparative fit index; RMSEA, root mean square error of approximation; TLI, Tucker–Lewis index;

Paper VI

Eating frequency and weight status in Portuguese children aged 3 to 9 years - results from the National Food, Nutrition and Physical Activity Survey 2015-2016

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[Submitted]

Abstract

Background: The association between eating frequency and adiposity measures in children is not consistent and requires more in-deep research.

Objective: To characterize eating frequency (daily number of main meals and snacks) in relation to weight status in children aged 3 to 9 years, representative of the Portuguese population.

Design: Cross-sectional study based on the National Food, Nutrition and Physical Activity Survey of the Portuguese population, 2015-2016.

Participants/setting: Portuguese children aged 3-9 years with complete dietary data and anthropometric measurements (n=518).

Main outcome measures: Dietary intake was estimated as the mean of two non-consecutive days of food diaries, followed by face-to-face interviews. Weight and height were measured by trained observers and were used to classify individuals into non-overweight and overweight/obese, according to the WHO criteria. Eating occasions (EO) were defined by the children's caregiver, and was considered as a separate EO if the time of consumption was different from other EO, and if provided at least 50kcal. Main meals defined as 'breakfast', 'lunch' and 'dinner' were unique and could only be selected once per day. The remaining EO were considered snacks.

Statistical analysis: The association between eating frequency and overweight and obesity were evaluated through logistic regressions weighted for the population distribution (odds ratio (OR) and 95%CI).

Results: Overall, the number of daily EO ranged from 2 to 11, and on average children had 5.7 daily EO. After adjustment for child's sex, age and total energy intake, and considering only plausible reporters of energy intake, having less than three daily snacks was positively associated with being overweight or obese (OR=1.98, 95%CI:1.00;3.90), comparing to having at least three daily snacks.

Conclusion*:* Lower daily frequency of EO was associated with increased odds of being overweight or obese in children. A higher eating frequency, maintaining the same energy intake, seems to contribute to a healthy body weight in children.

Keywords: eating frequency; snacks; children; obesity; dietary surveys

Introduction

The World Health Organization (WHO) estimates that over 340 million children and adolescent aged 5-19 are classified as overweight or obese (1). Among preschool children (<5 years of age), the global prevalence of excessive weight increased from 4.2% in 1990 to 6.7% in 2010. This trend is expected to reach 9.1%, representing 60 million children, in 2020 (2). Childhood obesity is a complex disease with several adverse consequences, including psychological, social and health consequences (3, 4). Poor self-esteem, depression and eating disorders have been described more extensively in obese children than in their normal weight peers (5-7). Furthermore, obese children are more susceptible to cardiovascular disease risk factors such as hypertension, dyslipidaemia, chronic inflammation, endothelial dysfunction and insulin resistance (8-12). Research has suggested that early childhood may be a particularly sensitive period for the development of obesity later in life (13), thus early life prevention of overweight and obesity should be valued.

There is evidence that meal characteristics, such as nutrient composition, eating frequency or regularity of meals, could influence adverse outcomes in health, such as obesity, insulin resistance, and metabolic syndrome, independently of other risk factors (14-20). Some studies among adults associate greater meal frequency with a healthier weight status (21, 22). On the other hand, increased eating frequency might actually lead to a higher exposure to energy-dense foods, such as fast food and soft drinks, and large-portion-size foods, resulting in an increased hunger, excess energy intake, and ultimately unhealthy body weight gain (23-25). The association between eating frequency and adiposity measures in children is not consistent, as a large number of cross-sectional studies among young populations have shown an inverse relationship (19, 26-30) but other showed the opposite relationship (20, 31).

There is no general agreement on the most appropriate definition of eating occasion, and in particular in the differentiation between what constitutes a main meal or a snack (32). The literature describes a variety of approaches to defining eating occasions (main meals and snacks), using, for example, the time of consumption ('time-of-day'), food groups or nutritional profile ('food-based classification') or identification by the participant ('participant-identified') (32). These approaches can be contradictory or complement one another depending on the context (31, 33).

A lack of public health recommendations on eating frequency or temporal distribution is common among European authorities, especially in children (34). There is a lack of a universal definition of what constitutes an eating occasion, and for the differentiation between a main meal and a meal snack-type (32). The characterization of meal patterns among children using European harmonized methodology and a national representative sample will support the improvement of the research field and support the development of specific dietary guidelines.

The present study aimed to characterize eating frequency (daily number of eating occasions, main meals and snacks) in relation to weight status in Portuguese children aged 3 to 9 years, using data from a national dietary survey.

Material and Methods

Subjects and design

The present study was conducted within the National Food, Nutrition and Physical Activity Survey of the Portuguese population, 2015-2016 (acronym: IAN-AF 2015-2016) (35).

The IAN-AF 2015-2016 aimed to collect nationwide and regional data on dietary habits and physical activity and to assess their relation with health determinants, namely socioeconomic factors. A representative sample of the Portuguese population, aged between 3 months and 84 years old, was selected from the National Health Registry, by multistage sampling, in each of the seven geographical regions (NUTs II) and weighed, according to sex and age groups. Data were collected by trained interviewers using Computer-Assisted Personal Interviewing (CAPI), in two interviews, during 12 months (35), distributed over the four seasons and including all days of the week, and following European standard guidelines (36). A total of 5811 participants completed two interviews, and 6553 completed only the first one. Participants' characteristics were compared with participants who refused to participate but have accepted to answer a short questionnaire by phone. Non-participants were older (>65 years: 22% vs. 13%) and less educated (>12 years: 19% vs. 27%). Regarding variables representing the main research areas of the survey, the differences between participants and non-participants were considered of small magnitude: fruit and vegetable consumption (≥5 portions/day: 18.6% vs. 18.1%), practice of regular structured physical activity (33% vs. 39%), and obesity (12.4% vs. 12.7%) (35). From the total children (<10 years) 1329 had complete data in the two face-to-face interviews. Our analysis included children aged 3-9 years of age (n=604), with complete data on key variables (n=522). Four children did not have data on measured weight and height and were excluded, achieving a final sample of 518 children.

Dietary Intake

For children under 10 years, dietary intake was obtained by two non-consecutive one-day food diaries followed by a face-to-face interview allowing the parent or another main caregiver to add details related to food description and quantification. The time between interviews was between 8 and 15 days and the days of reporting were selected (including all days of the week), but participants were able to change them, according to their own availability for the interview. All foods, including beverages and dietary supplements consumed during a 24-hour period, were recorded per food consumption occasions (and quantified and described as eaten). The place and time of consumption were also recorded for each food consumption occasion. For food and recipe quantification several methods were used, such as, standard unit method (for foods consumed in distinguishable units with more or less standard weights), food photographs (a digital colour food picture book was developed including 186 food photo series with six portions each food/recipe item and 11 household measures photo series) and household measures.

During the face-to-face interview, the food diaries completed by the caregiver were included in the 'eAT24' module (Electronic Assessment Tool for 24-hours recall) which allowed the assessment of dietary data by an Automated Multiple-Pass Method for 24-hour (35). The total children's energy intake was obtained through conversion of foods into nutrients by the software, using by default the Portuguese food composition table (37).

Eating frequency

The 'eAT24' comprises 12 predefined food consumption occasions to standardize the reporting of events (intake of foods and/or drinks) during the 24-hour period: 'before breakfast', 'breakfast', 'during morning', 'before lunch', 'lunch', 'after lunch', 'during afternoon', 'before dinner', 'dinner', 'after dinner', 'during evening/at night' and 'liquids intake'. All eating occasions reported in the food diaries were recorded and considered as separate intake occasions if the time of eating was different from another eating occasion; Events consisting of water only (zero-calorie foods) were excluded. To exclude negligible amounts of energy intake, eating occasions providing less than 50kcal were also excluded. Main meals defined as 'breakfast', 'lunch' and 'dinner' were unique and could only be selected once per day. The remaining eating occasions were considered as snacks, and they could have been selected more than once in case of having a snack on several occasions during the day.

Daily eating frequency describes the total number of separate eating occasions during a 24-hour period, as the result of the average of the two reported days.

Anthropometrics

Children's weight and height were measured by trained observers, according to standard procedures. Weight was measured in the same conditions, to the nearest tenth of a kilogram using a digital scale (SECA® 813, Hamburg, Germany). Height was measured to the nearest centimetre, with children in a stand position with light clothing and barefoot, using a portable wall stadiometer (SECA® 213, Hamburg, Germany). The child's age- and sex-specific body mass index (BMI) z-scores were calculated according to the WHO criteria (38). The BMI was categorized as underweight (<-2SD), normal weight (≥-2SD to ≤1SD) and overweight/obese (>1SD), according to the WHO cut-off points (38).

Co-variables

For socio-economic characterization, parental education and maternal age were considered. The parental education was defined as the maximum level of education of any of the parents categorized as 'no formal education or primary education' (less than 6 years), 'Secondary or post-secondary education' (between 6 and 12 years plus post-secondary education not including tertiary education) and 'tertiary education' (university degree, master and PhD). Parents also reported if their child had a regular practice of physical exercise (categorized as non-practitioners vs. practitioners) and the daily minutes spent watching TV or playing games during both week and weekend days. This variable was combined and the number of minutes were averaged between weekdays and weekend and converted into hours (categorized as less than 2 hours per day watching TV or playing games, between 2 and 3 hours per day and at least 3 hours per day), and used as an indicator of a more sedentary lifestyle. For children aged six years or older, physical activity was assessed by diaries adapted from a model previously proposed (39), including two consecutive days during the week and two days on the weekend.

Ethical Standards Disclosure

Ethical approval was obtained from the National Commission for Data Protection, the Ethical Committee of the Institute of Public Health of the University of Porto and from the Ethical Commissions of each one of the Regional Administrations of Health. Written agreements from the parents or legal representatives were required for children. All documents with identification data were treated separately and stored in a different dataset.

Statistical analysis

Misreporting evaluation

To evaluate the extent of misreporting of energy intake (EI) the ratio of proxy-reported EI to predicted the Basal Metabolic Rate (BMR) was used to classify the dietary report into under-reports, plausible reports and over-reports, based on Goldberg cut-offs (40). The BMR was estimated using the equations published by Schofield (41), considering age, sex, body weight and height. The physical activity level (PAL) was defined according to Torun et al (42) for children aged less than 6 years. For the remaining children, the individual PAL obtained by the diaries was used.

The characteristics of Portuguese children 3-9 years old were examined according to BMI (non-overweight vs. overweight/obese). Data were presented as prevalence estimates weighted by the inverse of its sampling probability for the Portuguese population distribution, according to the complex sampling design, considering to each region (NUTS II) a cluster effect for the Primary Health Care Unit selected and stratification by sex and age groups (35). Mixed weighting was used to correct proportions of the sample in order to coincide to the population proportions and to expand to the population size. The respective 95% confidence intervals (95% CI) were also obtained. Prevalence estimates according to socioeconomic variables, eating occasions (main meals and snacks), energy intake, physical activity and sedentary habits were compared using second-order Rao-Scott corrections to the Pearson Chi-squared test, for categorical variables and by generalized linear model with gauss family and identity link function for continuous variables. The probability of a child having a specific food consumption occasion was calculated using two trials binomial regression with logit link function.

To evaluate the association between eating frequency and overweight and obesity, weighted logistic regressions were used, obtaining crude and adjusted odds ratio (OR) and respective 95% CI. The significance of coefficients was estimated using Wald test. The following potential confounders were tested: children's sex and age, maternal age and education, parental education, NUTs II, child's practice of sports and sedentary lifestyle. Only children's sex and age (as a quadratic term) were significant to the models and included as confounders variables. We also adjusted for the child's energy intake averaged from the two days. Interactions between children' sex, sport and maternal education were also tested. Tests for interactions were not significant and therefore we present results for all children together.

Statistical analyses were performed using R software version 3.4.1 for windows and library "survey (43). A significance level of 5% was assumed.

Results

Table 1 presents characteristics of children, for the total sample and according to weight status (no overweight vs. overweight or obese). Only three children were classified as underweight and were added to the group of normal weight children. Comparing to normal weight children, overweight/obese children were older on average and the majority were girls (55% vs. 46%). The number of daily eating occasion ranged from 2 to 11, and on average children had 5.7 (95% CI: 5.5, 5.8) eating occasions per day. In the overweight/obese children, the average number of daily eating occasions [mean = 5.5 (95% CI: 5.2, 5.9)] was lower than in the normal weight children [mean = 5.8 (95% CI: 5.6, 5.9)], although the differences were not statistically significant. The number of main meals was similar in both groups, but normal weight children [mean = 2.8 (95% CI: 2.7, 2.9)] had on average more snacks than the overweight children [mean = 2.6 (95% CI: 2.2, 2.9)]. The number of daily eating occasions and snacks was categorized, with the reference category including the mean of daily frequency: middle category in the overall eating occasions (>5 and <7), and second category in the snacks frequency (≥3). The majority of overweight children had less than 3 snacks per day, comparing to normal weight children [66.9% (95%CI: 54.5, 79.4) vs. 48.9% (95%CI: 42.1, 55.7), p=0.024)]. On average, overweight children had an energy intake higher than normal weight children did [1798 kcal (95%CI: 1685, 1910) vs. 1659 kcal (95%CI: 1600, 1718)]. Normal weight children had younger mothers and parents' level of education higher than the overweight/obese children did. However, normal weight children practised less frequently sports and watch less TV or played games, than the other group. Regarding the misreporting evaluation, overweight children had a higher percentage of plausible reporters (93% vs. 90%), comparing to normal weight children, although the differences were not statistically significant.

Table 2 shows the percentage of children having eating occasions during the two recall days by food consumption occasions. For the three main meals, the percentage of children who consumed meals during both days were 95%, 97% and 96% for breakfast, lunch and dinner, respectively. Around 90% of the children did not have a meal before breakfast, before or after lunch, and before or after dinner. These results were similar across normal weight and overweight/obese children. The average time of day that each food consumption occasion occurred are shown in figure 1. Usually, children had breakfast between 7.30 am and 9.30 am; lunch was reported as early as 12 am until 1.30 pm and dinner between 7.30 pm and 8.45 pm. The period of time when usually meals occurred did not seem to be different between underweight/normal weight and overweight/obese children.

The energy intake increased significantly with increasing eating occasions and snacks in all children and in normal weight children. The overweight and obese children had an average energy intake higher than the non-overweight children (table 3).

Table 4 presents the results for the association between eating frequency and weight status. Considering all sample, and after adjustment for child's sex, age and total energy intake, having five or less eating occasions per day, was positively associated with being overweight or obese (OR=1.93, 95%CI: 1.00, 3.73). Having less than 3 snacks per day, compared to having at least 3 daily, was also positively associated with overweight or obesity (OR=2.17, 95%CI: 1.13, 4.16) (table 4). Due to the low number of misreporting reporters, we decided to perform the regression analysis excluding these children (n=34). Including only plausible reporters for energy intake, the association remained significant only for snacks frequency and overweight/obesity (OR=1.98, 95%CI: 1.00, 3.90, <3 snacks/d vs ≥3 snacks/d)

Discussion

Portuguese children aged 3 to 9 years old had on average six daily eating occasions: three main meals and three snacks. The distribution of the meals schedule throughout the day had a higher variation for 'before breakfast', 'breakfast', 'during afternoon' and 'before dinner'. Overweight or obese children had on average less eating occasions than normal weight children. Among these children, having five or less eating occasions, and in particular, having less than three daily snacks, increased the odds of having excessive weight, after adjustment for potential confounders. Taking into consideration misreporting, the positive association with body weight was only found for having less than three daily snacks.

We opted for not analysing main meals separated from snacks, as the variability was low and the majority of children had the three main meals. So our analyses included all eating occasions and then separated by daily snacks.

Several pathways might explain the association between a lower eating frequency and higher body weight. Theoretically, eating frequency could modify metabolism, increasing satiety and improving glucose and insulin metabolism (18, 44). Controlled feeding studies in adults (44) showed that reduced eating frequency had a negatively impact on the appetite control, increasing the perceived appetite and reducing the perceived satiety. In a randomized crossover trial among normal-weight women, meal regularity was associated with a greater thermic effect of food and lower glucose response, which might benefit weight management and metabolic health (45). Moreover, increased eating frequency has been associated with higher levels of physical activity an improved composition of snacks and breakfast in terms of fruit and vegetables, fat, fiber and carbohydrates, in healthy weight children aged 9-10 years (46).

Obesity is a complex disorder and some remaining uncontrolled confounding may not be discarded. However, we tested several potential confounders' variables, namely children´s sex and age, maternal age and education, parental education, geographic region, child's sports practice and sedentary lifestyle. Only children's sex and age remain significant in the models and were included as confounders' variables, as well as child's energy intake (averaged from the two days). Interactions between children' sex, sport and maternal education were also tested but no modifying effect was found.

Irregular consumption of energy intake at breakfast and between meals was previously associated with a higher cardiometabolic risk in adults, including an increased waist circumference and BMI (15); in children larger variability in eating frequency was already associated with a disruption in total- and LDLcholesterol concentrations (47). Although in this study, we did not assess these effects (since we evaluated dietary intake of only two days), we were successful in finding a significant association between eating frequency, namely snacks frequency, and BMI in children.

In a systematic review of observational studies among adults (48), fourteen studies reported an inverse association between eating frequency and body weight or body composition, while seven found a positive association. Although in men a potential protective effect was consistently described, the authors concluded that there was not enough evidence for establishing a clear association between eating frequency and body weight in adults. Among children and adolescents, a previous meta-analysis (30) described an overall inverse association between eating frequency and the likelihood of being overweight or obese. However, when stratified by sex, the effect remained significant only in boys. The presence of publication bias and heterogeneity of studies might in part explain these differences, and further research was advised. In the present study, we tested an interaction effect between sex and eating frequency in the association with obesity, as we did not find a statistically significant effect we decided to present the results for the entire sample, and not stratified by sex.

More recent, a previous study (20) failed to find any association between eating frequency and BMI in UK children aged 4-10 years, while another study (46) found an inverse association in healthy weight children aged 9-10 years, but not in centrally obese children. In a longitudinal study (49) in very young children, the daily eating frequency was not associated with the current or subsequent change in BMI. Authors highlighted that these null associations might be due to an improvement of internal energy self-regulation by young children, in comparison with older children (49).

Methodological differences between studies might explain the discrepancy of results regarding the association between eating frequency and weight. In the present study, the weight and height of the children were measured by trained examiners, following standard procedures, with the exception of three children in which we used the parent's report of weight and height. The BMI z-score were then calculated and categorized according to the WHO criteria. In the majority of previous research, children were categorized as overweight or obese according to other criteria, such as the International Obesity Task Force, hampering comparisons between different studies (30).

There is also variability in the dietary assessment method and in the definition of eating frequency, meal and snack frequency. Food diaries, 24h dietary recall and self-reported questionnaires (by children or by their caregivers) have been used to assess eating frequency. In the present study we defined eating occasions using the 'participant-identified' approach, as was the child's caregiver who decided the food consumption occasions in the food diaries, but were the researchers that categorized the food consumption occasions, that provided at least 50kcal, into main meals (breakfast, lunch and dinner) and snacks (the remaining food consumption occasions). We were also able to identify the time of day when children usually had each of the food consumption occasion. This information might be useful in future research on the topic of eating frequency among children if the researcher decides to use a time-of-day approach to define eating occasions.

Furthermore different studies used different cut-offs to determine high or low eating frequency or using the exposure as a continuous variable. This last approach might not be the best one as we believe the association between eating frequency and obesity is not linear, and the extremes are the ones most harmful; correspondingly, we used the middle category as the reference in the eating occasion variable.

The strengths of this study include a national representative sample of children and results weighted for the Portuguese population distribution regarding sex, age and region, following harmonized European methodology (36). We also used two non-consecutive one-day food diaries followed by a face-to-face interview to estimate children's eating frequency, which is an advantage in comparison to simply asking parents to specify the usually eating frequency of their child. Trained interviewers, following standard procedures, performed objectives measures of children's body size, which is also a strength comparing to parents reporting the weight and height of the children.

There are also some limitations that deserve discussion. First, under-reporting of dietary intake among overweight or obese people is largely described in the literature (50, 51), and might have an impact on the association between eating frequency and obesity. When we included only plausible reporters for energy intake, the association between daily eating occasions and body weight ceased to be statistically significant. This effect might be due to a loss of power, as we excluded some children, and not due to a bias regarding the dietary report. Literature has reported a mismatching in mothers' perception regarding their children's weight. For example, in a cohort study among children aged 6 to 8 years, the majority of mothers misclassified the child's weight and had the tendency to normalize their weight status (52). Mothers were also poor at recognizing overweight and obesity in their children at 4 or 6 years (53) or in pre-school children (54). As parents might fail to recognize their child as overweight or obese, it is not expected that they change their report based on the child's weight status.

A social desirability bias might have occurred, resulting in an over-reporting of healthy foods and/or under-reporting of unhealthy foods. However, this bias might have been minimized as our main exposure variable, eating frequency, was calculated independently of the quality or types of foods.

Another limitation is the cross-sectional nature of the study, which does not allow assessing the temporal relationship between eating frequency and obesity. Moreover, we could not exclude a potential reverse causality effect, since obese people could change the frequency of meals in an attempt to lose weight. However, this approach is more likely to occur in adults than in children.

Conclusion

Portuguese children aged 3 to 9 years had on average six daily eating occasions, divided by three main meals and three snacks. In these children, and after adjustment for potential confounders, lower daily snacks frequency (<3/day) was positively associated with being overweight or obese. This study supports that increasing the daily meals, maintaining the same energy intake, contributes to a healthy body weight in children.

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Figures legends

Fig. 1 Distribution of time of consumption (mean and SD) in hours of each of the food consumption occasions, weighted for the Portuguese population distribution

Table 1 Characteristics of the Portuguese 3-9-year-old children, for the total sample and according to body mass index categories*, weighted for the Portuguese population distribution

	All children (n=518)	**Normal weight** (n=375)	**Overweight/ Obese** (n=143)	***P* value**
Estimated population, n (%)	630 731 (100)	436 241 (69.2)	194 490 (30.8)	
Age (years), mean (95%CI)	5.8 (5.5-6.0)	5.4 (5.1-5.7)	6.5 (6.3-6.8)	<0.001
Sex (girls), % (95%CI)	49.1 (45.7-52.5)	46.4 (41.4-51.3)	55.1 (46.7-63.6)	0.134
Eating occasions (frequency per day), mean (95%CI)	5.7 (5.5-5.8)	5.8 (5.6-5.9)	5.5 (5.2-5.9)	0.201
Main meals (frequency per day), mean (95%CI)	2.9 (2.9-3.0)	2.9 (2.9-3.0)	2.9 (2.9-3.0)	0.695
Snacks (frequency per day), mean (95%CI)	2.7 (2.6-2.9)	2.8 (2.7-2.9)	2.6 (2.2-2.9)	0.212
Eating occasions, % (95%CI)				
Less than or equal to 5 per day	35.0 (29.2-41.1)	31.4 (24.9-37.9)	43.6 (30.6-56.6)	
More than 5 but less than 7 per day	49.9 (43.4-56.3)	53.8 (45.9-61.8)	40.9 (29.4-52.4)	
At least 7 per day	15.0 (10.6-19.4)	14.8 (9.7-19.8)	15.5 (6.9-24.1)	0.164
Snacks, % (95%CI)				
Less than 3 per day	54.4 (48.4-60.5)	48.9 (42.1-55.7)	66.9 (54.5-79.4)	
At least 3 per day	45.5 (39.5-51.6)	51.1 (44.3-57.9)	33.0 (20.6-45.5)	0.024
Energy intake (kcal/day), mean (95%CI)	1702 (1648-1756)	1659 (1600-1718)	1798 (1685-1910)	0.036
Mother's age (years), mean (95%CI)	37.2 (36.5-37.9)	36.8 (36.1-37.5)	38.0 (36.8.39.4)	0.091
Level maximum of parents' education, % (95%CI)				
No formal education or primary education	8.1 (4.8-11.4)	7.7 (3.8-11.5)	9.2 (29.8-15.4)	
Secondary or post-secondary education (but non- tertiary education)	47.4 (49.9-53.8)	46.7 (39.5-53.9)	48.8 (37.9-59.7)	
Tertiary education	44.5 (37.0-52.0)	45.6 (37.9-53.3)	42.0 (29.5-54.4)	0.812
Physical activity practice, % (95%CI)				
Yes	61.1 (53.5-68.7)	59.0 (51.2-66.8)	65.8 (53.4-78.2)	0.297
Watching TV/playing games, % (95%CI)				
Less than 2h per day	47.1 (40.1-54.0)	50.0 (42.1-57.8)	40.4 (28.4-52.4)	
More than 2h but less than 3h per day	28.7 (22.8-34.6)	27.4 (20.4-34.4)	31.5 (20.2-42.8)	
At least 3h per day	24.3 (18.1-30.4)	22.6 (15.8-29.4)	28.0 (15.4-40.7)	0.446
Misreporting				
Plausible	91.5 (87.7-95.3)	90.6 (85.8-95.4)	93.4 (86.7-100.0)	
Underreport	2.4 (0.0-5.1)	1.8 (0.0-4.6)	4.0 (0.0-10.0)	
Overreport	6.1 (3.2-9.0)	7.6 (3.6-11.6)	2.6 (0.0-5.9)	0.332

CI confidence interval, *h* hour

*BMI categories defined according to the World Health Organization criteria

P value are determined by generalized linear model with gauss family and identity link function for continuous variables and second-order Rao-Scott corrections to the Pearson Chi-squared test for categorical variables

Table 2 Percentage of children having eating occasions by specific food consumption occasions (FCO) for the total sample and according to body mass index categories*, weighted for the Portuguese population distribution

	Before Breakfast	Breakfast	During morning	Before lunch	Lunch	After Lunch	During afternoon	Before dinner	Dinner	After dinner	During evening
All sample											
Probability of having the FCO											
None of the day, %	92.81	0.00	20.92	95.28	0.00	96.40	0.03	87.81	0.12	93.06	52.99
One of the days, %	4.94	4.87	29.74	4.69	2.58	3.52	6.67	10.62	4.11	6.57	30.87
In both days, %	2.25	95.13	49.35	0.03	97.42	0.08	93.30	1.57	95.76	0.38	16.14
Normal weight											
Probability of having the FCO											
None of the day	92.58	0.00	18.01	94.61	0.00	97.31	0.05	84.11	0.18	91.64	53.51
One of the days	4.35	5.78	32.54	5.34	1.61	2.58	5.12	13.78	3.25	7.88	28.81
In both days	3.07	94.22	49.45	0.05	98.39	0.11	94.83	2.11	96.57	0.48	17.68
Overweight/ obese											
Probability of having the FCO											
None of the day	93.34	0.00	27.44	96.76	0.00	94.37	0.00	96.09	0.00	96.23	51.83
One of the days	6.25	2.83	23.46	3.24	4.75	5.63	10.14	3.54	6.05	3.63	35.49
In both days	0.41	97.17	49.11	0.00	95.25	0.00	89.86	0.37	93.95	0.14	12.68

sd standard deviation
*BMI categories defined according to the World Health Organization criteria
[a]Median and interquartile range

Table 3 Children's mean energy intake across categories of eating occasions and snacks events, for the all sample and according to body mass index categories*, weighted for the Portuguese population distribution

	All children		Normal weight children		Overweight/ Obese children	
	Mean Energy intake	95%CI	Mean Energy intake	95%CI	Mean Energy intake	95%CI
Eating occasions, per day						
Less than or equal to 5	1555	1459-1650	1478	1391-1564	1678	1461-1895
More than 5 but less than 7	1757	1693-1821	1711	1637-1784	1893	1748-2038
At least 7	1919	1773-2065	1886	1723-2050	1988	1779-2198
P value	<0.001		<0.001		0.080	
Snacks, per day						
Less than 3	1636	1551-1722	1553	1479-1627	1772	1605-1939
At least 3	1798	1724-1873	1769	1676-1862	1900	1790-2010
P value	0.011		0.002		0.201	

CI confidence interval
*BMI categories defined according to the World Health Organization criteria
P values were calculated with Likelihood Ratio Test working with Rao-Scott corrections and F test

Table 4 Association between eating frequency (daily eating occasions and daily snacks) and overweight and obesity, in a national representative sample of children aged 3 to 9 years of age, weighted for the Portuguese population distribution for all children (n=518) and excluding misreporting (n=484)

	OR for Overweight/ obesity (95% CI)					
	All children (n=518)			Only plausible reporters (n=484)		
	Model 1 (unadjusted)	Model 2	Model 3	Model 1 (unadjusted)	Model 2	Model 3
Eating occasions, per day						
Less than or equal to 5	1.83 (0.95-3.51)	1.78 (0.90-3.52)	**1.93 (1.00-3.73)**	1.81 (0.93-3.52)	1.64 (0.83-3.24)	1.91 (0.97-3.77)
More than 5 but less than 7	Reference	Reference	Reference	Reference	Reference	Reference
At least 7	1.38 (0.63-3.04)	1.48 (0.68-3.22)	1.40 (0.61-3.23)	1.79 (0.80-4.02)	1.88 (0.84-4.22)	1.40 (0.67-4.05)
Snacks, per day						
Less than 3	**2.12 (1.11-4.04)**	**2.00 (1.05-3.80)**	**2.17 (1.13-4.16)**	1.93 (0.99-3.76)	1.72 (0.88-3.35)	**1.98 (1.00-3.90)**
At least 3	Reference	Reference	Reference	Reference	Reference	Reference

OR odds ratio, *CI* confidence interval
Model 1, unadjusted; Model 2, adjusted for child's sex and age (quadratic term); Model 3, adjusted for child's sex and age (quadratic term) and daily children's energy intake (kcal).
Bold entries denote statistical significance.

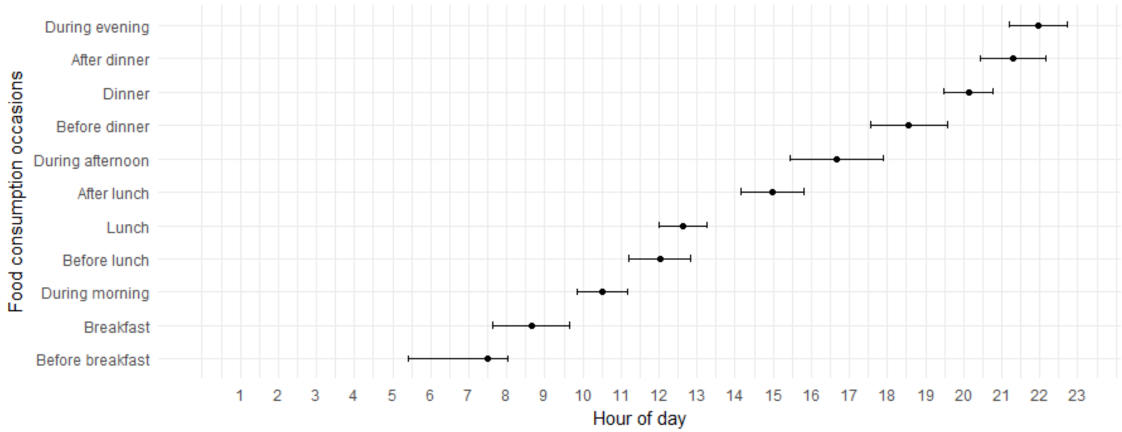
During evening
After dinner
Dinner
Before dinner
During afternoon
After lunch
Lunch
Before lunch
During morning
Breakfast
Before breakfast
Food consumption occasions
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hour of day

Fig 1

Paper VII

Chrono-nutrition: Relationship between time-of-day of energy and macronutrients intake and children's appetite and body weight status

Sofia Vilela, Andreia Oliveira, Milton Severo, Carla Lopes

[Submitted]

Abstract

Background/objectives: The time-of-eating has been considered as having an important role in weight regulation. However, there is a lack of evidence regarding the association between daily distribution of energy and macronutrients by meals and body mass index (BMI), especially in children. This study aimed to assess the effect of time-of-day of energy and macronutrients intake at 4 years on eating behaviours related to appetite and on BMI at 7 years of age.

Subjects/Methods: This study included 1961 singleton children from the population-based birth cohort Generation XXI (2005-2006) with data on 3-day food diaries at 4y and on the Children's Eating Behaviour Questionnaire (CEBQ) and body mass index (BMI) age- and sex-specific z-scores at 7y. *A posteriori-derived* dietary patterns were obtained for the distribution of energy and macronutrients across eating occasions.

Results: A higher intake of carbohydrates at the main meals but low at mid-afternoon at 4y was associated with lower food fussiness and satiety responsiveness but a higher enjoyment of food at 7y. Also, a higher intake of energy, protein and fat at lunch and dinner was positively associated with enjoyment of food. After adjusting for potential confounders, having a higher energy intake at lunch and supper (OR = 1.19, 95%CI:1.05;1.34) or at mid-afternoon (OR = 1.18, 95%CI:1.05;1.34) at 4y was associated with higher odds of developing overweight or obesity at 7y. A higher intake of fat at lunch was positively associated with later children's BMI (OR = 1.17, 95%CI:1.03;1.32). These associations were independent of the effect on children's eating behaviours related to appetite.

Conclusions: Considering all daily eating occasions, a higher proportion of energy and macronutrient intake at the main meals and a lower proportion at snacks events, especially in the evening meal, seems to be more beneficial for eating behaviours related to appetite and children's weight.

Keywords: feeding behaviours, time-of-day, childhood obesity, chrono-nutrition, cohort studies

Introduction

New research areas have emerged in an attempt to tackle the high prevalence of obesity. In that sense, accumulating evidence has suggested the importance of time of eating in weight regulation.[1-5] The circadian rhythm controls several physiological functions, including feeding. Parallel to the 'chrono-pharmacology', used to determine the best time to take a medication according to the circadian system of drug functions, the term 'chrono-nutrition' has been used to account for the relationship between food intake and the circadian clock.[6]

The suprachiasmatic nucleus (SCN) of the hypothalamus is believed to be the location of the master clock system in mammals, which rules the circadian rhythms.[7] The SCN receives light-dark information through the retinal-hypothalamic tract and organizes the peripheral clocks located in other parts of the body, such as pancreas, liver and adipose tissue.[8] In addition to light, other factors such as timing and type of nutrient intake, temperature or exercise can be a synchronizer for the circadian clock. Among these factors, food is the best synchronizer, as potent as the external light-dark signals.[7] In that way, poor nutrition (including unusual time of eating) can disrupt homeostasis, resulting in disturbances in leptin release, glucose and energy metabolism, as well as insulin sensitivity.[9,10] Hence, unusual feeding practices can cause disruption in both clock systems, and nutrient metabolism, which may increase the risk of development of diseases such as obesity or diabetes.[9,10]

An influence of the circadian clock on hunger and appetite has been previously suggested.[11] This endogenous control seems to be independent of other behaviours, such as duration of time since waking up.[11,12] Experimental studies also showed an effect by varying macronutrient intake of a meal or of the overall diet on appetite, with a more pronounced effect from proteins on suppressing appetite.[13,14]

A previous review of observational studies[1], on time-of-day of energy intake and its association with obesity, included evidence from eight cross-sectional studies (three among children) and two longitudinal cohort studies (one among children). Overall, the studies reported a positive association between evening energy intake and obesity. However, a more recent meta-analysis of observational and interventions trials among adults,[15] analysing the association between large evening meals and body mass index (BMI) in adults, showed conflicting evidence and failed to found a significant association between evening intake and BMI. Still in children, a cross-sectional study[16] also did not find any evidence that eating an evening meal after 8pm is associated with excess weight in UK children aged 4-18 years.

Almoosawi *et al.*[1] highlighted that it is still unclear if there are specific patterns of energy distribution that could be more beneficial for metabolic outcomes, especially obesity. Due to the little evidence that exists on how the macronutrient distribution influences BMI and given that the lipid and glucose metabolisms are influenced by the circadian clock, the authors stressed that the evaluation of time-of-day of macronutrient intake is a critical point to consider in future studies.[1]

Taking into consideration the gaps in the literature, this study aims to assess prospectively the association between time-of-day of energy and macronutrients intake at 4 years on appetite and BMI at 7 years of age. Moreover, we intended to assess if the effect of time-of-day intake on obesity is independent of children's appetite.

Subjects and Methods

Participants are from the population-based birth cohort Generation XXI (G21), assembled in the public maternity Units of the Porto Metropolitan Area (Portugal), during 2005/2006 (n=8647 newborns).[17] These maternity units were responsible, at enrollment, for 91.6 % of the deliveries in the whole eligible population.

The present study included a subsample of children evaluated at both 4 and 7 years of age by face-to-face interviews, providing complete 3-day food diaries data from age 4 and objective measures of weight and height at 7 years (n=2035). We excluded twins (n=60) and children with congenital anomalies or diseases that might influence dietary intake (n=14), achieving a final sample of 1961 children. In all evaluations, the information regarding socioeconomic characteristics, dietary intake, and medical history were collected by trained interviewers.

The project G21 was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee of the São João Hospital/ the University of Porto Medical School. The project was approved by the Portuguese Authority of Data Protection. Parents or legal guardians of each participant received an explanation on the purposes and design of the study and gave written informed consent at baseline and follow-up evaluations.

Dietary intake

At 4-year follow-up evaluation, parents or another main caregiver were asked to provide a detailed description of all foods and drinks consumed by the child, in 2 weekdays and 1 weekend day, in a format of a food diary.[18] Oral and written instructions were given for the correct completion of food diaries, including information on estimation of food portions size. Caregivers were also asked to provide a description of the method of food preparation, recipes, place and time of consumption, whenever possible. In the case the child was out of the home during the day, parents were advised to ask for the help of other caregivers.

As previously described,[18,19] a team of trained nutritionists was responsible for the codification process, using an age-specific food coding manual previously developed by our research team. Nutrient intake was estimated using the software Food Processor SQL (2004-2005 ESHA Research, Salem, Oregon), based on the Food Composition Table of the United States of America Department of Agriculture.[20] For typically Portuguese foods or culinary dishes, new codes were created with national nutritional information.

Meal definition

The meal definition used was described previously.[21] Briefly, an 'eating occasion' was any occasion when food or drink was consumed 30min apart and provided a minimum of 210kj (50Kcal) of energy. As caregivers did not distinguish the meals in the food diary, the team of trained nutritionist classified and distinguished meals (e.g. breakfast, supper) according to the type of foods, the period of time and place of consumption. Using this variable, a decision tree[22] was applied to define the best period of time for breakfast, mid-morning, lunch, mid-afternoon, dinner and supper. Breakfast was defined between 6-9.30am; lunch and dinner were considered between 11am and 2.30pm, and between 7-9.30pm, respectively. The other periods were considered for mid-morning, mid-afternoon and supper. Patterns of time-of-day of energy and macronutrient intake according to the meals were identified by factor analysis using the standard principal component analysis (PCA).

Anthropometrics

Children's anthropometric measurements were performed by a team of trained examiners, according to standard procedures. Children's weight was measured in underwear and without shoes using a digital scale and was recorded to the nearest 0.1kg. Height was measured as the distance from the top of the head to the bottom of the feet without shoes, using a fixed stadiometer to the nearest 0.1cm. Children's BMI, defined as weight in kg divided by height in meters squared, is often used as a proxy of adiposity in children.[23] Age- and sex-specific z-scores were established according to the WHO criteria,[24] allowing us to have indirect age and sex-specific measure of relative adiposity.

Children's eating behaviours related to appetite

A Portuguese version of the Children's Eating Behaviour Questionnaire (CEBQ),[25] originally developed by Wardle *et al*[26], was used to evaluate parental report (94% of the questionnaires were answered by mothers) regarding their child's eating behaviour at 7 years. This 35-item instrument is rated on a 5-point Likert scale ("never", "seldom", "sometimes", "often" and "always"), scored 1 to 5 and includes eight subdimensions of eating behaviours, containing 3 to 6 items each. The subdimensions 'Enjoyment of Food', 'Food Responsiveness', 'Satiety Responsiveness' and 'Food Fussiness' were included in the present study and analyzed independently. In individuals with missing data for less than 50% of the items, missing data (around 3%) were handled by imputation, replacing the average of the remaining questions within each subdimension.

Parental concerns regarding children's problematic eating behaviours assessed at 4 years of age, were used as proxies of the subdimensions of the CEBQ (since this was not assessed at 4 years) as described previously[27]. In the present analysis, the following questions were included: "my child does not eat enough", "my child eats very slowly" and "my child eats too much". Parents reported if any of these behaviours happened during the previous year and what was their level of concern (very concerned, somewhat concerned, no concern).

Statistical analysis

Characteristics of normal weight or underweight children were compared with those children with overweight/obesity, using the chi-square test for categorical variables and the student's t-test for two independent samples. Pearson's correlation was used to evaluate the relationship between the proportions of energy consumed across meals (as the percentage of total energy intake).

Patterns of distribution of daily energy and macronutrient according to the meals were identified by factor analysis using PCA without rotation and using the co-variance matrix. This methodology was applied separately for energy, protein, carbohydrate and fat. The criteria used was the eigenvalue (>1 times the mean of eigenvalue), scree plot, and interpretability of the components. Higher loadings (absolute value) indicate a stronger positive or negative correlation between the intake at a specific meal and the pattern. Energy or nutrient intake at specific meals with loadings above +/-0.3 on a component was considered to have a strong correlation with that component. Labelling of the patterns was based on the highest component loadings within each pattern. Factor scores were calculated using regression method.

The association between the energy and macronutrient intake throughout the day, summarized as the meal pattern score, at 4 years and eating behaviours related with appetite at 7 years was assessed through linear regression models, obtaining regression coefficients and respective 95% confidence intervals ($\beta^{\wedge}$ (95%CI)). Models were adjusted for children's sex, maternal age and education, child's BMI, total daily energy intake and parental concerns regarding children's problematic eating behaviours at 4 years. The association between meal patterns at 4 years and the children's z-score BMI (non-overweight vs. overweight/obese) at 7 years was examined using logistic regression models – obtaining *Odds Ratio* and respective 95% Confidence Intervals (OR (95%CI)). The following potential confounders were tested as potential influencers of either food intake (meal patterns) or BMI and test statistically in the final models: children's sex, maternal age and education, child's BMI, number of meals, total daily energy intake, practice of structured physical activity and sedentary lifestyle at 4 years (model 1), and CEBQ sub-dimensions' score at 7 years (model 2). Model 2 aims to test the potential mediating effect of children's eating behaviours related to appetite on the association between time-of-day intake and BMI. The sub-dimensions included as confounders in the model 2 were the ones statistically significant for the model ($p<0.05$).

The software Statistical Package for the Social Sciences (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.), and the R language and software environment for statistical computation (version 3.4.1, R Foundation for Statistic Computing, Austria, 2010) were used. For the student's t-test, a significance level of 1% was assumed to allow for multiple testing.[28] For all other analysis, a p-value of <0.05 was used to determine significance.

Results

The prevalence of overweight/obese children at 7 years was 36.7%. Characteristics of normal/underweight and overweight/obese children are presented in Table 1. The mean energy intake was higher in the overweight/obese group in comparison with the normal weight children, although it did not achieve statistical significance (p=0.069). The proportion of energy intake at dinner was higher in the non-overweight children in comparison with overweight/obese children (p=0.006). Maternal education was in average higher among children with normal/underweight, comparing to children with excessive weight (p<0.001).

At 4 years, table 2 shows a negative correlation between energy intakes in each of the meals, being the stronger correlation between adjacent meals. This shows a compensation of energy intake between meals. A similar approach analyzing the macronutrient intake in the different meals did not show any trend.

An overview of the component loadings identified by PCA per meal pattern (according to energy and macronutrient distribution by meals) is shown in table 3. Three distinct patterns were identified for energy intake, explaining 67% of the variance in the total. The first pattern labelled 'Main Meals', is characterized by higher energy intake at lunch and dinner. The second pattern was labelled 'Mid-afternoon' based on its high loadings of energy intake in the mid-afternoon. The last pattern was named 'Lunch&Evening' and is characterized by higher energy intake at lunch and supper but lower energy intake at dinner. For protein intake, the two patterns identified explained 63% of the total variance. The patterns were labelled: 'Main Meal', characterized by higher intake of protein at lunch and dinner, and 'Lunch', characterized by higher intake at lunch but lower intake at dinner. Three distinct patterns were identified for carbohydrate intake, named: 'Afternoon', 'Main Meal' and 'Morning', and together they explained 67% of total variance. The first pattern is characterized by a higher intake of carbohydrate at lunch, mid-afternoon and dinner. The second pattern is characterized by a higher intake of carbohydrate at all main meals but low intake at mid-afternoon. Finally, the last pattern is characterized by a higher intake at mid-afternoon and lunch but lower intake at breakfast. Two patterns were identified for fat intake, similar to the patterns observed for protein intake: higher fat intake at lunch and dinner ('Main Meal') and higher intake at lunch but lower intake at dinner ('Lunch'). These two distinct patterns explained 67% of total variance.

Associations between meal patterns for energy and macronutrient distribution at 4 years and eating behaviours related to appetite at 7 years are described in table 4. Overall the patterns characterized by higher energy intake or higher protein or fat intake at lunch and dinner ('Main Meals') were positively associated with 'Enjoyment of Food' but inversely associated with 'Satiety Responsiveness', after adjustment (model 2). On the other hand, the 'Lunch&Evening' pattern for energy intake was inversely associated with 'Enjoyment of Food' (β=-0.04,95%CI:-0.07;-0.01) only. Regarding carbohydrate intake, the pattern 'Main Meal' was positively associated with 'Enjoyment of Food' (β=0.04,95%CI:0.00;0.07) but inversely associated with 'Satiety Responsiveness' (β=-0.04,95%CI:-

0.07;-0.01) and 'Food Fussiness' (β=-0.07,95%CI:-0.13;-0.02). Children with higher loadings in the 'Lunch' protein intake pattern at 4 years had lower scores in the 'Food Responsiveness' subdimension (β=-0.03,95%CI:-0.06;-0.00), while children with higher loadings in the 'Mid-afternoon' energy intake pattern had higher scores in the 'Satiety Responsiveness' subdimension (β=0.04,95%CI:0.01;0.06).

Table 5 shows the associations with overweight/obesity. After adjustment, higher scores in the pattern 'Mid-afternoon' (OR=1.18,95%CI:1.05;1.34) and 'Lunch&Evening' (OR=1.19,95%CI:1.05;1.34) for energy intake at 4 years was associated with higher odds for overweight/obesity at 7 years (model 1). Higher fat intake at lunch but lower intake at dinner ('Lunch pattern') at 4 years was positively associated with overweight/obesity at 7 years (OR=1.17,95%CI:1.03;1.32). After adjustment, neither carbohydrate nor protein daily distribution at 4 years was associated with overweight/obesity at 7 years (model 1). The adjustment for sub-dimensions of the CEBQ (model 2) did not change significantly the associations described before.

Discussion

To our knowledge, this is the first study considering meal patterns of energy and macronutrients' distribution and their association with children's appetite and body weight, using a prospective approach. After adjusting for potential confounders, a higher intake of carbohydrates at the main meals but lower at mid-afternoon was associated with lower scores in 'Food Fussiness' and 'Satiety Responsiveness' but with higher scores in 'Enjoyment of Food' at 7 years. Additionally, a higher intake of energy, protein and fat at lunch and dinner was positively associated with 'Enjoyment of Food'. Having a higher energy intake at lunch and supper or at mid-afternoon was positively associated with later children's BMI. Similarly, a higher intake of fat at lunch at 4 years increased the odds of developing overweight/obesity at 7 years. These associations were independent of the effect on children's eating behaviours related to appetite. No association was found between time-of-day of protein or carbohydrate intake and children's BMI.

Another interesting finding was the compensatory regulation of energy intake in a meal depending on the previous intake. These might be the result of a self-regulation, as previously described in young children, by adjusting their portion size according to the number of meals,[29] or a regulation imposed by the caregiver. This trend was similar between non-overweight and overweight/obese children. The weakest correlation for adjacent meals was between mid-morning and lunch, in both groups. Other studies also described that the energy or nutrient consumed at a certain time of the day influences subsequent eating occasions.[1,2] For example, in a longitudinal study among girls aged 8-12 and 11-19 years, the mean energy consumed in the evening period during weekdays was negatively correlated with the mean energy consumed in the mornings and afternoons during weekdays.[2] The overall results of this study indicated that the mean percentage of daily energy intake at night was positively associated with changes in the girls' BMI.[2]

Studies among shift workers suggest that nocturnal eating could result in metabolic disruption,[30-32] partly explained by a decreasing in glucose tolerance and insulin resistance at night.[33,34] Accordingly, changes in the levels of circulating of leptin or ghrelin, by circadian disruption could influence energy intake and expenditure. Moreover, research also suggests that energy metabolism is less efficient during the evening, as morning-diet induced thermogenesis is significantly higher than night-induced thermogenesis.[35] In that way, eating late in the day or at night may disrupt circadian rhythms leading to excessive weight gain. One of the meal patterns described in our study and positively associated with odds of overweight/obesity was the one higher in energy intake at lunch and supper.

A more holistic approach has been encouraged to explore complex issues, such as nutrition-related health outcomes, before addressing any specific question to explain the whole.[36,37] Traditional analysis of isolated foods or nutrients has been relevant, for instance in decreasing prevalence of hypovitaminosis, however, due to the complex nature of the diet, this approach seems to fail in describing adequately the diversity of metabolic effects on the entire organism.[38] The analysis at meal patterns level allows assessing the combined effect of food compounds synergy and to be translated into meaningful results, such as dietary guidelines.[39,40] In the present study, patterns of distribution of energy and macronutrients by meal were examined in relation to the obesity likelihood and appetite control in children, as previously recommended.[1]

This study has examined the prospective association between timing of energy and macronutrient intake and obesity in children, using all eating occasions. Previous studies have focused on breakfast[41,42] or evening meals only.[16] Contrary to earlier research,[43,44] we did not find any link between energy or macronutrient intake at breakfast and children's BMI. Obese children have been shown to skip breakfast more frequently than non-overweight children.[45-47] However, Portuguese children have a very low prevalence of skipping breakfast; data from the National Food, Nutrition and Physical Activity Survey 2015-2016, shows that around 98% of children had breakfast in the evaluated days.[48] This overall regularity towards breakfast might explain the null associations found in our sample, between the breakfast's nutrient composition and the occurrence of overweight/obesity. Additionally, the breakfast only represents 11% of daily energy in our sample of children, and so did not seem to have a large impact on weight regulation. A previous cross-sectional study[49] also showed a positive link between lunch's energy intake and the likelihood of overweight in preschool Chinese children.

Previous research has shown that specific appetite-related traits (e.g. 'Food Responsiveness') are positively associated with child's overweight, while other traits, such as 'Satiety Responsiveness' are inversely associated with child's weight.[50] Interestingly, the energy distribution patterns associated with obesity ('Mid-afternoon' and 'Lunch&Evening') were inversely associated with 'Enjoyment of Food' but positively with 'Satiety Responsiveness'. Previously, children with higher scores in 'Enjoyment of Food' were the ones with a higher intake of fruit and vegetables whereas children with higher scores in 'Food Responsiveness' were having a higher consumption of fruit but also had a higher liking for energy-dense foods.[51,52] The further adjustment of CEBQ's sub-dimensions in the association between meal patterns and children's BMI showed that the effect of time-of-day of energy and macronutrient intake on obesity was independent of the effect on children's appetitive behaviours, and thus these behaviours do not seem to be mediating factors in that associations.

The meal pattern with an equal distribution of carbohydrates at the main meals but low at mid-afternoon at 4 years was associated with lower scores in 'Food Fussiness' at 7 years. Eating problems, such as a low interest in food or fussy eating, are common among children.[53] A higher intake of energy and macronutrient at main meals in childhood seems to be related to better eating behaviours a few years later.

Another study using dietary pattern based on the time-of-day of eating events described that Brazilian children having a traditional lunch with rice and beans as the main meal of the day had the lowest obesity risk.[5] These results highlighting the importance of considering both what and when is eating for weight regulation. Meal irregularity of energy intake, especially at breakfast and between meals, appeared also to be related to a higher cardiometabolic risk in adults, including an increased waist circumference and BMI.[54] Time-of-day and nutrient composition of meals were also associated with other chronic diseases, such as metabolic syndrome and its individual components, including abdominal obesity.[4] Results from this research show that carbohydrate intake at breakfast or mid-morning is protective against abdominal obesity.[4] In our study, we found a positive association between consuming carbohydrate at afternoon and BMI in children, but after adjustment for total daily energy intake, the association was no longer significant. Nevertheless, the pattern with higher carbohydrates intake at breakfast was associated with healthier eating behaviours in children.

Similar to our results, a previous study showed that the timing of the lunch in a Mediterranean adult population influenced the weight loss during a 20-week dietary intervention in adults with overweight or obesity.[55] Late lunch eaters lost less weight, independently of the total energy intake, dietary composition, estimated energy expenditure, appetite hormones and sleep duration.[55] Parallel, our results showed an effect of time-of-day of energy and fat on adiposity independently of the appetite control (using CEBQ as proxies). Changes in the chronotype, genetic background and/or circadian system function may be implicated in these associations.[56]

Some strengths of this study were the prospective design based on a large population-based birth cohort, the age frame considered, detailed measures of dietary intake and objective measures of children's weight and height. The use of a prospective cohort also allowed testing a wide set of potential confounders.

Some limitations of this study need further discussion. Misreporting of dietary intake among overweight or obese people has been previously described in the literature,[57,58] which might affect the association between eating habits and BMI. However, there is no evidence of a misreporting depending on the time of eating, and we believe that in this particular study misreporting might have been minimized as the parents were the ones reporting the children's dietary intake, and not the children themselves. Additionally, selection bias may have occurred due to the high burden related to completion of the food diaries. Comparing the children included in our study with the remaining cohort, we did not find statistically significant differences regarding child's sex and BMI (at 7 years), although mothers included in this study were slightly older [30.0±5.09 vs. 28.7±5.70 years, $p<0.001$] and more educated [11.5±4.25 vs. 10.1±4.21 school years, $p<0.001$] in comparison with the remaining mothers included at baseline. However, these differences are more likely to be due to large sample size rather than to considerable differences. Despite adjustment for relevant confounders, some residual confounding cannot be excluded.

In summary, considering all daily eating occasions, our results suggest that a higher proportion of energy and macronutrient intake at the main meals and a lower proportion at snacks events, especially in the evening meal, are more beneficial for children's eating behaviours related to appetite and body weight. These results emphasise the important role of the of food intake circadian rhythm on children's appetite control and overweight/obesity development.

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Potential conflict of interest and source of funding

The authors declare no conflicts of interest.

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Table 1 – Characteristics of the children according to body mass index categories

	All children	Normal weight/ Thinness (%) 7y	Overweight/ obese (%) 7y	p-value*
Children's characteristics at 4y				
Sex (girl), n (%)	926 (48.9)	577 (48.1)	349 (50.2)	0.380
Structured Physical activity practice, n (%)				
Yes	1320 (69.9)	839 (70.2)	481 (69.4)	0.716
Total energy intake (kcal/d), mean (SD)	1633 (293.7)	1623 (291.5)	1649 (296.9)	0.069
Protein (g),	76 (15.2)	75 (15.3)	77 (15.0)	0.039
Protein (% energy)	19 (2.5)	19 (2.5)	19 (2.5)	0.419
Carbohydrates (g)	200 (40.6)	199 (40.5)	202 (40.7)	0.112
Carbohydrates (% energy)	49 (4.6)	49 (4.7)	49 (4.6)	0.986
Fat (g)	59 (13.2)	58 (13.0)	59 (13.6)	0.134
Fat (% energy)	32 (3.6)	32 (3.7)	32 (3.6)	0.940
Energy (%), mean (SD)				
Breakfast	11 (5.6)	11 (5.6)	11 (5.6)	0.936
Mid-morning	8 (5.1)	8 (5.1)	8 (5.1)	0.129
Lunch	29 (6.1)	29 (6.0)	30 (6.3)	0.403
Mid-afternoon	21 (5.9)	20.7 (5.83)	21.4 (6.06)	0.021
Dinner	25 (6.6)	26 (6.7)	25 (6.5)	0.006
Supper	5 (4.9)	5 (5.0)	5 (4.7)	0.099
Protein (%TEI), mean (SD)				
Breakfast	18 (6.8)	18 (6.8)	19 (6.6)	0.059
Mid-morning	14 (9.0)	14 (8.9)	14 (9.0)	0.450
Lunch	19 (4.3)	19 (4.3)	19 (4.3)	0.821
Mid-afternoon	15 (4.0)	15 (4.0)	15 (4.0)	0.469
Dinner	20 (5.5)	20 (5.4)	20 (5.6)	0.935
Supper	16 (11.8)	15.7 (11.6)	16.0 (12.1)	0.642
Carbohydrate (%TEI), mean (SD)				
Breakfast	52 (15.1)	51 (15.6)	52 (14.1)	0.569
Mid-morning	57 (23.6)	57 (23.4)	56 (23.8)	0.585
Lunch	43 (7.5)	43 (7.5)	43 (7.6)	0.334
Mid-afternoon	59 (8.7)	59 (8.6)	59.2 (8.8)	0.865
Dinner	41 (9.4)	41 (9.3)	40 (9.5)	0.904
Supper	36 (25.4)	37 (25.2)	36 (25.7)	0.577
Fat (%TEI), mean (SD)				
Breakfast	20 (8.0)	20 (8.2)	20 (7.6)	0.839
Mid-morning	19 (11.0)	19 (11.0)	19 (11.0)	0.747
Lunch	36 (6.1)	36 (6.0)	36 (6.2)	0.295
Mid-afternoon	24 (7.0)	24 (6.9)	24 (7.1)	0.320
Dinner	37 (7.4)	37 (7.3)	37 (7.3)	0.571
Supper	16 (12.0)	16 (12.1)	15 (11.7)	0.135
Mother's characteristics, mean (SD)				
Education at 4-year-follow up (years)	12 (4.2)	12 (4.2)	11 (4.1)	**<0.001**
Age at 4-year-follow up (years)	34 (5.1)	34 (5.1)	34 (5.1)	0.102

TEI, total energy intake

* p<0.01 (Bonferroni correction)

Table 2 – Correlation between the percentages of energy consumed in each meal, in all children and according to body mass index categories at 4 years.

%Energy	Breakfast	Mid-morning	Lunch	Mid-afternoon	Dinner	Supper
All children						
Breakfast	1	**-0.380****	-0.188**	-0.081**	-0.163**	-0.198**
Mid-morning		1	-0.185**	-0.070**	-0.193**	-0.028
Lunch			1	**-0.363****	-0.170**	-0.172**
Mid-afternoon				1	**-0.368****	-0.091**
Dinner					1	**-0.309****
Supper						1
Normal weight						
Breakfast	1	**-0.380****	-0.182**	-0.073*	-0.186**	-0.186**
Mid-morning		1	-0.187**	-0.065*	-0.190**	-0.030
Lunch			1	**-0.333****	-0.159**	-0.212**
Mid-afternoon				1	**-0.386****	-0.098**
Dinner					1	**-0.294****
Supper						1
Overweight/obese						
Breakfast	1	**-0.380****	-0.199**	-0.094*	-0.123**	-0.222**
Mid-morning		1	-0.183**	-0.084*	-0.194**	-0.021
Lunch			1	**-0.416****	-0.188**	-0.099**
Mid-afternoon				1	**-0.332****	-0.072
Dinner					1	**-0.347****
Supper						1

*p<0.05; **p<0.001

Table 3- Factor loadings of distribution of energy and macronutrients by meals identified by Principal Component Analysis without rotation (loadings above +/- 0.3 are shown in bold)

	Energy			Protein		Carbohydrate			Fat	
	'Main Meals'	'Mid-afternoon'	'Lunch & Evening'	'Main meals'	'Lunch'	'Afternoon'	'Main Meals'	'Morning'	'Main Meals'	'Lunch'
Variance explained	31.4%	18.7%	16.9%	41.4%	21.8%	26.9%	20.7%	19.4%	41.5%	25.8%
Energy or macronutrient intake at										
Breakfast	0.119	0.203	-0.223	0.058	-0.050	0.285	**0.360**	**-0.828**	0.037	0.019
Mid-morning	0.044	0.085	0.140	0.054	0.051	0.134	-0.276	**0.452**	0.023	-0.050
Lunch	**0.639**	-0.180	**0.721**	**0.626**	**0.779**	**0.365**	**0.554**	**0.404**	**0.517**	**0.854**
Mid-afternoon	0.251	**0.952**	0.098	0.049	-0.119	**0.879**	**-0.453**	-0.052	0.103	0.074
Dinner	**0.897**	-0.101	**-0.404**	**0.922**	**-0.385**	**0.462**	**0.625**	0.262	**0.935**	**-0.354**
Supper	-0.007	0.115	**0.280**	-0.025	0.065	0.090	-0.177	0.109	0.000	0.068

Table 4 - Associations between meal patterns at 4 years and eating behaviours related to appetite at 7 years of age.

	Enjoyment of Food (EF)		**Food Responsiveness (FR)**		**Satiety Responsiveness (SR)**		**Food Fussiness (FF)**	
	Crude model $\hat{\beta}$ (95% CI)	Adjusted model[a] $\hat{\beta}$ (95% CI)	Crude model $\hat{\beta}$ (95% CI)	Adjusted model[a] $\hat{\beta}$ (95% CI)	Crude model $\hat{\beta}$ (95% CI)	Adjusted model[a] $\hat{\beta}$ (95% CI)	Crude model $\hat{\beta}$ (95% CI)	Adjusted model[a] $\hat{\beta}$ (95% CI)
Energy patterns								
'Main Meals'	**0.07 (0.04;0.11)**	**0.05 (0.02;0.08)**	**0.04 (0.00;0.07)**	0.02 (-0.01;0.06)	**-0.06 (-0.09;-0.03)**	**-0.04 (-0.07,-0.01)**	**-0.08 (-0.13;-0.02)**	0.01 (-0.04;0.06)
'Mid-afternoon'	-0.02 (-0.05;0.02)	-0.02 (-0.05;0.02)	0.02 (-0.02;0.05)	0.02 (-0.02;0.05)	**0.04 (0.01;0.07)**	**0.04 (0.01;0.06)**	0.03 (-0.02;0.08)	-0.04 (-0.09;0.01)
'Lunch&Evening'	-0.01 (-0.04;0.03)	**-0.04 (-0.07;-0.01)**	0.01 (-0.03;0.04)	-0.02 (-0.06;0.01)	0.00 (-0.03;0.03)	0.02 (-0.01;0.04)	0.01 (-0.04;0.06)	0.02 (-0.05;0.05)
Carbohydrate patterns								
'Afternoon'	0.02 (-0.01;0.06)	-0.00 (-0.05;0.05)	0.03 (-0.01;0.06)	0.03 (-0.02;0.08)	-0.02 (-0.05;0.01)	-0.01 (-0.05;0.04)	-0.02 (-0.07;0.03)	0.01 (-0.08;0.09)
'Main meals'	**0.04 (0.00;0.07)**	**0.04 (0.00;0.07)**	0.01 (-0.03;0.04)	0.01 (-0.02;0.05)	**-0.04 (-0.07;-0.01)**	**-0.04 (-0.07;-0.01)**	**-0.08 (-0.14;-0.03)**	**-0.07 (-0.13;-0.02)**
'Morning'	0.02 (-0.01;0.06)	0.01 (-0.02;0.04)	0.03 (-0.00;0.06)	0.02 (-0.01;0.05)	**-0.03 (-0.06;0.00)**	-0.02 (-0.05;0.01)	-0.01 (-0.06;0.04)	0.00 (-0.05;0.06)
Protein patterns								
'Main Meals'	**0.06 (0.03;0.10)**	**0.04 (0.00;0.08)**	0.01 (-0.02;0.05)	-0.02 (-0.06;0.02)	**-0.07 (-0.10;-0.04)**	**-0.06 (-0.09;-0.02)**	**-0.06 (-0.11;-0.01)**	-0.04 (-0.10;0.02)
'Lunch'	0.01 (-0.02;0.05)	-0.03 (-0.06;0.01)	0.01 (-0.03;0.04)	**-0.03 (-0.06;-0.00)**	**-0.04 (-0.07;-0.01)**	-0.02 (-0.05;0.01)	-0.02 (-0.07;0.03)	-0.01 (-0.06;0.04)
Fat patterns								
'Main Meals'	**0.06 (0.03;0.10)**	**0.06 (0.02;0.10)**	**0.04 (0.00;0.07)**	0.02 (-0.02;0.06)	**-0.05 (-0.08;-0.02)**	**-0.05 (-0.09;-0.01)**	**-0.05 (-0.11;-0.00)**	-0.05 (-0.12;0.02)
'Lunch'	0.02 (-0.01;0.06)	0.00 (-0.03;0.04)	0.01 (-0.03;0.04)	-0.02 (-0.05;0.02)	-0.01(-0.04;0.02	0.00 (-0.03;0.03)	-0.02 (-0.07;0.03	-0.02 (-0.07;0.04)

[a]model adjusted for maternal age and education, children's sex and BMI, and parental concerns regarding child's problematic eating behaviours at 4 years; carbohydrate, protein and fat patterns were further adjusted for total daily energy intake.

Table 5 – Association between meal patterns at 4 years and BMI at 7 years

	Normal weight vs. overweight/obese (OR (95%CI)		
	Model 0	Model 1	Model 2
Energy patterns			
'Main Meals'	1.04 (0.94-1.14)	1.13 (0.99-1.27)	1.05 (0.92-1.19)[a]
'Mid-afternoon'	**1.12 (1.02-1.23)**	**1.18 (1.05-1.34)**	**1.21 (1.07-1.38)**[a]
'Lunch&Evening'	**1.14 (1.04-1.25)**	**1.19 (1.05-1.34)**	**1.21 (1.06-1.37)**[a]
Carbohydrate patterns			
'Afternoon'	**1.11 (1.01-1.22)**	1.20 (0.99-1.46)	1.19 (0.97-1.46)[a]
'Main Meals'	0.93 (0.85-1.02)	0.98 (0.86-1.12)	0.94 (0.81.1.08)[a]
'Morning'	1.04 (0.95-1.14)	1.06 (0.94-1.19)	1.02 (0.90-1.16)[a]
Protein patterns			
'Main Meals'	1.03 (0.94-1.13)	0.93 (0.81-1.08)	0.94 (0.80-1.09)[b]
'Lunch'	**1.13 (1.03-1.24**)	1.10 (0.98-1.24)	1.11 (0.98-1.27)[b]
Fat patterns			
'Main Meals'	1.02 (0.93-1.12)	0.96 (0.81-1.13)	0.94 (0.78-1.13)[b]
'Lunch'	**1.12 (1.02-1.23)**	**1.17 (1.03-1.32)**	**1.19 (1.05-1.36)**[b]

Model 0: crude model;

Model 1: adjusted for maternal age and education, children's body mass index (BMI), number of eating occasions , and total daily energy intake (except energy patterns) at 4 years

Model 2: Model 1 plus CEBQ sub-dimensions; [a]SR and EF; [b]SR and FR

GENERAL DISCUSSION AND CONCLUSIONS

In this thesis, we examined the tracking of different dimensions of eating habits (diet quality, diet variety and eating frequency) from preschool to school age and its association with children's eating behaviours related to appetite and with their weight status. We also evaluated methodological issues associated with the collection of children's dietary data and how early life factors influence the acquisition of eating habits in childhood. We used data from two large well-designed epidemiologic studies: the population-based birth cohort Generation XXI (G21) and the most recent National Food, Nutrition and Physical Activity Survey of the Portuguese general population (IAN-AF).

In order to improve children's eating habits, it is fundamental to have accurate data on their current consumption. Additionally, the use of methods with low validity seriously attenuates (or incorrectly reverse) the associations between dietary intakes and outcomes in health (267). The development of validated age-specific instrument is a cornerstone in the evaluation of usual food and nutrient intake.

Considering this, we evaluated the performance of a short non-quantitative food frequency questionnaire (FFQ) and tested a new method to convert the food frequency into food quantity in grams and subsequent conversion into nutrients (z-score calibration) at both 4 and 7 years of age [paper I]. We then compared the dietary results – grams of food and nutrients - obtained using the z-score method and using the standard method (frequency*standard portion) with 3-day food diaries (FD) and key serum biomarkers. In general, the mean daily food intake estimated by the z-score method had a higher agreement with the FD, than the standard method. Significant correlation coefficients were observed for energy intake and all nutrients evaluated (n=19) and the correlation coefficients' mean was 0.39 at 4 years and 0.42 at 7 years of age. The classical method of converting an FFQ, using specific standard portions equal to all participants, showed an overall overestimation of dietary intake, comparing to the FD. There is extend literature describing a general trend of the FFQ's dietary intake overestimation, in comparison with other dietary methods (80-84). This trend can be partly explained by the inclusion of a large number of foods in the FFQ, or by an inaccurate report of the frequency of more often consumed foods. Another hypothesis, supported by our results, is the use of portion sizes incorrectly high or that increasing frequency could result in decreasing the portion size. Previously, it was described an effective self-control in young children's energy intake as children adjust their portion sizes depending on the daily number of eating occasions (85). On the other hand, the caregiver could execute this control by decreasing the portion size given to the child with increasing eating frequency.

Our results suggest that the FFQ used to evaluate the dietary intake of G21 children aged 4 and 7 years performed well and it seems to be a useful instrument for evaluating a wide range of food groups and key nutrient intake in these children. The results also support that adjusting the portion size when converting an FFQ, by using a z-score method, increase the accuracy of dietary data in young children. The inclusion of FD in a subsample in future studies assessing dietary intake through FFQ will be an advantage, as these results showed that a calibration of the FFQ is useful, decreasing the exposure measurement error.

Using dietary data collected by different instruments (FFQ and FD) and applying distinct approaches we found some level of stability of eating habits throughout childhood, however, there was still scope for changes in children's eating behaviours.

From 2 to 4 years, a positive tracking was found for the consumption of energy-dense foods (EDF) [paper II]. The consumption of EDF increases from 2 to 4 years of age and a higher consumption at 2 years was associated with a higher consumption at 4 years. An important result was the association of a higher consumption of EDF at 2 years with lower intakes of F&V at 4 years of age (but not with other food groups) and consequently with lower scores in the healthy eating index. These results raise special concern since diet quality scores are inversely related to health outcomes (268). Children consuming soft drinks more often at 2 years of age were approximately three times more likely to consume soft drinks at 4 years of age, and this was the strongest association found. This finding deserves special attention, as SSB have been reported to be significantly associated with childhood obesity due to both a high glycaemic index and a weak compensatory response to beverages (269).

The development of a healthy eating index to summarise the overall diet quality of children represents a complementary approach to the study of single foods and accounts for cumulative and interactive effects of nutrients and foods. Thus, may provide a more comprehensive approach. The same healthy eating index was adapted and used to evaluate the tracking of children's dietary intake from 4 to 7 years of age [paper III]. Even though having a better diet quality influence positively later consumption, some negative changes occurred, namely, the increase of sweets and soft drinks, matching a decrease in consumption of dairy. These trends are in accordance with the increase of westernized diets occurring in the modern societies (30, 31). While the consumption of fruit and vegetables remain the same between 4 and 7 years of age, less than half of the children were consuming the recommended five portions a day. It seems there is a trend of increase in the consumption of foods high in sugar and energy (soft drinks and sweet snacks) throughout childhood, as our results at 2, 4 and 7 years confirm it [paper II, III]. Attention should be given to this trend since the consumption of EDF is associated with adverse health outcomes, namely a higher risk of obesity in both adults and children (270-274).

The maintenance of a healthy diet in childhood was associated with a better socioeconomic context and with maternal behaviours [paper III]. Higher maternal education and a better maternal diet quality were independently associated with a higher adherence to a healthy eating index score at 7 years. These results are in agreement with previous research, that shows that healthier eating habits among pre-school and school children are associated with higher maternal education (49, 275-279). Low maternal levels of education increase the probability of a poorer food quality diet in children, as this socio-demographic condition probably limits the purchasing power and the ability to obtain adequate nutritional information (275). This association is due to the fact that parents are important role models for children's dietary intake. There is considerable evidence that children's eating behaviours, including food preferences, are very similar to that of their parents (46, 280). Family factors play an important role in affecting children's eating behaviours, which was partly reflected in the resemblance in diets between parents and their children, as described in our research. In that way, a better maternal dietary intake seems to influence positively the children's diet. These findings were consistent with other studies (48, 281). The correlation between parent-child dietary intakes may be due to shared genetics and environments. A previous study also showed that younger mothers, less educated and with a lower income are more likely to provide 4-year-old children with an obesogenic home environment (282).

The contribution of genetic factors to individual differences in food intake has been described in studies among twin adults (283-285). One study among UK 4-5-year-old twins assessed the heritability of food preferences, ranging from 20% for dessert to 78% for meat and fish (286). Among 3-year-old children, the genetic influence for food preferences was higher for vegetables (54%) and protein (48%), but lower for starches (32%), snacks (29%) and dairy (27%) (287). Another study, based on one day 24h dietary recall, found also some degree of genetic effect in US twins aged 7 years, varying by food groups and sex (288).

Maternal behaviours during pregnancy and during the first year of child's life may influence the development of the child's dietary intake. In particular, the fetal and newborn environment may alter the development of appetite and satiety pathways (289). In fact, children whose mothers smoked during pregnancy are more likely to be overweight throughout their lives (290). Nicotine can have a profound impact on the developing fetal brain and has been linked to abnormal hypothalamic gene expression of appetite regulators (291). These modifications on the development of appetite and satiety mechanism could lead to unhealthy eating behaviours (such as preferences for junk food) in the offspring. Breastfeeding is also one of the factors that play a significant role in establishing long-term child's eating behaviours (65), and its duration is inversely associated with the child's risk of being overweight later (292). Results from the European Habeat project, that used data from four European cohorts (EDEN, ALSPAC, G21 and EuroPrevall) described that the breastfeeding duration was positively associated with later F&V intake in two of the four cohorts but not consistently in other two (EuroPrevall and G21) (66, 67). These mixed results could be partly explained due to the great differences across the European countries in early feeding practices and in F&V's intake in pre-schoolers; G21 had the longer breastfeeding duration and higher intake of F&V at 4 years (66).

Nevertheless, the results from this thesis found no significant associations between diet quality at 7 years and early life factors, namely pregnancy characteristics, child's birth weight or duration of breastfeeding [paper III]. In this age, the socio-economic context, as well as maternal behaviours seems to be the main determinants of children's diet quality. Previous results from the Gemini twin birth cohort among young children also described the shared environmental influences as the predominant determinants of children's dietary intake (293).

Parents and caregivers are important targets for education and interventions to improve the diets of children and our results highlighted the importance of specific interventions toward food education for less educated mothers, encouraging them to adopt healthy eating behaviours (280, 294, 295).

In this thesis, we also evaluated tracking of other dimensions of eating habits, such as the consumption of a variety of healthy foods in childhood [paper IV]. From 4 to 7 years, a higher varied diet predicted a fair to moderate tracking, with a high tracking of F&V consumption. To the extent of our knowledge (131), this was the first study analysing the tracking of the overall food variety throughout childhood, using the same validated instrument. Although a certain level of tracking was observed there was a general trend for a decrease in diet variety over time. Further research is warranted to test whether this trend for a decrease in diet variety extends into late childhood and transition to adolescence. Our results are in accordance with a previous study, thus early food variety is a predictor of later food variety in childhood (296).

Multi-level models of eating behaviours include genetic, biological, behavioural, psychological and environmental factors. Although the focus of this thesis was on the behavioural level, biological factors underlining these behaviours can also be pointed out. For example, traits associated with perceived fullness (e.g. 'Satiety Responsiveness') reflect the homeostatic appetite control system that regulates satiety and hunger according to energy levels. If a children's satiety response is weak following food consumption, then their risk for overconsumption is higher. On the other hand, the tendency to eat more in response to sight or smell of palatable food (e.g. 'Food Responsiveness') is governed by the hedonic appetite control system.

Obesity has been recognized as a heritable psychobiological phenotype caused by an interaction between genetic vulnerability and an obesogenic environment (297). Interestingly, in similar environmental conditions, not all individuals become obese; some remain lean despite increased food intake. Differences in psychological traits affecting eating behaviours may explain a greater predisposition or resistance to weight gain observed among individuals (297). Several authors have found that children's BMI was positively associated with dimensions of children's eating behaviours such as food responsiveness and enjoyment of food and were inversely associated with satiety responsiveness and slowness in eating (237, 238, 298), while others have reported no association (299-301).

Eating behaviours related to appetite show moderate tracking from early to late childhood, indicating that they are relatively stable traits, however, it was also described that appetitive traits related to higher satiety (satiety responsiveness, slowness in eating) decrease as children get older, while those associated with food responsiveness and up-regulation of eating tend to increase (302). The small-to-moderate changes in the magnitude of these traits throughout childhood could reflect changes in the food environment that children are exposed. The results of this thesis support that hypothesis, as a higher diet variety from 4 to 7 years was associated with higher scores in enjoyment of food and lower scores in food fussiness and desire to drink at 7 years [paper IV]. These aspects were also previously explored in younger ages: a beneficial effect was reported for an early introduction of vegetables (on complementary feeding period) on fussy eating behaviours around 4 years of age (246, 247).

Understanding the development of less healthy eating behaviours related to appetite, such as a more fussiness behaviour, that might put these children at risk of an unbalanced diet will help parents to adopt feeding practices in a way that fosters healthier eating behaviours. The assessment of appetitive traits in childhood could help identifying children that would benefit most from practical and targeted interventions to promote healthy diets in early life.

We have explored the influence of eating frequency on children's appetite and weight status using different definitions [paper V, VI]. Using data from 3-day food diaries, we defined 'eating occasion' as any occasion when food or drink is consumed with 30 min apart and provided a minimum of 210kJ (50 kcal) of energy (neutral approach) [paper V]. One of the advantages of using a neutral approach is that it can be standardized and comparable over time and across different studies. Afterwards, a time-of-day approach was used to define a 'main meal' and a 'snack' event. Main meal was defined as the eating occasion providing more energy content between 6 am and 9.30 am (breakfast), between 11 am and 2.30 pm (lunch) and between 7 and 9.30 pm (dinner). All other eating occasions were considered as snacks events. One of the limitations of using a time-of-day approach is that it does not capture unusual time of feeding, such as among shift workers, however, as we evaluated eating frequency of young children, a more typical pattern of feeding is likely to occur. Another limitation is the use of periods influenced by local or cultural factors, which might hamper comparison across different studies.

Using data from Portuguese 3-9-year-old children, we tested the 'participant-identified' approach to define an 'eating occasion' [paper VI]. The parents or another main caregiver identified 12 predefined food consumption occasions (ranging from 'before breakfast' to 'during evening/at night'). All eating occasion reported in the food diaries were considered as separated intake occasions if the time of eating was different from another eating occasion and providing at least 210Kj (50kcal) of energy. However, the differentiation between 'main meal' and 'snacks' events was performed a *posteriori* by the researchers: the main meals were defined as breakfast, lunch or dinner (respondents could only select one of each) and all the other eating occasions were classified as snacks. With this methodological decision, we were able to overcome some limitations of the 'participant-identified' approach, such as participants' subjectivity of what constitutes a main meal and what constitutes a snack.

Using the neutral definition we obtained a daily frequency of eating occasions ranging from 4 to 11 at 4 years of age (mean=6.5) and from 4 to 9 at 7 years of age (mean=5.8). Using the 'participant-identified' definition, children aged 3-9 years were having between 2 to 11 eating occasions daily (mean=5.8). The eating frequency obtained using different definitions seems to be similar, and the differences in the minimum interval might be due to the inclusion of older children (up to 9 years), as children seem to decrease eating frequency as their get older. Moreover, comparing the time-frames of the main meals using the two approaches (´time-of-day' and 'participant-identified') and, despite the 'time-of-day' approach had a larger interval (G21), all periods of time described in the 'participant-identified' (IAN-AF) were included in the 'time-of-day' approach definition (G21). This might indicate that our 'time-of-day' approach was successful in identifying the meal with higher energy content in these periods of time.

Our results suggest that eating frequency is somewhat stable during childhood as a moderate correlation between the number of eating occasions at 4 and 7 years of age was described [paper V]. In addition, using a prospective analysis, a bidirectional relationship was described between eating frequency and children's eating behaviours related to appetite. The stronger association found was between snack frequency at 4 years and the eating behaviours at 7 years of age. These results add relevant knowledge to previous gaps in the literature, as the majority of previous studies assume that the relationship is unidirectional, eating behaviours influencing eating frequency. A recent study also showed that other factors, as children's body composition, could influence appetitive traits over time (303). Our results showed that a higher frequency of main meals in preschool age was associated with higher scores in food-approach traits (e.g. 'Enjoyment of Food' and 'Food Responsiveness') a few years later, while a higher frequency of snacks was associated with food-avoidance traits (e.g. 'Satiety Responsiveness' and 'Slowness in Eating').

As a higher frequency of daily snacks enhanced scores in food avoidance traits, this might protect these children from developing overweight or obesity. Our results also showed that in Portuguese children, and considering only plausible reporters of energy intake, a lower daily frequency of snacks was positively associated with children being overweight or obese [paper VI]. Regardless of the results, the quality of snacks should not be neglected. We have compared the macronutrient composition of the main meals and the snacks events. Snacks were proportionally richer in carbohydrates and contained a lower percentage of fats and proteins than main meals. Comparing to the dietary recommendation for macronutrient in children (4-18y) (166) the snacks events are closer to the recommendations. It seems that, at least, in Portugal, the foods given to children between meals are healthier compared to other industrialized countries, such as the USA.

As the variety and the quality of the children's diet are important factors of the children's food environment, we hypothesized that the dietary variety could modify the relationship between the eating frequency and eating behaviours related to appetite. The healthy diet variety index calculated to assess children's diet variety was included in the analysis, but no significant interaction was found in the association between eating frequency and children's eating behaviours.
Another aspect that might impact on appetite control and weight status is the time of feeding. Experimental studies show that when animals eat at different times than the normal they become obese, despite being fed the same diet (304, 305).

The circadian rhythm controls many physiological functions, such as feeding, motor activity, endocrine secretion and autonomic nerve, and is governed by the hypothalamus, through the suprachiasmatic nucleus (SCN) (306). The SCN receives light-dark information through the retinal-hypothalamic tract and organizes the peripheral clocks located in other parts of the body, such as pancreas, liver and adipose tissue (307). Food is one potent external synchronizer of the peripheral clocks (306). A regular food intake pattern can entrain the peripheral circadian clock, whereas peripheral clock systems can control the absorption distribution, metabolism and excretion of nutrients, suggesting mutual interactions between circadian clocks and nutrition/food. For instance, an unusual time of feeding might over-ride the SCN so that the peripheral clock becomes desynchronized from the master clock. This chrono-disruption has been hypothesized to lead to adverse health effects, through a disruption of homeostasis, disturbance in leptin response, glucose and energy metabolism, and in insulin sensitivity (308, 309). Hence, poor eating habits, including unusual time of eating, can disrupt the clock system, increasing the risk of development of disease such as obesity or diabetes (308, 309).

Our results showed that indeed, the time-of-day of macronutrient and energy intake impacts on the development of overweight and obesity in children, independently of the effect on children's appetitive behaviours [paper VI]. This was the first study assessing patterns of energy and macronutrient distribution throughout the day and its influence on children's appetite and the likelihood of developing excessive body weight. Similar, a previous study showed that the timing of the main meal (lunch time before or after 3:00 pm) in a Mediterranean population influenced the weight loss during a 20-week dietary intervention in overweight or obese adults (310). Late lunch eaters lost less weight, independently of the total energy intake, dietary composition, estimated energy expenditure, appetite hormones and sleep duration (310). The mechanism linking meal timing and development of obesity (or weight loss) may be in part explained by the involvement of satiety hormones. Changes in the levels of circulating of leptin or ghrelin, by circadian disruption could influence energy intake and expenditure. However, as in the intervention study among Mediterranean adults, our results have shown an effect of the time-of-day of energy and fat on adiposity independently of the appetite control (using eating behaviours as proxies). Changes in the chronotype, genetic background and/or circadian system function may be implicated in these associations (311).

The results from this thesis increase the understanding of the potential importance of the circadian rhythm of food intake on childhood overweight/obesity development. Although the mechanisms associated with meal timing and weight regulation are not fully understood yet, our results suggest that new therapeutic strategies, as well as preventive measures, should consider not only the nutrient composition of the diet but also the timing of feeding.

While some children's appetitive traits, such as 'Food Fussiness' and 'Satiety Responsiveness' may appear to be protective against excessive caloric intake and subsequent weight gain, a nutritionally adequate and well-balanced diet should not be compromised. For example, previous studies have described a link between higher scores in 'Satiety Responsiveness' and 'Slowness in Eating' and a higher consumption of sweets (242) and a lower preference for F&V (243). Children with higher scores in 'Food Fussiness' sub-dimension, characterized by a picky eating behaviour, tend to accept only a limited number of foods which might compromise their nutrition status (199-201). On the other hand, higher scores in 'Enjoyment of Food' (a food-approach trait associated with weight) have been associated with higher intake of F&V (242, 243).

Moreover, the comparison between the means scores obtained in each of the sub-dimensions of CEBQ in G21 7-year-old children with the scores obtained in other studies has shown a rather different range of values. Taking the example of two traits associated with a lower body weight, 'Satiety Responsiveness' and 'Slowness in Eating', we obtained a mean of 2.7 and 2.9 respectively. Previous studies found similar values in underweight 10-year-old children (312) and low-normal BMI in 8-11-y-old (241). Also, another trait associated with higher weight but also with higher intake of F&V (242, 243), 'Enjoyment of Food', obtained a mean value of 3.0 in our sample of 7-year-old children far below the mean score of 4 described in low-normal BMI 8-11-y-old children (241). In particular, in our sample, practices that decrease food-avoidance scores and increase food-approach scores might be encouraged, such as increasing diet variety, higher frequency of main meals, and a higher proportion of energy and macronutrients at the main meals and a lower proportion in the remaining eating occasions.

Strengths and limitations

Major strengths of this thesis include the population-based design with detailed children's dietary habits, the age frame considered and objective measures of children's weight and height. Children from G21 are part of a population-based birth cohort regularly followed, which has enabled to study our hypothesis by using a longitudinal approach. In addition, the IAN-AF study, despite its cross-sectional nature, allowed us also to have updated data representative of Portuguese children, following harmonized European methodology. Moreover, several important factors besides eating habits were evaluated, such as socioeconomic circumstances, the practice of structured physical activity and sedentary lifestyle variables, allowing the adjustment for several potential confounding factors.

Nonetheless, potential limitations of the present research exist. The inclusion of a subsample of the G21 cohort may have introduced some selection bias. Comparison of baseline characteristics with the remaining cohort showed that mothers included in our sample were slightly older and more educated. However, given that Cohen's effect size values are not high (<0.35), these differences are more likely to be due to large sample size rather than to substantial differences between participants.

A social desirability bias may also have occurred, resulting in an over-reporting of healthy foods and/or under-reporting of unhealthy foods. Dietary intake was assessed either in face-to-face interviews (FFQ) or by food diaries completed by parents or another main caregiver, who might not be aware of all foods children have. The recorded consumption could reflect attitudes about what should be consumed as opposed to what was really consumed. If a lower report of less healthy foods and/or a higher report of more healthy foods have occurred, our associations could be underestimated and be even stronger.

Misreporting of food consumption is a possible limitation that needs to be addressed. Underreport of dietary intake among overweight or obese people is largely described in the literature (191, 192), and might affect the association between eating habits and children's weight status. We have performed a sensitivity analysis including all children and then including only plausible reporters of energy intake for the association between eating frequency and BMI [paper VI]. In the analysis including only plausible reporters, the association between the number of main meals and children's BMI ceased to be statistically significant. This effect might be due to a loss of power, as we excluded some children, and not due to a bias regarding the dietary report. Moreover, this bias might have been minimized, as the parents were the ones reporting the dietary intake of the children, and not the children themselves. Literature has reported a mismatching in mothers' perception regarding their children's weight. For example, in a cohort study among children aged 6 to 8 years, the majority of mothers misclassified the child's weight and had the tendency to normalize their weight status (313). Mothers were also poor at recognizing overweight and obesity in their children at 4 or 6 years (314). This phenomenon was also described in mothers of pre-school children, who failed to perceive their overweight child as different from their peers (315). As parents might fail to recognize their child as overweight and obese, it is not expected that they change their report based on the child's weight status. Additionally, there is no evidence of a misreporting depending on the time of eating.

Another limitation of our research is the cross-sectional nature of the study assessing the association between eating frequency and obesity, which does not allow assessing a clear temporal relationship. Moreover, we could not exclude a potential reverse causality effect, since obese people could change the frequency of meals in an attempt to lose weight. However, this issue is more likely to occur in adults than in children.

Concluding remarks

Overall, some level of tracking of children's eating habits was observed. The continuity of these habits can be assumed as a positive, as in the case of diet quality and diet variety, but also as a negative behaviour regarding energy-dense foods tracking. Eating habits, evaluated through different dimensions, emerge early in the development pathway and show levels of individual stability. The socioeconomic circumstances as well as healthy maternal behaviours strongly influence the establishment of healthy eating behaviours since early ages and their maintenance throughout childhood. From 4 to 7 years of age, maintain or increase a high diet variety, higher frequency of eating occasion, and a higher proportion of energy and macronutrients at the main meals, were associated with healthier eating behaviours related to appetite, namely lower scores in desire to drink, food fussiness and higher scores in enjoyment of food. The beneficial effect of a higher intake of energy and macronutrients at main meals on children's weight status seems to be independent of the effects on appetite control.

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