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

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Abbreviations

EF, Eating Frequency; CEBQ, Children's Eating Behaviour Questionnaire

The examination of specific characteristics of eating habits in childhood and its influence on eating behaviours is needed to better understand the establishment of appetite-related eating behaviours. This study aimed to assess the association between eating frequency (EF) (main meals vs. snacks) at 4 and 7y and problematic eating behaviours and appetite-related eating behaviours at 7y. The analysis included 1359 children from the birth cohort Generation XXI who provided 3-day food diaries, at both ages, and complete information on the Children's Eating Behaviour Questionnaire, at 7y. Problematic eating behaviours were assessed using parental perception of child's poor eating and respective level of concern (including not eating enough, eating too slow or overeating) at both ages. 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 parental concerns regarding problematic eating behaviours. Associations between EF and appetite-related eating behaviours were evaluated through linear regression models. Between 4 and 7y a moderate tracking of EF was described as well as a strong tracking of parental concerns. We found a bidirectional relationship between EF and parental concerns. Prospectively, a higher frequency of snacks at 4y was independently associated with higher scores in 'Desire to Drink' at 7y (β=0.051, 95%CI: 0.009;0.093). At 7y, a higher EF had a positive association with 'Satiety Responsiveness' but an inverse association with 'Enjoyment of Food' and 'Food Responsiveness'. A higher number of main meals had a positive association with 'Enjoyment of Food' and 'Food Responsiveness'. In conclusion, children who had more eating occasions seem to have more food-avoidance behaviours, traits that might protect these children from gaining excessive weight.

1. 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].

The prevalence of problem eating behaviours in childhood is increasing, ranging from picky eating to overeating and disinhibited eating, including a high food responsiveness, avid appetite, rapid eating and eating without hunger, which ultimately can lead to excess weight gain [4-6]. Parents are frequently concerned about their child's picky eating behaviour, due to a possible dietary inadequacy, which in turn, might lead the parents to pressure the child to eat [7]. Feeding difficulties that arise early in childhood may persist as maladaptive eating later in life, compromising their future health [8].

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 a high satiety responsiveness showed a graded negative association with weight, while a high response to external food cues ('Food Responsiveness' subscale), was positively associated with children's weight [9-11]. Certain dietary components can influence mechanisms of appetite, namely those related to satiety [12, 13]. For example, dietary proteins induce satiety, increasing secretion of gastrointestinal hormones and diet-induced thermogenesis [14]. Previous evidence has suggested that characteristics of the meals, such as nutrient composition [15, 16] and frequency of meals [17-19], could influence outcomes in health, such as obesity, insulin resistance, and metabolic syndrome, independently of other risk factors. The examination of meal characteristics provides an interesting approach by expressing cumulative and interaction effects of food and meal frequency alongside nutrient intakes, overcoming the limitations of studying specific foods or nutrients [20].

Regular eating frequency could have a beneficial effect on metabolism, by increasing satiety and improving glucose and insulin metabolism [17, 21]. On the one hand, controlled feeding studies in adults [21] showed that reduced eating frequency negatively influence appetite control, increasing the perceived appetite and reducing the perceived satiety. On the other hand, increasing the number of daily meals might lead to a higher exposure to energy-dense foods, and large-portion-size foods, resulting in increased hunger, excess energy intake, and ultimately body weight gain [22, 23]. 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 [24], snacking events have been described as increasing in recent years, particularly among children [25, 26]. The literature describes a variety of approaches to define a snack, using, for example, the time of consumption, food groups or nutritional profile [27, 28]. Most associations found between eating habits and appetite-traits have focused on 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 [6, 29]. On the topic of eating frequency, a previous study [30] has described a positive association between higher scores for the 'Food Responsiveness' subscale at 16 months and frequency of meals at 21 months. However, these results cannot be generalized across childhood, as these relations could change as children get older. In another cross-sectional study [31] the authors described a positive association between a general interest in food and the daily number of main meals in 6-8-year-old children. These previous studies have focused on a unidirectional relationship between children's eating behaviours 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 eating behaviours, has not been studied to date. Therefore we aimed to assess the association between daily eating frequency (main meals vs. snacks) from pre-school to school-age and parental concerns regarding child's problematic eating behaviours, as well as, with appetite-related eating behaviours at 7 years of age.

2. Methods

2.1. Study design and participants

This study was conducted within Generation XXI, a population-based birth cohort that has been previously described [32]. 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. 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 were 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 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.

The present study included all 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 from the Child 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 z-score body mass index (BMI) and 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) years of age, $p<0.001$) and more educated (mean (SD): 11.7 (4.27) vs. 10.2 (4.21) years of education, $p<0.001$).

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 World Health Organization criteria [33].

2.2. Dietary intake

As previously described [34], 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, prior 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 [35]. For typically Portuguese foods or culinary dishes, new codes were created with national nutritional information. [34].

2.2.1.Meal definition

Based on the meal patterns reviewed by Leech et al [24], 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 [36] 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 the highest energy content (minimum of 210kj) was defined as the main meal and all other eating occasions separated by 30 minutes were considered as snacks.

Breakfast was defined as the eating occasion with the highest energy content between 6 am and 9.30 am. Lunch and dinner were considered as the eating occasion with the highest 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 percentage of eating occasions providing <210kj was 3.4 % at 4 years and 1.8% at 7 years of age.

2.3. Eating behaviours

A number of psychometric instruments were developed to evaluate eating behaviour in children, including the CEBQ [37]. 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 [38]. This instrument is a parent-report questionnaire that measures appetitive traits in children. The subscales '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. The original item number 3 (belonging to the subscale SR) and item number 4 (belonging to the subscales 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 subscale.

Data on parental concerns regarding children's problematic eating behaviours were collected at both 4 and 7 years of age through a questionnaire based on a previous one developed by the Avon Longitudinal Study of Parents and Children (ALSPAC) study [39] . Caregivers (94% were mothers), were asked to rate specific perceived eating problems observed in their child on a four-point Likert scale. 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 occurred during the previous year and their level of concern (very concerned, somewhat concerned, no concern). These questions were derived from some subscales of the CEBQ, namely FF, SE and FR. The point-biserial correlations between the three questions and the corresponding subscales in the original CEBQ were moderate to high [5]. As used before [40], data on parental concerns were included as potential confounders of the association between eating frequency and subscales of the CEBQ at 7 years.

2.4. Statistical analysis

Continuous variables were summarized by means and standard deviations (SD) and categorical variables by proportions. Mean (SD) and frequency differences were compared through the paired sample t-test (for continuous variables) and McNemar test (or the marginal homogeneity test) (for categorical variables).

Parental perception about and concern for their child's problem eating comprised the questions "my child does not eat enough", "my child eats very slowly" and "my child eats too much" (including the four original responses), and was used as an indicator of a higher concern of poor eating at both ages. A higher score in the variable parental concern represents a higher concern for the child not eating enough or eating slowly, and lower concern for the child eating too much. A cross-lagged panel design analysis [41] was performed using the overall parental concern regarding problematic eating behaviour variable 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 snack frequency, and parental concern across the two periods of time; and the cross-lagged analysis of eating frequency at 4 years on parental concern at 7 years, as well as of parental concern at 4 years on eating frequency at 7 years. Mean eating frequency and parental concern 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 eating frequency of the same eating occasion (main meal or snack) 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 z-score 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 children's sex, and between eating frequency and diet variety (previously developed for these children, based on food frequency data [40]) 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 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.

3. Results

3.1. Participants and meal characteristics

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 respective mothers. Almost half of the sample (47%) were 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 occasions 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 regarding children's problematic eating behaviours no statistical differences were found

between the two periods of time, with the exception of an increase in concern regarding the child eating too much, from 4 to 7 years (somewhat concerned/very concerned: 8.3% vs. 14.9%, p<0.001, respectively).

3.2. Parental concerns regarding problematic eating behaviours/CEBQ

The mean scores in the CEBQ subscales 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 regarding children's problematic eating behaviours and scores for the CEBQ subscales at 7 years (Table 2). For example, children with the higher scores for 'Slowness in Eating' were the ones with a higher level of parental concern of 'eating too slow'. Parents who were more concerned with 'eating too much' had children with higher scores for 'Enjoyment of Food' and 'Food Responsiveness' but lower scores for ''Satiety Responsiveness' and 'Slowness in Eating' (Table 2).

In order to explore the direction of the associations between parental concerns regarding children's problematic eating behaviours 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 statistically significant (Figure 1). Problematic eating behaviours as perceived by the parents at 4 years had a strong association with parental concerns regarding problematic eating behaviours at 7 years ($\beta_{standardized}$=0.779; p<0.001). The cross-lagged association showed a positive bidirectional relationship between eating frequency and parental concerns regarding children's problematic eating behaviours. A higher level of concern with not eating enough or slow eating at 4 years was positively associated with snack frequency at 7 years ($\beta_{standardized}$=0.081; p=0.026), but not with main meal frequency ($\beta_{standardized}$=0.039; p=0.321). The strongest direction found was for snack frequency linked to parental concern ($\beta_{standardized}$=0.108; p=0.002) (Figure 1).

3.3. Prospective analysis

Total eating frequency at 4 years was not significantly associated with 'Satiety Responsiveness', 'Enjoyment of Food', 'Food Responsiveness' or 'Slowness in Eating' (Table 3). Similar to a higher eating frequency, 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=0.079, 95% confidence interval: 0.038; 0.119) and independently of other potential confounders (Model 2, β=0.051, 95%CI: 0.009; 0.093) (Table 3). After adjustment, no association

was found between a higher frequency of daily main meals at 4 years and children's appetite at 7 years (Supplementary Table 1).

3.4. Cross-sectional analysis

In the cross-sectional analysis at 7 years, and after multivariate adjustment, a higher eating frequency and a higher number of daily snacks had a statistically significant positive relationship with 'Satiety Responsiveness' but inversely associated with 'Enjoyment of Food' (Table 3, model 2). A higher number of daily snacks at 7 years was independently and positively associated with 'Slowness in Eating' (β=0.056, 95%CI: 0.001; 0.112) only after adjustment for energy intake (Table 3, model 3). The additional adjustment for total energy intake (model 3) did not change significantly the other association described between eating frequency and children's eating behaviours. A higher frequency of daily main meals had a statistically significant positive relationship with 'Enjoyment of Food' and 'Food Responsiveness' [Supplementary Table 1].

4. Discussion

The study assessed cross-sectional and prospectively the association between eating frequency (main meal and snack frequency) and children's eating behaviours. 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

The prevalence of problematic behaviours perceived by the parents (defined as any behaviours reported, independently of the level of concern) ranged from 12% to 51% at 4 years and between 19% and 52% at 7 years of age. Previous studies have found similar prevalence rates, ranging between 3 to 66% [4-6]. The present study indicated also that the parental perception of problematic behaviours increased as the child gets older. This might indicate that parents might start to be more aware of children's weight (prevalence of overweight has increased from 31% to 37%) and consequently more preoccupied with over-eating (has increased from 12% to 19%). Indeed, feeding problems seem to persist throughout childhood as shown in the present study by the strong tracking of problematic eating behaviours between 4 and 7 years of age and as described in previous studies [4, 42]. This might have long-term effects on children's health, namely worse eating habits and unhealthy body weight later in life.

A bidirectional association between eating frequency and parental concern with child feeding difficulties was described for the first time in this study, as the majority of previous studies

assume that the relationship is unidirectional, eating behaviours influencing eating frequency. This supports the proposal that parents are aware of their children's eating behaviours as they grow and then change their concerns (and consequently behaviours) accordingly. Indeed, children perceived by the parents as eating large amounts of food were more likely to be overweight or obese and their parents were more likely to report concern about children's eating behaviour and weight [5]. On the other hand, children who eat very slowly or small quantities were less likely to be overweight or obese and accordingly the parents were less likely to show concern about their weight [5]. Moreover, a moderate correlation was found between the number of eating occasions at 4 and 7 years of age, which suggests that eating frequency is somewhat stable during childhood.

An increasing number of daily snacks at 4 years was associated with higher scores for 'Desire to Drink' at 7 years, independently of the number of snacks eaten at 7 years. Previously, higher scores for 'Desire to Drink' was associated with a higher consumption of sugar-sweetened beverages in 11-year-old children [43] but not in younger children [31]. 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 skim milk [31]. 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 of age a higher eating frequency, and particularly a higher number of daily snacks had a positive association with scores for 'Satiety Responsiveness' and an inverse association with 'Enjoyment of Food'. In contrast, a higher number of daily main meals was positively associated with 'Enjoyment of Food' and 'Food Responsiveness'.

The positive link between higher eating frequency and the degree to which the child regulates food intake based on internal cues (assessed through the 'Satiety Responsiveness' subscale) might be explained partly by adopting a more regular eating pattern, compared to children with fewer eating occasions. A regular feeding pattern has been linked to increased internal satiety and improved glucose and insulin metabolism [17, 21]. Indeed, controlled feeding studies in adults [21] showed that a reduced eating frequency had a negative impact on appetite control, by increasing perceived appetite while reducing perceived satiety. In addition, children with a high sensitivity to internal cues of satiety might fill up quickly, requiring small amounts eaten during each occasion but eating more frequently.

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' [37]. In our analyses at 7 years, a higher eating frequency,

mainly through daily intake of snack, was linked to more food avoidance traits, while a higher frequency of main meals was linked to more food approach traits. Higher scores for food approach traits have been positively associated with children's weight, whereas more food avoidance traits have been negatively associated with children's weight [10]. Eating more frequently might protect these children from gaining excess weight. 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) [44] it seems that the nutritional profile of the children's diet was close to the recommendations. Also, young children seem to self-regulate their energy intake by adjusting their portion sizes, depending on the number of daily meals [45], meaning that a higher frequency of snacks in early life does not necessarily translate into an excess energy intake.

While higher scores for 'Satiety Responsiveness' and 'Slowness in Eating' have been linked with a lower risk of overweight [10], previous studies have found an association between higher scores for these eating behaviours and higher consumption of sweets [31] and lower preferences for vegetables and fruit [46]. Children with these appetitive traits may not only be consuming less in general, but also eating disproportionally fewer nutrient-dense foods.

A recent study [30] analysed the associations between eating behaviour related to appetite, such as 'Food Responsiveness' and 'Satiety Responsiveness', when the children were 16 months old, and they also assessed eating frequency and meal size at 21 months. Higher 'Food Responsiveness' was associated with greater frequency of meals, while lower 'Satiety Responsiveness' was associated with a larger meal size but not with eating frequency. 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 [31]. 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 for 'Enjoyment of Food'. Moreover, a higher interest for food in general ('Enjoyment of Food') have been associated with higher intake of fruit and vegetables whereas a higher responsiveness to external stimulus ('Food Responsiveness') have been associated with higher consumption of fruit but also with a higher liking for noncore foods [31, 46]. These children seem to have a diet which is close to the 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 [10], these children might benefit from interventions to avoid excessive food consumption, especially from larger meal sizes and more energy dense foods.

An earlier study has proposed that aspects of eating behaviour track throughout childhood [47]. 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 [6, 29].

Strengths and limitations of the present study deserve further discussion. We define the exposure, children's eating frequency, based on previously used approaches [24]. First, 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 distinguish 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) [48], may reduce caloric intake later in that day [49]. 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. 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.

Similar to other children feeding studies [4, 5, 50], we used parental report on infant feeding including eating habits and eating behaviours which might be prone to misreporting and social desirability bias. Food diaries were completed by parents or other main caregivers, who might not be with the child all day. However, parents were advised to obtain 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., foods high in energy density) and/or over report of more healthy foods (e.g., fruits and vegetables). Moreover, 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 three main meals. Children's eating behaviours were assessed subjectively by parental report; nevertheless, the CEBQ has shown good psychometric properties in this population [38] and good correspondence with objective measures [51]. The questions used to assess parental concerns regarding problematic eating behaviours were originally developed by the cohort within the Avon Longitudinal Study of Parents and Children ALSPAC [39] and several studies have used it to evaluated children's feeding difficulties [4, 5, 50, 52]. Moreover, these

questions have shown moderate-to-high correlations with the original subscales of the CEBQ, giving more robustness to the data regarding problematic eating behaviours [5]. Literature has reported a mismatching in mothers' perception regarding their children's weight [53, 54]. As parents might not recognize their child as overweight or obese, they might not be concerned about their child's tendency to overeat. In this case, this specific problematic eating behaviour might be underestimated in the present study; however, the parental concern about overeating increased from 4 to 7 years of age, concomitant with an increase of prevalence of children's overweight/obesity which might indicate an increase in parental awareness and concern about their children's problematic eating behaviours.

Another limitation of this study was the collection of the CEBQ data only at 7 years of age. However, the use of parental perception and concern with children's problematic eating behaviours allowed us to uncover a bidirectional association between eating frequency and parental concerns regarding problem eating. Moreover, these variables were included in the final models to adjust for previous eating problems.

In the present study, we assessed in a cross-sectional and longitudinal analysis the association between eating frequency and eating behaviours related to appetite, overcoming the limitations of previous cross-sectional studies. We also assessed the cross-sectional association of eating frequency and appetite at 7 years, adjusted for the previous eating frequency.

5. Conclusions

A moderate association between the number of eating occasions at 4 and 7 years of age was found. Although it was described a strong tracking of parental concerns regarding problematic eating behaviours between 4 and 7 years of age, there was an increase in their prevalence. A bidirectional association was described between eating frequency and parental concern between the two periods of time, with a stronger link between snack frequency at 4 years and parental concern regarding child's eating difficulties at 7 years. A higher eating frequency, including a higher number of daily snacks, was associated with food-avoidance traits. The food-avoidance behaviours identified, including a higher sensitivity to satiety signals and a healthy eating pace should be encouraged to prevent problematic eating behaviours without resulting in excess 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 eating behaviours.

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Potential conflict of interest and source of funding

The authors declare no conflicts of interest.

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Figure legends

Figure 1. Cross-lagged models for the associations between parental concerns regarding children's problematic eating behaviours and 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;

Table Legends

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

Table 2. Mean differences in subscales of the Child Eating Behaviour Questionnaire (CEBQ) by the parental level of concern with problematic eating behaviours at 7 years.

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

Supplementary Table 1. Associations between daily number of main meals at 4 and 7 years and eating behaviours related to appetite at 7 years of age.

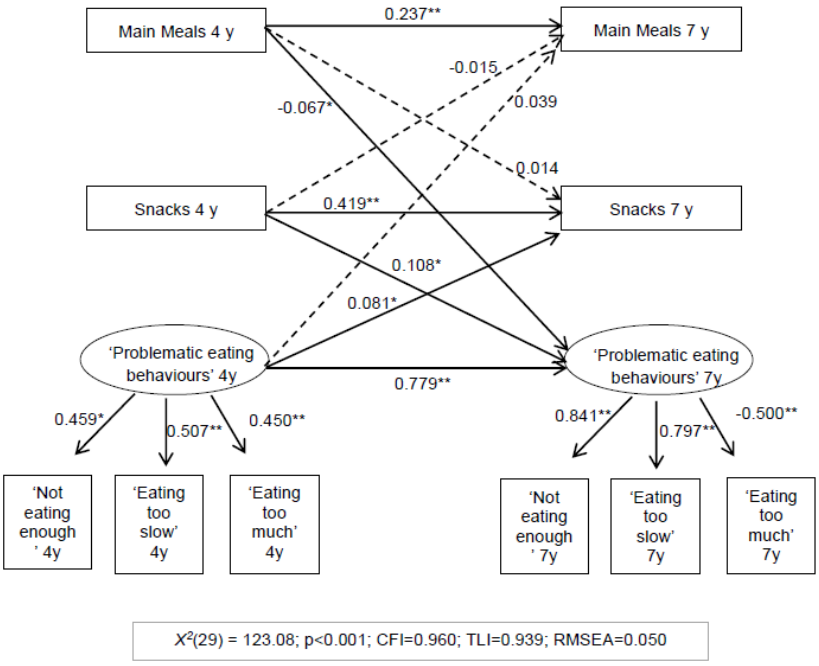
Main Meals 4 y
Main Meals 7 y
0.237**
-0.015
-0.067*
0.039
0.014
Snacks 4 y
Snacks 7 y
0.419**
0.108*
0.081*
'Problematic eating behaviours' 4y
'Problematic eating behaviours' 7y
0.779**
0.459*
0.507**
0.450**
0.841**
0.797**
-0.500**
'Not eating enough ' 4y
'Eating too slow' 4y
'Eating too much' 4y
'Not eating enough ' 7y
'Eating too slow' 7y
'Eating too much' 7y
$X^2(29) = 123.08$; p<0.001; CFI=0.960; TLI=0.939; RMSEA=0.050

Figure 1

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)		
Weight status, n (%)			
Normal weight	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 of problematic eating behaviours, 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 is 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 subscales of the Child Eating Behaviour Questionnaire (CEBQ) by the 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 between 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)					
EO (frequency/day)					
Model 0	0.020 (–0.011;0.052)	–0.005 (–0.041;0.031)	–0.007 (–0.041;0.027)	0.031 (–0.009;0.071)	**0.067 (0.031;0.104)**
Model 1	–0.001 (–0.035;0.033)	0.017 (–0.022;0.056)	0.006 (–0.032;0.043)	0.011 (–0.032;0.055)	**0.070 (0.030; 0.109)**
Model 2	0.015 (–0.016;0.047)	–0.006 (–0.042;0.030)	–0.004 (–0.039;0.030)	0.025 (–0.015;0.064)	**0.044 (0.003;0.085)**
Model 3	0.019 (–0.013;0.051)	–0.009 (–0.046;0.027)	–0.006 (–0.041;0.029)	0.026 (–0.013;0.066)	0.041 (0.000;0.082)
Snacks (frequency/day)					
Model 0	0.030 (–0.002;0.062)	–0.012 (–0.048;0.025)	–0.004 (–0.039;0.031)	0.040 (–0.001;0.080)	**0.078 (0.041;0.115)**
Model 1	0.004 (–0.031;0.039)	0.019 (–0.021;0.059)	0.014 (–0.024;0.053)	0.018 (–0.026;0.062)	**0.079 (0.038;0.119)**
Model 2	0.017 (–0.015;0.049)	–0.001 (–0.038;0.036)	0.004 (–0.032;0.039)	0.028 (–0.012; 0.068)	**0.051 (0.009; 0.093)**
Model 3	0.021 (–0.011; 0.054)	–0.006 (–0.043; 0.031)	0.002 (–0.034;0.037)	0.030 (–0.011; 0.070)	**0.048 (0.006;0.090)**
Eating frequency at 7y (cross-sectional analysis)					
EO (frequency/day)					
Model 0	**0.070 (0.029;0.111)**	**–0.061 (–0.109;–0.014)**	–0.039 (–0.084;0.007)	**0.070 (0.018;0.123)**	0.029 (–0.019;0.077)
Model 1	**0.070 (0.025;0.116)**	**–0.070 (–0.122;–0.019)**	–0.042 (–0.091;0.008)	**0.064 (0.007;0.122)**	–0.008 (–0.060;0.044)
Model 2	**0.044 (0.004;0.084)**	–0.039 (–0.085;0.008)	–0.005 (–0.050;0.039)	0.037 (–0.013;0.088)	–0.002 (–0.054,0.051)
Model 3	**0.068 (0.024;0.111)**	**–0.058 (–0.108; –0.009)**	–0.015 (–0.063;0.033)	0.047 (–0.008;0.101)	–0.002 (–0.077,0.036)
Snacks (frequency/day)					
Model 1	**0.085 (0.038;0.131)**	**–0.099 (–0.152; –0.045)**	**–0.061 (–0.112;–0.009)**	**0.069 (0.010;0.129)**	–0.002 (–0.057;0.052)
Model 2	**0.060 (0.018;0.101)**	**–0.071 (–0.119;–0.023)**	–0.027 (–0.073;0.019)	0.046 (–0.006;0.099)	0.000 (–0.054;0.055)
Model 3	**0.083 (0.039; 0.128)**	**–0.093 (–0.143;–0.042)**	–0.038 (–0.087;0.011)	**0.056 (0.001;0.112)**	–0.016 (–0.073;0.042)

95%CI, 95% confidence interval

Model 0: unadjusted; model 1: adjusted for the eating frequency of 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 z-score, 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

Supplementary Table 1. Associations between daily number of main meals 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)
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)

95%CI, 95% confidence interval

Model 0: unadjusted; model 1: adjusted for the eating frequency of 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 z-score, 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