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Determinants of dietary habits and adiposity during early childhood

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II. Durão C, Severo M, Oliveira A, Moreira P, Guerra A, Barros H, Lopes C. Association of maternal characteristics and behaviours with 4-year-old children's dietary patterns. *Matern Child Nutr* 2016 Apr 3. doi: 10.1111/mcn.12278. [Epub ahead of print]

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IV. Durão C, Severo M, Oliveira A, Moreira P, Guerra A, Barros H, Lopes C. Association between dietary patterns and adiposity from 4 to 7 years of age. [Submitted]

V. Durão C, Oliveira A, Santos AC, Severo M, Guerra A, Barros H, Lopes C. Protein intake and dietary glycemic load of 4-year-olds and association with adiposity and fasting serum insulin at 7 years of age: sex-nutrient and nutrient-nutrient interactions. [Submitted]

Esta tese foi realizada no Departamento de Epidemiologia Clínica, Medicina Preditiva e Saúde Pública da Faculdade de Medicina da Universidade do Porto e na Unidade de Investigação em Epidemiologia do Instituto de Saúde Pública da Universidade do Porto, sob orientação da Professora Doutora Carla Maria de Moura Lopes e coorientação do Professor Doutor António José Mónica da Silva Guerra.

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I hereby declare that I have contributed to this thesis by collection of part of the data and by handling the data collected. I also participated in the conceptualization of the research questions, performed statistical analysis and wrote the first draft of all manuscripts included. No part of this work has been submitted as application for other degree or qualification of this or any other university or institution of learning.

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I. Durão C, Andreozzi V, Oliveira A, Moreira P, Guerra A, Barros H, Lopes C. Maternal child-feeding practices and dietary inadequacy of 4-year-old children. *Appetite* 2015; 92: 15-23.

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ABSTRACT

Objectives

The general aim of this research was to examine determinants of children's dietary habits and, in turn, to evaluate the association between these habits and later adiposity. To accomplish this, five studies were performed answering to five specific objectives:

i) to estimate the association between maternal child-feeding practices and children's dietary inadequacy relatively to consumption of specific food groups;
ii) to examine the association of maternal socioeconomic, demographic and behavioral characteristics with children's dietary patterns;
iii) to assess the association of consumption of energy-dense micronutrient-poor foods and beverages (EDF) at 2 years with Body Mass Index (BMI) at 4 years;
iv) to evaluate the association of dietary patterns with adiposity from 4 to 7 years of age and to examine if child's sex modified this association;
v) to evaluate the association of protein intake and dietary glycemic load at 4 years with adiposity and serum insulin 3 years later, and to examine the possible interaction between protein intake and dietary glycemic load on these associations, by sex.

Participants and Methods

Participants are part of the population-based birth cohort Generation XXI (Porto, Portugal, 2005-2006). At 2 years of age, a subsample of 855 children was reevaluated, 621 of whom were also evaluated 2 years later. At 4 and 7 years of age, the entire cohort was invited to reevaluations with participation proportions of 86% and 80%, respectively. For families not able to participate in-person (20% at 4 years and 15% at 7 years), information was collected by telephone. Complete questionnaires on maternal child-feeding practices and 3-days food diaries were available for subsamples of children (*n*=4873 and *n*=2493, respectively). This study complied with the principles expressed in the Declaration of Helsinki and signed informed consent was required for all participants.

The main exposures of interest were determinants of children's dietary habits: maternal characteristics [socioeconomic position at mothers' 12 years of age, and demographic and socioeconomic characteristics at child's delivery (age, education, income, marital and working statuses)]; family characteristics (dimension, siblings and caregivers) at child's 4 years of age; and maternal behaviors (practice of physical exercise, smoking habits, diet, and child-feeding practices) at child's 4 years of age.

Different methodological approaches were used to assess children's dietary intake. At child's 2 years of age, a 17-items Food Frequency Questionnaire (FFQ) assessing consumption of EDF was used. At 4 and 7 years of age, the two FFQ aimed to assess overall diet. For the subsample of children whose diet was evaluated by 3-days food diaries, nutrient intake was estimated.

The child's diet was considered both as an exposure and as an outcome, using different approaches; specific food groups; *a posteriori* dietary patterns identified by Latent Class Analysis; and protein intake plus dietary glycemic load [both energy-adjusted by the residuals method and converted into sex-specific tertiles (T)].

Besides children's diet, the outcome of major interest was their adiposity. At 2, 4 and 7 years of age anthropometrics were evaluated by trained professionals. Age- and sex-specific BMI standard deviation scores (BMI z-scores) were defined according to the World Health Organization.

Outputs from tetrapolar bioelectric impedance were converted by Schaeffer's equation into fat-free mass, further used to calculate Fat Mass Percentage (FM%) and Fat Mass Index (FMI). Waist-to-Height (W/Ht) ratio was calculated as waist in cm divided by height in cm. These adiposity measures were converted into z-score units, using the sex-specific means and standard deviations observed in the current sample.

Fasting Serum Insulin (FSI) at child's 7 years was also considered. After an overnight fast, a venous blood sample was collected according to standard procedures. Insulin was measured using electrochemiluminescence immunoassay, was expressed in µUI/ml, further converted into z-scores using the sex-specific means and standard deviations observed in the present sample.

The association of maternal child-feeding practices with children's dietary inadequacy, and of family and maternal characteristics and behaviors with dietary patterns, were estimated by odds ratio (OR) and respective 95% confidence intervals (95%CI) derived from multinomial logistic regression models.

The association between EDF and BMI from 2 to 4 years of age was estimated by regression coefficients (β) and respective 95%CI obtained from both linear regression and cross-lagged panel design models. Linear regression models were also used to evaluate associations of children's diet (dietary patterns, protein intake, and dietary glycemic load) with adiposity from 4 to 7 years of age.

Results

Maternal restriction, monitoring, overt and covert control were associated with 11-18% lower odds of children consuming fruit and vegetables below the recommendations. Yet, overt control was also associated with 24% higher odds of children consuming these foods above the recommendations and pressure-to-eat was associated with 8% higher odds of dairy consumption above recommendations. With the exception of pressure-to-eat, maternal child-feeding practices were inversely associated with children's consumption of EDF.

Three dietary patterns were identified in children: high in EDF (named EDF dietary pattern); low in foods typically consumed at main meals and intermediate in snacks (named Snacking dietary pattern); higher in vegetables and fish and lower in unhealthier foods such as EDF (named Healthier dietary pattern, used as reference category in all analyses).

Lower socioeconomic position of the mother at 12 years of age had the overall effect of increasing the odds of children consuming unhealthier dietary patterns, and maternal education at child's delivery was directly inversely associated with the odds of children following these patterns. Children with only older siblings were more likely to follow a dietary pattern high in EDF, while those having only younger siblings and being cared by parents during day-time were more likely to follow the Snacking dietary pattern.

Two maternal child-feeding patterns (perceived monitoring and restriction) were inversely associated with children's EDF and Snacking dietary patterns. Children whose mothers had worse dietary quality (1^{st} *vs.* 4^{th} quartile of maternal dietary score) were significantly more likely to follow unhealthier patterns (EDF, OR=9.94, 95%CI 7.35-13.44, *p*-trend<0.001; Snacking, OR=4.21, 95%CI 2.94-6.05, *p*-trend<0.001). After considering upstream factors, maternal diet accounted for one-third of the determination coefficient of the fully adjusted model, and was the factor most strongly associated with children's diet.

Consumption of EDF at 2 years was significantly positively associated with consumption of the same foods at 4 years of age (β=0.522, 95%CI 0.432-0.612) and BMI z-scores tracked in time (β=0.747, 95%CI 0.688-0.806). In the cross-lagged analysis, consumption of EDF at 2 years was not associated with subsequent BMI z-scores. Likewise, the Snacking pattern at preschool age was not associated with later adiposity. Child's sex modified the association between diet and adiposity (*p*=0.046). The EDF pattern increased all adiposity indicators only in girls (BMI z-score, β=0.075, 95%CI 0.009-0.140; FMI z-score, β=0.071, 95%CI 0.000-0.142; and W/Ht ratio z-score, β=0.094, 95%CI 0.023-0.164).

Protein intake was positively associated with BMI z-scores in girls (T2 *vs.* T1, β=0.187, 95%CI 0.015-0.359) and boys (T3 *vs.* T1, β=0.205, 95%CI 0.003-0.406), being associated with fasting serum insulin z-scores only in boys (T3 *vs.* T1, β=0.207, 95%CI 0.011-0.404, *p*-interaction=0.026). Dietary glycemic load was also associated with BMI z-scores only in boys (T3 *vs.* T1, β=0.362, 95%CI 0.031-0.693, *p*-interaction=0.006). Also only in boys, significant interactions between dietary glycemic load and protein intake were found on the associations with FMI z-scores (*p*=0.019) and W/Ht ratio z-scores (*p*=0.039); boys in the 3rd tertile of both glycemic load and protein intake at 4 years of age, when compared to those in the 1st tertile for both dietary factors, had higher z-scores of FMI (β=0.505, 95%CI 0.085-0.925) and W/Ht ratio (β=0.428, 95%CI 0.022-0.834) at 7 years of age.

Main Conclusions

Socioeconomic position of the mother at 12 years of age indirectly increases the possibility of exposing the child to unhealthier dietary patterns and maternal education at child's delivery is directly associated with this possibility, findings that may reflect a transgenerational influence of less favorable socioeconomic conditions on children's diet.

Distinct family characteristics were associated with different dietary patterns; older siblings seem to act as models, increasing consumption of a pattern high in EDF, while having only younger siblings and being cared by parents during day-time both increase a pattern low in foods typically consumed at main meals and intermediate in snacks.

Some maternal child-feeding practices may be beneficial as they are inversely associated with children's unhealthier dietary habits or patterns. Maternal diet was a key factor to children's diet showing the highest magnitude of association with children's dietary patterns, above and beyond demographic and socioeconomic characteristics.

Child's sex seems to interact with dietary factors on the association between diet and adiposity. Early consumption of EDF tracks during early childhood and a dietary pattern high in this food group followed at 4 years increases later adiposity in girls. Protein intake is positively associated with BMI in both girls and boys but is associated with increased serum insulin only in boys. Also, dietary glycemic load is positively associated with adiposity only in boys, in whom it seems to interact with protein intake enhancing increased adiposity.

RESUMO

Objetivos

O objetivo geral desta dissertação foi o de examinar determinantes da alimentação da criança e avaliar a associação dos seus hábitos alimentares com posterior adiposidade. Desenvolveram-se cinco estudos com os seguintes objetivos específicos:

i) estimar a associação entre práticas maternas de controlo alimentar e inadequação alimentar da criança relativamente ao consumo de grupos alimentares específicos;
ii) examinar a associação de características familiares e maternas com os padrões alimentares da criança;
iii) avaliar a associação de alimentos e bebidas de elevada densidade energética e/ou baixa densidade nutricional (AEDE) com Índice de Massa Corporal (IMC) dos 2 para os 4 anos de idade;
iv) estimar a associação de padrões alimentares da criança com adiposidade dos 4 para os 7 anos de idade e avaliar se o sexo da criança modifica esta associação;
v) avaliar a associação da ingestão proteica e da carga glicémica alimentar aos 4 anos de idade com adiposidade e insulina sérica 3 anos mais tarde, e examinar a possível interação entre ingestão proteica e carga glicémica nestas associações, por sexo.

Participantes e Métodos

Os participantes integram a coorte Geração XXI (Porto, 2005-2006). Aos 2 anos, uma subamostra de 855 crianças foi reavaliada, 621 das quais também foram avaliadas aos 4 anos. Aos 4 e 7 anos, toda a coorte foi convidada para reavaliação (86% e 80% de participação, respetivamente) e famílias que não puderam comparecer presencialmente (20% aos 4 e 15% aos 7 anos) foram avaliadas por telefone. Questionários completos de práticas maternas de controlo alimentar (*n*=4873) e diários alimentares de 3 dias (*n*=2493) foram recolhidos para subamostras. O estudo obedeceu aos princípios da Declaração de Helsínquia e consentimento informado foi assinado por todos os participantes.

As principais exposições de interesse foram determinantes dos hábitos alimentares da criança: características maternas [posição socioeconómica da mãe aos 12 anos e características maternas no momento do parto (idade, escolaridade, rendimento, estado marital e ocupação)]; características familiares (dimensão do agregado, irmãos, cuidadores) aos 4 anos de idade da criança; e comportamentos maternos (exercício físico, tabagismo, alimentação, e práticas de controlo alimentar) aos 4 anos de idade da criança.

Diferentes metodologias foram utilizadas para avaliar a alimentação da criança. Aos 2 anos, foi usado um Questionário de Frequência Alimentar (QFA) de 17 itens com o intuito de avaliar o consumo de AEDE. Aos 4 e 7 anos, os QFA tiveram o objetivo de avaliar a globalidade da alimentação. Para a subamostra de crianças avaliada por diários alimentares, o aporte de energia e nutrientes foi derivado da média dos 3 dias de registo.

A alimentação da criança foi considerada como desfecho e exposição, usando-se diferentes abordagens na sua avaliação; grupos de alimentos; padrões alimentares identificados por modelos de análise de classes latentes; ingestão proteica e carga glicémica alimentar [ajustadas para a energia pelo método dos resíduos e categorizadas em tercis específicos para cada sexo (T)].

Para além da alimentação da criança, a sua adiposidade também foi considerada como desfecho de interesse. Aos 2, 4 e 7 anos de idade, profissionais treinados procederam à avaliação antropométrica. Desvios padrão de IMC específicos para sexo e idade (*z-scores* de IMC) foram definidos de acordo com a Organização Mundial de Saúde.

Os resultados da bioempedância tetrapolar foram convertidos pela equação de Schaeffer em massa magra, usada para calcular Percentagem de Massa Gorda (%MG) e Índice de Massa Gorda (IMG). Foi ainda considerada a razão Cintura/Altura (C/A). Estas medidas de adiposidade foram convertidas em *z-scores* usando as médias e desvios-padrão observados em cada sexo.

A insulina sérica em jejum aos 7 anos de idade da criança foi determinada por imunoensaio de eletroquimioluminescência, expressa em µUI/ml, sendo convertida em *z-scores*, usando as médias e desvios-padrão observados na amostra em cada sexo.

A associação entre práticas maternas de controlo alimentar e inadequação alimentar das crianças, bem como das características familiares e maternas com os padrões alimentares, foi estimada por *odds ratio* (OR) e respetivos intervalos de confiança a 95% (IC95%) obtidos de modelos de regressão logística multinomial.

A associação entre AEDE e IMC dos 2 para os 4 anos de idade foi avaliada por coeficientes de regressão (β) e respetivos IC95% estimados por modelos de regressão linear e por modelos *cross-lagged panel design*. As associações dos padrões alimentares, da ingestão proteica e da carga glicémica alimentar aos 4 anos com a adiposidade e insulina sérica aos 7 anos de idade também foram avaliadas por modelos de regressão linear.

Resultados

Práticas maternas de restrição, monitorização, controlo explícito e controlo encoberto verificaram-se associadas a uma possibilidade 11-18% menor de as crianças consumirem fruta e hortícolas abaixo do recomendado. Contudo, o controlo explícito também se associou a 24% maior possibilidade de as crianças consumirem estes alimentos acima do recomendado e a pressão-para-comer associou-se a uma possibilidade 8% superior de consumo de lacticínios acima das recomendações. Excetuando esta última prática, as práticas maternas mostraram-se inversamente associadas ao consumo de AEDE.

Identificaram-se três padrões alimentares na idade pré-escolar: rico em AEDE (denominado de padrão AEDE); baixo em alimentos normalmente consumidos nas refeições principais e intermédio nos tipicamente consumidos nas refeições intercalares (denominado de padrão *Snacking*); e rico em produtos hortícolas e peixe, bem como mais pobre em alimentos menos saudáveis como AEDE (denominado de padrão Mais Saudável, definido como classe de referência nas análises).

Menor posição socioeconómica aos 12 anos de idade da mãe teve o efeito global indireto de aumentar a possibilidade de a criança seguir padrões alimentares menos saudáveis (AEDE e *Snacking*), enquanto a escolaridade da mãe no momento do parto se associou direta e inversamente a estes padrões.

Crianças com apenas irmãos mais velhos apresentaram maior possibilidade de seguir um padrão alimentar rico em AEDE, enquanto crianças com apenas irmãos mais novos e cujo principal cuidador diurno era um dos pais seguiram sobretudo o padrão *Snacking*.

Dois padrões de práticas maternas de controlo da alimentação da criança (monitorização e restrição) associaram-se inversamente aos padrões alimentares menos saudáveis dos filhos. Crianças cujas mães apresentaram pior qualidade alimentar (1º *vs.* 4º quartil de *score* alimentar materno) apresentaram maior suscetibilidade de seguir os padrões alimentares menos saudáveis (AEDE: OR=9,94 IC95% 7,35-13,44 *p-trend*<0.001; *Snacking*: OR=4,21 IC95% 2,94-6,05 *p-trend*<0.001).

Após consideração de fatores a montante, a alimentação da mãe foi responsável por um terço do coeficiente de determinação do modelo final, bem como foi o fator que apresentou maior magnitude de associação com a alimentação da criança.

O consumo de AEDE aos 2 anos associou-se significativa e positivamente ao consumo dos mesmos alimentos aos 4 anos de idade (*β*=0,522 IC95% 0,432-0,612), bem como os *z-scores* de IMC persistiram no tempo (*β*=0,747 IC95% 0,688-0,806). Na análise *cross-lagged*, não se verificou uma associação entre consumo de AEDE e adiposidade dos 2 para os 4 anos. O padrão *Snacking* seguido aos 4 anos de idade também não se mostrou associado à adiposidade aos 7 anos de idade. O sexo da criança modificou a associação entre alimentação e adiposidade (*p*=0,046). O padrão AEDE associou-se significativa e positivamente a todos os indicadores de adiposidade aos 7 anos de idade apenas nas raparigas (*z-score* de IMC: *β*=0,075 IC95% 0,009-0,140; *z-score* de IMG: *β*=0,071 IC95% 0,000-0,142; e *z-score* de razão C/A: *β*=0,094 IC95% 0,023-0,164).

A ingestão proteica mostrou-se positiva e significativamente associada a *z-scores* de IMC nas raparigas (T2 *vs.* T1: *β*=0,187 IC95% 0,015-0,359) e nos rapazes (T3 *vs.* T1: *β*=0,205 IC95% 0,003-0,406), estando positivamente associada aos *z-scores* de insulina sérica apenas nos rapazes (T3 *vs.* T1: *β*=0,207 IC95% 0,011-0,404 *p*-interação=0,026). A carga glicémica aos 4 anos de idade também se mostrou associada aos *z-scores* de IMC apenas nos rapazes (T3 *vs.* T1: *β*=0,362 IC95% 0,031-0,693 *p*-interação=0,006). Adicionalmente, apenas nos rapazes, observaram-se interações significativas entre carga glicémica e ingestão proteica na associação com *z-scores* de IMG (*p*=0,019) e *z-scores* de razão C/A (*p*=0,039); rapazes nos tercis mais elevados de carga glicémica e de ingestão proteica, mostraram z-scores mais elevados de IMG (*β*=0,505 IC95% 0,085-0,925) e de razão C/A (*β*=0,428 IC95% 0,022-0,834) aos 7 anos de idade.

Conclusões principais

Menor estatuto socioeconómico durante a juventude da mãe aumenta indiretamente a possibilidade de expor os filhos a padrões alimentares menos saudáveis e menor escolaridade materna no momento do parto aumenta diretamente essa possibilidade, o que pode estar a refletir uma influência transgeracional de condições socioeconómicas menos favoráveis na alimentação da criança.

Diferentes características familiares associam-se a distintos padrões alimentares praticados pela criança; irmãos mais velhos parecem agir como modelos aumentando o consumo de um padrão alimentar rico em AEDE, enquanto ter apenas irmãos mais novos ou ser cuidado por um dos pais durante o dia aumentam o consumo do padrão *Snacking*.

Algumas práticas maternas de controlo alimentar podem ser benéficas já que estão inversamente associadas a consumo de alimentos/padrões alimentares menos saudáveis. A alimentação da mãe é um fator-chave para a alimentação da criança, sendo aquele mais fortemente associado com os padrões alimentares da criança, acima e para além de características demográficas e socioeconómicas.

O sexo da criança parece ser um modificador de efeito na associação entre alimentação e adiposidade. O consumo de AEDE e o IMC persistem durante idades precoces e um padrão alimentar rico em AEDE seguido aos 4 anos aumenta subsequente adiposidade nas raparigas. A ingestão proteica associa-se positivamente ao IMC em ambos os sexos, mas associa-se ao aumento dos níveis séricos de insulina apenas nos rapazes. A carga glicémica alimentar associa-se positivamente a posterior adiposidade apenas nos rapazes, nos quais parece interagir com a ingestão proteica aumentando o efeito sobre a adiposidade.

LIST OF ABBREVIATIONS

BMI	Body Mass Index
BMI z-scores	Age- and sex-specific Body Mass Index Standard Deviation Scores according to the World Health Organization
EDF	Energy-Dense micronutrient-poor Foods and beverages
FFQ	Food Frequency Questionnaire
FM%	Fat Mass Percentage
FMI	Fat Mass Index
FSI	Fasting Serum Insulin
G21	Generation XXI birth cohort
GI	Glycemic Index
GL	Glycemic Load
IGF-I	Insulin-like Growth Factor I
SEP	Socioeconomic Position
UK	United Kingdom
US	United States of America
WHO	World Health Organization
W/Ht	Waist-to-height

INTRODUCTION

1. Global and national trends in prevalence of childhood overweight

Although childhood obesity may be reaching a plateau in some regions (1, 2), prevalence of overweight and obesity in children has generally increased worldwide in the last four decades (3). As depicted in figure 1, prevalence of overweight is high (4) and – in 2014 – 41 million children below the age of 5 years were estimated to be overweight (5).

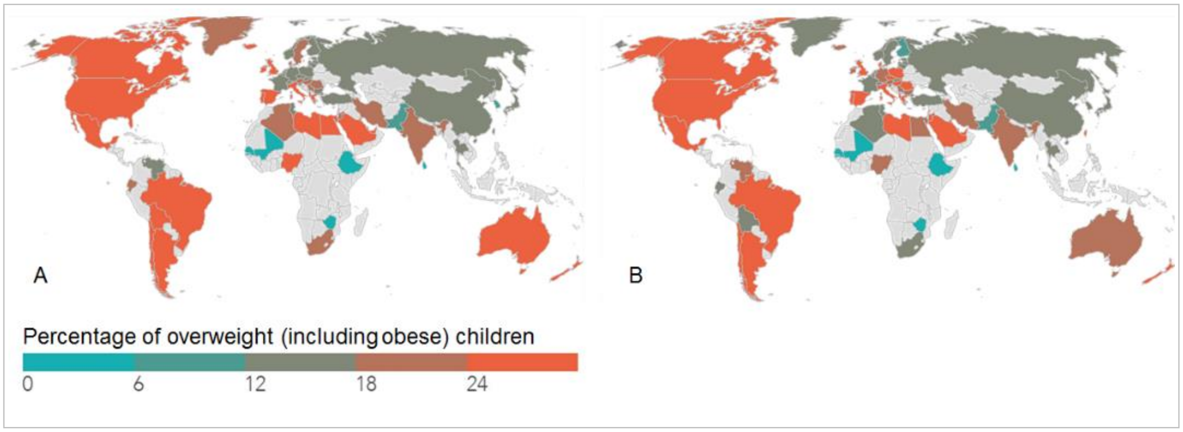


Figure 1. Worldwide prevalence of overweight in girls (A) and boys (B) aged 2 to 18 years, in the latest available year [source: World Obesity Federation (4)].

Data published in 2010 (6) relative to European preschool children reported no clear trends towards increasing prevalence of overweight in the last two-three decades [with the possible exception of the United Kingdom (UK)], and showed that higher prevalence was observed in Mediterranean countries. Concerning European children below 10 years of age, results published in 2014 (7) – according to Cole's cut-offs (8) – also showed higher prevalence of overweight in southern countries such as Portugal.

A review published in 2007 (9) examined studies in Portuguese children and adolescents, concluding that the general picture was alarming with prevalence of overweight above 30% in several regions. In children aged 6-8 years, data from the Childhood Obesity Surveillance Initiative published in 2012 (10) reported a national overweight prevalence of 37.9% [according to the criterion of the World Health Organization (WHO) (11)] with some differences in distinct Portuguese regions (Figure 2). In 2014, the Childhood Obesity Surveillance Initiative (12) reported that overweight prevalence in Europe ranged from 18% to 57% in boys and from 18% to 50% in girls. In addition, higher prevalence in southern European countries was also reported, and higher prevalence was observed in girls when compared to boys in some countries (e.g., Portugal with 36% in girls and 32% in boys) as depicted in the following figure.

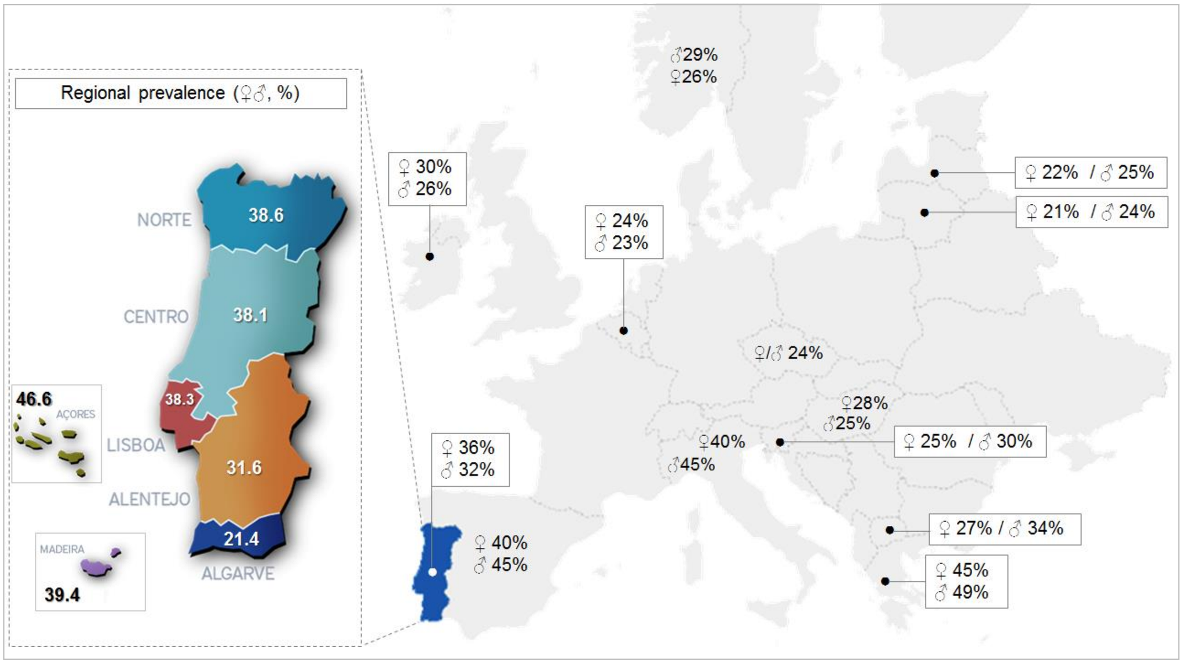


Figure 2. Prevalence of overweight (including obesity) in girls and boys aged 7-8 years in Europe according to the WHO's criterion [Adapted from references (10, 12)].

Overweight and obese children have increased risk of obesity-related morbidities (e.g., hyperinsulinemia, type 2 diabetes, endocrine and orthopedic disorders, hypertension, psychiatric problems) and are at increased risk of becoming obese adults which – in turn – carries increased probability of chronic illness (13). Hence, the burden of childhood obesity for current and future demands on health services is expected to be high (3).

Reviews published in 2012 and 2013 (14-16) showed that, although the most effective interventions on obesity prevention are usually multiple approaches including home and community components, most intervention studies have been implemented in the school setting. Evidence supports the need of following the life-course framework for obesity prevention (17) and to give particular consideration to important time periods in life (preconception, pregnancy, infancy, early childhood), since waiting until children begin school might be too late. In addition, addressing childhood obesity requires consideration of the environmental context in which children are embedded, notably the obesogenic environment to which they are currently exposed to (18).

2. A brief overview of children's diets: secular trends in dietary intake

Daily energy intake in children aged 2-18 years from the United States of America (US) has significantly increased from 1989 to 2010 (19) showing increased intake from 1989 to 2004, followed by declines or stabilization. Preschool children and children in low-income families were among those with largest increases in energy intake from 1989 to 2004 and – in contrast to other subgroups – their total energy intake in 2009-2010 was higher than in 1989-1991. Consistently major contributors to energy intake were; sugar-sweetened beverages, pizza, full-fat milk, grain desserts, breads, pasta dishes, and savory snacks (19).

Over the end of the 20th century, a decrease in fat intake in children from Europe and the US was reported, coupled with increased intake of carbohydrates (20, 21). These trends were mainly characterized by increased consumption of processed convenience foods and sugar-sweetened beverages (22-24), and tendencies observed in the Glycemic Index (GI) of children's diet (25) may be reflecting those changes. Indeed, trends in children's consumption of sugar-sweetened beverages have been repeatedly highlighted (23, 26-28) and were associated with decreased nutritional quality in their diet (29-31).

These tendencies have been similar to those observed in consumption of foods such as fast food and savory snacks, which – as depicted in Figure 3 – are major contributors to children's energy intake.

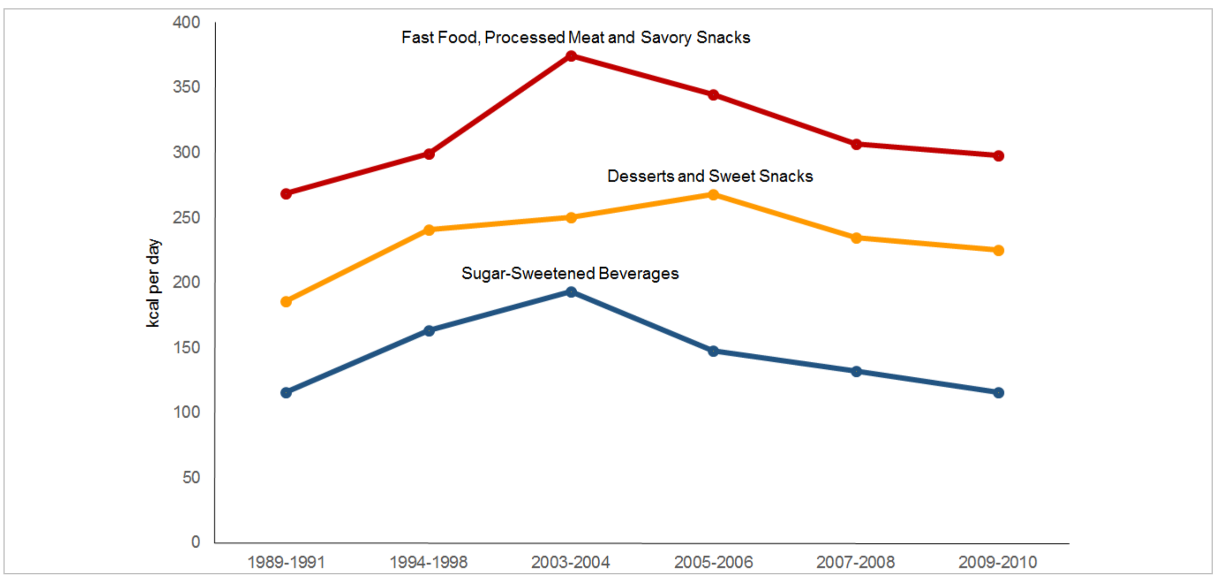


Figure 3. Trends in per capita energy from foods high in energy density and sugar-sweetened beverages in US children aged 2-18 years [source: adapted from reference (19)].

Grain desserts such as cakes, cookies, donuts or pies were among the top five sources of energy in children's diet, while pizza, whole milk, cheese, and fatty meats were major sources of solid fat (32). In a national random sample of US children (33), 47% of children at preschool age were below the acceptable distribution range for fat, although intakes provided more saturated fat than recommended. In addition, average daily intake of energy from added sugars was high (365 kcal) with sodas, fruit drinks, desserts and candies as major sources of added sugar (32), while fiber intake was lower than recommended (33).

Among European children, consumption of sugars has been shown to be high and higher dietary GI was associated with a less favorable diet with higher intake of sugar and lower intake of fiber (24, 25). In preschool children from Belgium, mean energy percentage from saturated fatty acids was high and few children reached the recommended dietary allowances for fiber, vitamin D and iron, while everybody exceeded the tolerable upper intakes for sodium (34). Also, daily intakes of fruit and vegetables were below recommendations and consumption of high-energy foods was excessive in children from both Belgium and Germany (35, 36), while a substantial percentage of preschool children from Greece showed intakes of fat and carbohydrate outside the recommended distribution range. Furthermore, in European children aged 2-9 years, a 'processed' dietary pattern characterized by high consumption of pizza, hamburgers, savory pastries and crisps was identified in all countries included in the Dietary- and lifestyle-induced health Effects in Children and InfantS' (IDEFICS) study (37).

In Portugal, national representative published data on children's diet are lacking. In preschool children enrolled in the population-based birth cohort Generation XXI (G21), only 45% showed daily consumption of vegetables on a plate and the proportion of those consuming sweets (cakes, chocolate, candies, and sugar) on a daily basis was high (65%) (38). Also, consumption of foods high in energy density tracked during preschool age and was associated with lower dietary quality at child's 4 years of age (39). In addition, children reporting consumption of <80% of meals at home and the remaining meals out-of-home (e.g., restaurants, coffee shops) were those with higher intake of saturated fat, caffeine, consumption of foods high in energy density, and lower dietary adequacy index (40).

Changes in children's diet coupled with increased prevalence of overweight, led to an increased interest of research on the contextual determinants of children's diet and childhood overweight.

3. Contextual models of childhood obesity

Obesity results from an imbalance between energy consumed and energy expended (41). However, its etiology is far more complex than this sentence implies, as obesity is consequence of an interplay between a large range of determinants set within larger social and environmental contexts (42). Yet, research on determinants of childhood overweight traditionally lacked comprehensive models considering the environment in which the child develops and where parenting takes place, such as the family (43).

The Ecological Systems Theory faces the family ecology as a context for human development (44), considering that change in individual characteristics cannot be effectively understood without taking into account the individual's ecological context, both distal and proximal. In the case of a child, proximal contexts comprise the family and the school, both included in broader social contexts. As illustrated in figure 4, child's risk factors (e.g., diet) interact with child's characteristics (e.g., gender) and are shaped by contextual factors which are influenced by more distal factors such as community and societal characteristics (43).

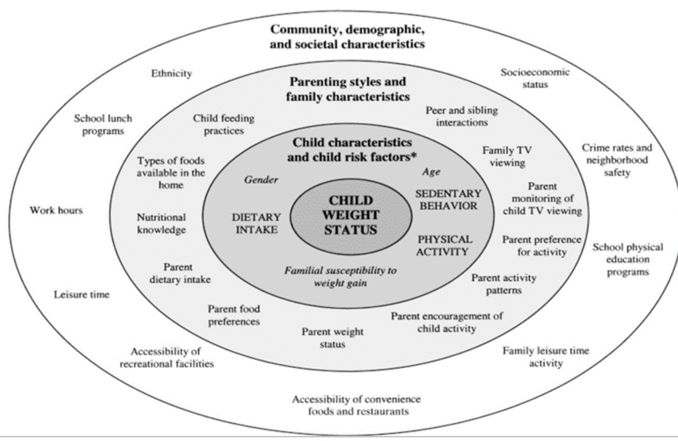


Figure 4. Ecological model of determinants of children's lifestyle and of childhood overweight [source: Davison and Birch, 2001 (43)].

The Ecological Systems Theory and Social Ecological Theory (44, 45) consist in general models of environmental influences on behavior and, besides the framework depicted in figure 4, other frameworks have been used to describe specific environmental factors associated with children's weight and nutritional status (42, 46-48). These models usually distinguish interpersonal (peers and family), organizational (schools), community and societal influences on behavior (49). The family context consists of a determinant with particular importance, especially at preschool age.

4. Determinants of children's diet within the family context

The family is the first social structure to which children are exposed and it is critical in their general socialization (50). Within this structure, parenting affects children's social development (51), being crucial for survival and well-being of the offspring (52). The act of parenting may be defined as caring for the young, including aspects such as food provision, nursing, protection, and skills' teaching (52). Since parents provide environments for their children's early food experiences, it is likely that many modifiable risk factors for childhood overweight (e.g., unhealthy diets) are rooted within the family context (53, 54).

Family environments include parental behaviors such as parenting eating styles, parenting child-feeding practices or parental diet that influence child's weight indirectly through the effect on the child's diet (53). However, research has mostly focused on risk factors of childhood obesity and much less so on more upstream determinants of children's behaviors (55). It has been emphasized (56) that the child's diet is likely to be strongly influenced by environmental factors, since children have less autonomy in food choice.

Considering that increased prevalence of obesity has been observed in very young children, even before the school environment starts to exert its effect, and that preschool children are starting to respond to environmental familial cues and are beginning to develop eating habits that track into later childhood, when examining determinants of young children's diet the family context is particularly important especially parenting child-feeding practices and parents' eating behavior.

4.1. Parenting child-feeding practices

As literature often fails to make a distinction between parenting style and parenting practices (53), it is important to clarify these terms. General parenting style refers to all domains of the parent-child relation, while feeding styles refer to the specific domain of feeding children, a part of the broader construct of general parenting style. The typology of parenting styles mostly used by nutrition researchers was developed by Maccoby and Martin (57) based on prior work by Baumrind (58) and is operationalized in two dimensions: demandingness (number and types of control and expectations parents place on their children) and responsiveness (extent to which parents demonstrate consideration for the child emotional needs and involvement). From these dimensions, four styles emerge: Authoritative, Authoritarian, Indulgent or Permissive, and Neglectful or Uninvolved.

Authoritative style seems to support positive food-related behaviors (59-61), but other studies do not suggest this association (62, 63). In contrast, indulgent, neglectful and authoritarian styles are usually linked to negative food-related behaviors and to higher Body Mass Index (BMI) (64). However, authoritative styles have also been related to lower intakes of fruit and vegetables when compared to indulgent or neglectful styles (65).

Although related to parenting styles, parenting child-feeding practices are distinct and are defined as specific strategies used by parents to control what, when and how much their children eat, such as pushing the child to eat, restricting access to certain foods and monitoring children's diet (53, 66). It has been hypothesized that parenting child-feeding practices increase the risk of overweight through the disruption of the child's capacity of self-regulating energy intake (67). Child-feeding practices have been studied mainly as to the central topic of parental control and its association with childhood overweight.

Parental control has also been examined relatively to dietary intake, but findings are contradictory. Albeit restriction is the parenting practice more consistently associated with variation in children's dietary intake (68), some evidence suggests that it is linked to higher consumption of palatable snacks (69-72) and lower consumption of fruit and/or vegetables (73, 74), while other evidence suggests the opposite, linking it to lower consumption of palatable snacks (75, 76) and higher consumption of fruit and/or vegetables (75, 76). Likewise, findings relative to the parenting practice of pressuring the child to eat (pressure-to-eat) are also conflicting, some studies supporting an association with higher consumption of snacks (74, 76, 77) and lower consumption of fruit and/or vegetables (74, 76-78), while others suggest the opposite (79, 80). Monitoring has also shown some conflicting results. It has been associated with higher fiber and lower sugar intakes (79), higher fruit and vegetables consumption (81), higher healthy (82) and lower unhealthy eating (81, 82), but also with higher consumption of foods high in energy density in the presence of a permissive parenting style (83) and it was associated with higher overweight (84) and lower BMI (85).

The conflicting and rather confusing literature about parental control and children's dietary intake can be explained by several aspects; the use of different tools, the use of different items to measure the same construct, the occasional use of a single item to represent quite complex constructs, studies carried out in different populations, limitations inherent to self-reported information and lack of distinction between practices and styles, being important to notice that conflicting findings might also be related to the possibility that practices are dependent on parents' more general parenting style (86).

Furthermore, as highlighted by other authors (87), inconsistencies may reflect the contradictory nature of parental control, a concept that might be more complex than previously acknowledged, being possible that instruments are measuring some aspects of control that are positive and some aspects that are just the opposite. Hence, Ogden *et al*. (87) proposed two new dimensions to distinguish different aspects of restriction – 'overt control' and 'covert control'. Overt control consists of limiting consumption of unhealthy foods in a way perceived by the child (e.g., being firm about how much is eaten) and covert control consists of limiting consumption of unhealthy foods in a way undetected by the child (e.g., not buying unhealthy foods) (87). Higher degrees of covert control were associated with lower consumption of unhealthy snacks (78, 87), while higher overt control was related to higher healthy snacks consumption (87), but also to lower consumption of chocolate candies (88). Both overt and covert control were positively associated with consumption of fruit and vegetables (78).

Research on parenting child-feeding practices has mainly focused on fruit and vegetables (73-75, 77, 80, 89-91) and foods or snacks high in energy density (74, 75, 77, 91, 92), which may be producing only a partial picture of their association with children's diet. Few studies have considered broader ranges of foods or children's dietary patterns (76, 92), and those considering dietary guidelines for preschool children or patterns of child-feeding practices are practically non-existing (93). Also, the number of studies on overt and covert control is limited (78, 87, 88, 94) and more research is needed to better clarify the association of these specific child-feeding practices with children's diet.

4.2. Parental diet

Besides engaging in practices to control the child's diet, parents determine food availability and accessibility and act as role models shaping children's diet (43, 95). Beyond child-feeding practices, most studies on environmental determinants of dietary behaviors in children have focused on availability and accessibility (49, 56). Research on household availability and accessibility has particularly focused on fruit and vegetables, showing a positive association with children's consumption (96-104). Yet, it has been highlighted (56) that studies showing an association only slightly outnumber those not showing any association between availability and/or accessibility and dietary intake.

Studies on parental food consumption also focused predominantly on consumption of fruit and vegetables, showing consistent positive associations between parents' and children's consumption for fruit and vegetables (105-111) or fruit (112-116). Concerning other foods, positive associations between parental and child consumption were reported for milk (105, 117, 118), sugar-sweetened beverages (107, 111, 119-122) and foods or snacks with high energy density (107, 112, 114, 115, 123).

Notwithstanding this considerable evidence, studies on broader ranges of foods are scarce and those with overall dietary measures are even scarcer. Brown and colleagues (114) studied a broader set of foods, classifying them into healthy (fruits, yogurt, toast) or unhealthy (e.g., chocolate and pastries) snacks and showed positive correlations between parents' and children's consumption. Another study (116), assessing an even wider array of foods, reported several positive parent-child associations, while a study focusing on nutrient-dense and foods with high energy density (123) found a positive association between mothers' and children's diet.

Fisk and colleagues (124) evaluated dietary consumption of mothers and their 3-year old children, using Principal Component Analyses. A prudent dietary pattern – characterized by high intake of fruit and vegetables and low intake of crisps and white bread – was identified in both mothers and children. Maternal dietary quality was the key influence on children's dietary quality. Besides the paucity of studies with overall measures of diet (124), relatively few studies focused on parental and child's diet at preschool age (105, 106, 109, 123, 125).

Studies in preschool children are particularly warranted because parental influence on dietary behaviors at this age is likely to be especially important. Considering that early diet tracks into later ages and influences current and future health (126-128), prevention of unhealthy dietary habits in early childhood might be a major step in the promotion of health.

However, evidence base for interventions in preschool children is limited (129) and research on determinants of preschool children's diet is particularly scarce (55, 130). Additionally, research seems somewhat disconnected between studies assessing socioeconomic factors and those mostly focused on parental behaviors such as child-feeding practices or parental diet (54, 55, 95, 130).

4.3. Other sociocultural influences

Besides parents, children's food preferences and dietary habits are modified under the influence of other caregivers, peers and siblings (43, 131). As consequence of changes in employment patterns and family structure, children are routinely fed by someone other than a parent, namely grandparents, other relative or caregivers in organized childcare (95).

Limited evidence reported family composition (family dimension, and one- or two-parent families) and peers' diet to be associated with children's dietary intake (49, 56). Positive associations with siblings' energy and fat intake have been reported in children aged 12-18 years (56), but in preschool children research is scarce (130).

In 2-year-old Portuguese children (132), having older siblings was positively associated with consumption of foods with high energy density, similarly to findings reported in preschool children from the UK and from France (133, 134).

Although number of hours of maternal work, frequency of family meals, out-of-home meals, and childcare meals have been examined (49, 56, 130), evidence on day-time caregivers is scarce.

4.4. Socioeconomic position

Factors within the family context, particularly parents, are expected to influence children's dietary habits development, especially during preschool years (43, 53, 54, 95). In turn, this proximal family context is influenced by the more distal context of socioeconomic characteristics.

It is recognized that socioeconomic position (SEP) plays a central role in the social production of health and disease (135) and that it tracks in time. Lower SEP during pediatric ages has been associated with higher prevalence of overweight as well as poorer dietary habits (136).

However, the behavioral pathways that link SEP to health and the length needed for socioeconomic exposures to exert their influence, warrant particular attention (136). Hence, studies examining parental determinants of children's diet should take into account both distal and proximal indicators of SEP.

SEP does not exert a direct effect on health or on children's dietary habits. Instead, it is expected to operate through intermediary determinants of health in a sequence of interlinked events (137, 138). In a systematic review published in 2014 (139) maternal intake of specific foods have been considered as a SEP-associated determinant of children's diet, but this review also concluded that too little research has been conducted in this area.

Conceptually (138), SEP may influence maternal education attainment at child's delivery, maternal occupation, family income and structure, child's caregivers, as well as maternal child-feeding practices and maternal behaviors. Then, maternal behaviors may influence children's diet that – in turn – can have an effect on adiposity. Yet, some factors may exert their influence above and beyond socioeconomic factors, and some may be more important than others.

Although increased out-of-home childcare has been observed in the last decades, preschool children still consume high proportions of their daily energy intake at home (140), a finding also reported in Portugal (40). As preschool children have less autonomy in food choice, parenting behaviors are expected to have a strong influence on their diet, above and beyond family characteristics and socioeconomic determinants.

In addition – keeping in mind that, in mammals, mothers usually take the role of parenting (52) as well as that, in humans and in most families, women still have the primary responsibility for feeding children (95) – maternal characteristics and behaviors are of particular interest. Still, changes in women's employment patterns and in family structure seem to have altered children's dietary exposures.

5. Dietary determinants of childhood obesity

5.1. Specific food groups

The study of the role of individual foods is important and several specific foods or food groups have been examined as to their relationship with childhood obesity. Research has mainly focused on fruit, vegetables and sugar-sweetened beverages, while examination of other food groups (e.g., cereal products, or foods with high energy density) is more limited.

Fruit and vegetables – due to their low energy density, to their contribution to satiety and to the possibility of displacing foods with high energy density from the diet – have been proposed to be protective against obesity. Earlier reviews (141, 142) on the association between fruit and vegetables or plant-based diets and childhood obesity have concluded that evidence is limited and does not suggest a protective effect of fruit and vegetables on risk of childhood obesity. A review published in 2010 (143) also did not find evidence of an association between fruit and vegetables and childhood obesity.

Other plant foods such as legumes and nuts were inversely associated with obesity in children (143). Likewise, cereal products were significantly inversely associated with childhood obesity (141-143), but evidence is limited and studies considering the GI of these foods are needed.

Animal foods such as meat and eggs have been positively associated with childhood obesity (143). Dairy foods have also been positively associated with risk of overweight in children (143), but other studies found an inverse association of dairy products, milk or calcium with adiposity in children (141, 144, 145).

Most evidence on consumption of specific food groups and childhood obesity refers to sugar-sweetened beverages and several systematic reviews have evaluated their association with children's weight status, reaching distinct conclusions.

A systematic review published in 2006 (146) concluded that research provided strong evidence for the independent role of the consumption of sugar-sweetened beverages in the promotion of obesity in children and adolescents, but another review of the same year (147) concluded that a variety of inconsistencies and methodological difficulties precluded definite conclusions. In 2007, a systematic review and meta-analysis (148), including nearly the same studies, reported that there was a clear association between consumption of sugar-sweetened beverages and weight.

A subsequent meta-analysis, addressing only longitudinal studies and randomized controlled trials among children and adolescents (149), reported that there was practically no association between consumption of sugar-sweetened beverages and BMI. Four years later, a meta-analysis of randomized experiments found that evidence did not show conclusively that sugar-sweetened beverages contributed to obesity (150). Some of these reviews were criticized as to its quality (151) and a posterior revision emphasized that the relation between intake of sugar-sweetened beverages and obesity was convincing (152).

A meta-analysis published in 2013 (153) concluded that consumption of sugar-sweetened beverages promotes weight gain in children and adults but, although results from prospective cohort studies in children support an association between consumption of these beverages and change in BMI, the overall pooled estimate of results from five randomized controlled trials in school-aged children and adolescents was not statistically significant.

Considering that sugar-sweetened beverages are frequently consumed with other foods and are associated with other behaviors, the focus of research on these beverages may be giving a very narrow image of the effect that foods and beverages low in nutrient density and/or high in energy density may have on childhood obesity. Yet, evidence on the association of foods with high energy density with obesity in children is far more scarce (141, 144, 154).

Increased consumption of meals away-from-home may contribute to childhood obesity as foods commercialized are usually higher in fat and/or sugar and lower in fiber than those prepared at home (141). In longitudinal studies, food purchased away-from-home, consumption of fast-food and consumption of fried foods away-from-home have been positively associated with weight status in children (144, 154, 155), but in other studies fast food was not associated with weight (156, 157). In addition, prospective studies have not shown an association between energy-dense snacks and weight gain in children (144, 158).

There is a paucity in studies on dietary behaviors in preschool children (145), not only where it concerns consumption of foods with high energy density and sugar-sweetened beverages, but also regarding overall measures of diet, and research is particularly scarce on the association of *a posteriori* dietary patterns at preschool age with later adiposity.

5.2. Dietary patterns

Traditionally, nutritional epidemiology examined specific nutrients and foods (159). Dietary scores or indices were the first methods used to assess the association of a combination of foods with health-related outcomes (160). A variety of indices have been proposed to assess overall dietary quality, usually defined as a composite score of nutrients, foods or both, based on nutrition knowledge (*a priori*) to evaluate adherence to guidelines (160). Another approach, usually known as '*a posteriori*', explores collected dietary data *post hoc*, and identifies relevant dietary patterns of the study population by specific statistical analyses (159, 161).

Various statistical methodologies have been used to analyze dietary data and to empirically identify dietary patterns, mainly factor and cluster analysis. Factor analyses includes both principal component analysis and common factor analysis, and identifies common underlying dimensions of food consumption aggregating food items on the basis of the degree to which these items are correlated with each other – being considered a food-centered approach. In contrast, cluster analysis is a person-centered approach that aggregates individuals into groups (clusters) with relatively similar diets (159).

Latent class analysis is an example of a person-centered approach that can be used to describe how the probabilities of a set of observed categorical variables (e.g., items in a food frequency questionnaire) vary across groups of individuals (clusters, homogeneous within groups) in order to find the smallest number of unobserved variables (latent classes) (162). Dietary profiles can be compared across clusters to interpret patterns identified.

Traditional analysis of foods and specific nutrients has been very important, but has several limitations related to the relationship between nutrients and/or foods. The effect of a single nutrient or a single food may be too small to detect, and many conditions cannot be attributed to a single item but rather to multiple nutrients and foods. Hence, analysis of dietary patterns is essential in order to account for the complexity of human diet as these overall measures of diet use the correlated nature of dietary data and may be easier to translate into meaningful dietary guidelines (159, 161, 163).

Published studies examining the association of empirically-derived dietary patterns practiced at preschool age with later adiposity are scarce and have not found an association of dietary patterns with adiposity from 3 to 7 or from 5 to 9 years of age (164, 165).

In older children, dietary patterns have been associated with subsequent higher adiposity (165), higher trunk adiposity, and higher BMI (166). Among adolescents, results are conflicting (163) and one study (167) reported slight differences between sexes on the association of a 'starchy foods' pattern with overweight. Yet, among preschool children, prospective studies have not reported formal examination of an interaction between child's sex and dietary patterns on the association with adiposity. Evidence on energy intake, macronutrient distribution and dietary Glycemic Load (GL) is also sparse.

5.3. Dietary composition: energy, fat, protein and carbohydrates

As the gap between energy intake and energy expenditure results in storage of excess energy as adipose tissue (144), energy intake has been of major interest in research focused on childhood obesity. But lack of consistency between studies has raised methodological questions concerning its measurement in epidemiological studies (141, 144).

Energy density as a property of food volume or weight (kcal/g) was identified as a determinant of energy intake in laboratory studies (168-170), where it was shown that increased energy density of meals significantly increased daily energy intake, independently of the macronutrient composition. Few studies examined the role of energy density on childhood overweight during early childhood and, despite increases in energy density over time in young children, energy density was not significantly associated with weight (171) or daily energy (172). In addition, albeit energy density at 7 years of age has been associated with excess adiposity at 9 years of age, at the preschool age of 5 years it was not (173).

The dietary macronutrient distribution may also have a role on childhood overweight which may be independent of energy intake, but the role that particular macronutrients play in the etiology of childhood obesity is still unclear, mainly because of methodological limitations related with the co-occurrence of nutrients in foods and meals and the complex interrelation between macronutrients (141, 144).

As fat is twice as energy dense than carbohydrates and protein, this macronutrient received more attention in its relationship to obesity. Increased fat intake has been significantly positively associated with adiposity in children (174-177), but in other studies (178-182) findings did not suggest an association between fat intake and childhood overweight.

In preschool children, positive associations between fat intake and adiposity were reported (183, 184), although in other studies no association was found (185-187) and a review published in 2006 (188) concluded that the role of type of fat as early determinant of childhood obesity is insufficiently understood. A systematic review from 2014 (189) included seven trials that compared a low-fat diet to an isocaloric or an *ad libitum* low-carbohydrate diet and concluded that improved weight status can be achieved by energy reduction, rather than by changes in macronutrient distribution.

Rolland-Cachera *et al*. (190) were the first to report that high protein intake was positively associated with later adiposity (through early adiposity rebound) and with increased height and accelerated growth, suggesting that an early diet rich in protein could increase the risk of obesity in later life. Subsequent prospective observational studies (191-193) supported this hypothesis. The 'Early protein hypothesis', advanced by Koletzko and colleagues (194) and driven by the inverse association between breastfeeding and obesity, further proposed that the markedly higher protein intake in formula-fed infants could play a role in predisposing infants to an increased obesity risk later on (195) and clinical trials (196, 197) conducted in the same sample of infants have supported this hypothesis

Several factors such as availability of amino acids and energy, growth hormone, insulin and insulin-like growth factor-I (IGF-I) regulate early growth, IGF-I being essential for mediating the metabolic, endocrine and anabolic effects of growth hormone (198, 199). It has been proposed (200) that a reduction of the protein content in infant formula results in lower concentration of insulin-releasing amino acids, decreasing circulating levels of insulin and IGF-I and resulting in lower early weight gain. Results from the European Childhood Obesity trial (196) have shown that essential amino acids (particularly branched-chain ones - leucine, isoleucine and valine), IGF-I and urinary peptide-C/creatinine ratio were higher in infants fed high protein infant formula (201).

Research on the association between protein and adiposity has mainly addressed protein intake in the first 2 years of age (191-193, 196, 199, 201-204). Although mean protein intake from 2 to 8 years has been associated with higher BMI at 8 years (205) and current protein intake has been related to BMI at 4 years (206), very few prospective studies (207, 208) addressed protein intake at preschool age and later BMI or growth. These studies (207, 208) suggest that there may be sensitive periods other than infancy and more research on protein intake at preschool age is warranted.

As highlighted in a systematic review published in 2006 (209), the lack of effectiveness of low-fat diets and the continuing increase in obesity prevalence has led to a resurgence of interest in low-carbohydrate and low-glycemic diets, concluding that low-carbohydrate or low-GI diets seem as effective as low-fat diets for short-term weight loss in children, although evidence on long-term weight control is limited.

The concept of GI was proposed more than 30 years ago (210) as a classification system of the blood glucose-raising potential of carbohydrate-containing foods. It is defined as the incremental area under the blood glucose response curve elicited by 50 g available carbohydrate of a test food expressed as percentage of the response produced by 50 g available carbohydrate of a standard (glucose or white bread) (210, 211). Dietary GL was proposed later (212) to quantify the overall glycemic effect of food, incorporating both the quality (i.e., GI) and quantity of dietary carbohydrates. Low-carbohydrate diets have been reported to be associated with a significant short-term beneficial effect on BMI (189). As well, diets low in GI or GL have been suggested as beneficial in overweight or obese children and adolescents (213). However, most studies examining the association between dietary GI or GL and later adiposity have concentrated on pre-pubertal children and adolescents (213, 214) and prospective evidence in early childhood is very scarce (215).

The effect of amino acids on glucose metabolism is not independent of glucose itself. The majority of amino acids attenuate the response to glucose ingestion probably through stimulation of insulin secretion (216). Glucose also stimulates insulin secretion, and IGF-I shows a glucose lowering effect reminiscent of insulin (217). Moreover, both insulin and IGF-I, along with amino acids and glucose, activate the mammalian target of rapamycin [(mTOR1)] – a cell signaling pathway that regulates cell growth and proliferation (218, 219). Hence, carbohydrate intake should be considered together with protein and their possible interaction on the association with adiposity should be examined.

In addition, sex-differences in the association between macronutrient intake and adiposity have been observed (141, 144). For instance, some studies (220, 221) found a positive association between fat intake and adiposity only in boys, while percentage of energy from carbohydrate has been inversely associated with adiposity, significantly only in boys (222) or only in girls (223). Likewise, higher protein intake was associated with higher adiposity only in boys (192) or only in girls (224, 225). Hence, the possible interaction of child’s sex on the association between diet and adiposity should also be evaluated.

6. A conceptual framework of determinants preschool children's diet and subsequent adiposity

Although many potential determinants have been studied for a variety of children's dietary behaviors, only few studies have examined the same specific combination of environmental factor and dietary behavior (56).

Specifically in preschool children, determinants of energy-balance related behaviors were mostly studied regarding physical activity and sedentary behavior, while determinants of dietary intake have been much less examined (130, 226). Additionally, albeit intervention studies show higher effects at preschool age, evidence base for intervention in this age group is scarce (227).

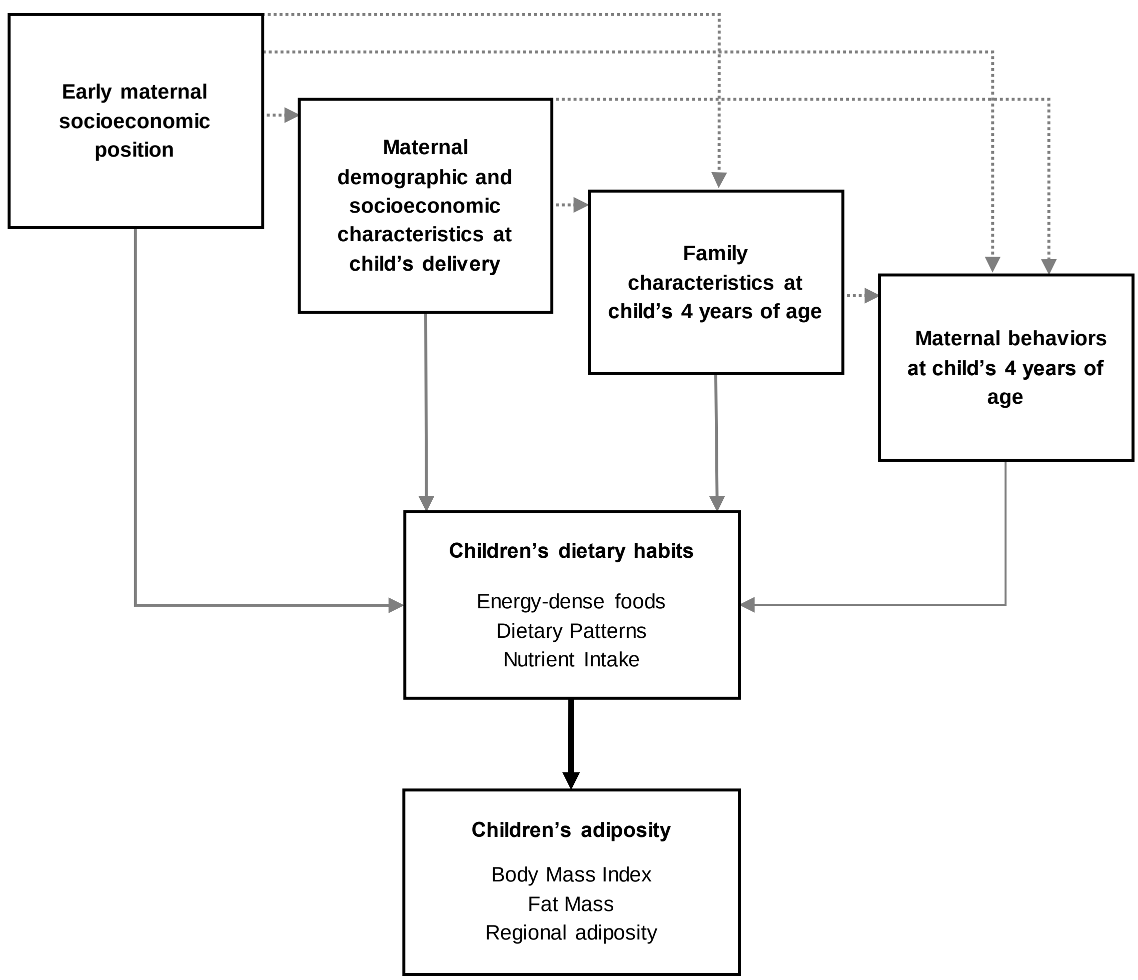
Considering this paucity of evidence, as well as that eating habits start to be established in early years tracking into later life (126, 127) and that preschool age may include a sensitive period as it is when adiposity rebound is likely to occur (228), studies on determinants of diet and adiposity are particularly needed during early childhood.

There is an underlying complexity to both the development of dietary habits and childhood obesity. As emphasized by other authors (42), this means that tackling childhood obesity requires a multifaceted approach that is based in conceptual models representing a set of attributes together with relationships between them to help unravel some of the associations and relative importance of various determinants.

Likewise, complex interlinked factors may influence children's dietary habits. For instance, early socioeconomic conditions may influence maternal education attainment at child's delivery, subsequent family income and family structure, as well as maternal behaviors such as dietary habits. These habits are expected to influence children's diet that – in turn – can have an effect on adiposity. Also, some factors may exert their influence above and beyond socioeconomic factors, and some may be more important than others.

Hence, studies based on conceptual frameworks able to better contribute to the knowledge about possible causal mechanisms and that may enable a better understanding of upstream and downstream factors, as well as which environmental factors are more influential are especially warranted (49, 56, 229).

In addition, consideration of different approaches to evaluate diet (dietary patterns, specific food groups, and nutrients) and distinct measures of adiposity is particularly needed. Hence, a conceptual framework of determinants of preschool children's diet as well as of the association between diet and later adiposity was developed for this research (Figure 5).



Arrows represent theoretical relationships of determinants of diet and adiposity during early childhood. Dotted grey lines indicate possible indirect effects in the pathway between levels of determinants and solid grey lines represent possible direct effects. The solid thick black line represents the association between children's diet and later adiposity. Upstream factors influence downstream ones that, in turn, influence the child's diet which – ultimately – may exert its effect on subsequent adiposity.

Figure 5. Conceptual framework defined for examining determinants of preschool children's diet and the association between diet and subsequent adiposity

OBJECTIVES

The general objective of this research was to examine determinants of preschool children's dietary habits and to evaluate the association of these habits with subsequent adiposity. As such, this thesis aimed to answer to the following research questions:

1. What is the association of maternal child-feeding practices with children's consumption of specific food groups, when dietary inadequacy relatively to guidelines for preschool children is considered? [Paper I]
2. Considering a theoretical conceptual framework of determinants of children's eating habits, how are family and maternal characteristics associated with children's dietary patterns and which factors are more important? [Paper II]
3. What is the association of eating habits during preschool age with adiposity later on, using the following approaches to measure diet;
    - 3.1. Specific food groups (e.g., foods high in energy density, sugar-sweetened beverages)? [Paper III]
    - 3.2. Overall measures of diet (*a posteriori* dietary patterns)? [Paper IV]
    - 3.3. Macronutrients (protein, dietary GL). Is there an interaction between these factors on the association of children's diet with adiposity? [Paper V]
4. Is there an interaction between child's sex and dietary intake on the association of diet with adiposity? [Papers IV and V]

In order to answer to these research questions, five studies – corresponding to five papers – were conducted with the following specific objectives:

- i) to estimate the association between maternal child-feeding practices and children's dietary inadequacy relatively to consumption of specific food groups;
- ii) to examine the association of maternal socioeconomic, demographic and behavioral characteristics with children's dietary patterns;
- iii) to assess the association between consumption of foods and beverages high in energy density and/or low in nutrient density at 2 years and BMI at 4 years of age;
- iv) to evaluate the association of dietary patterns with adiposity from 4 to 7 years of age and to examine if child's sex modifies this association;
- v) to evaluate the association of protein intake and dietary GL at 4 years with adiposity and serum insulin 3 years later, and to examine the possible interaction between protein intake and dietary GL on these associations, by sex.

METHODS

1. Setting and research design

This PhD thesis is founded on the population-based birth cohort G21 (230), assembled between April 2005 and August 2006 in the metropolitan area of Porto. The project G21 aims to characterize prenatal and postnatal development, identifying its determinants to understand the state of health in childhood and in later life.

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.

This research uses data mostly collected at the ages of 2, 4 and 7 years. Main exposures of interest were determinants of children's diet. The child's diet was also considered as exposure of subsequent adiposity (figure 6).

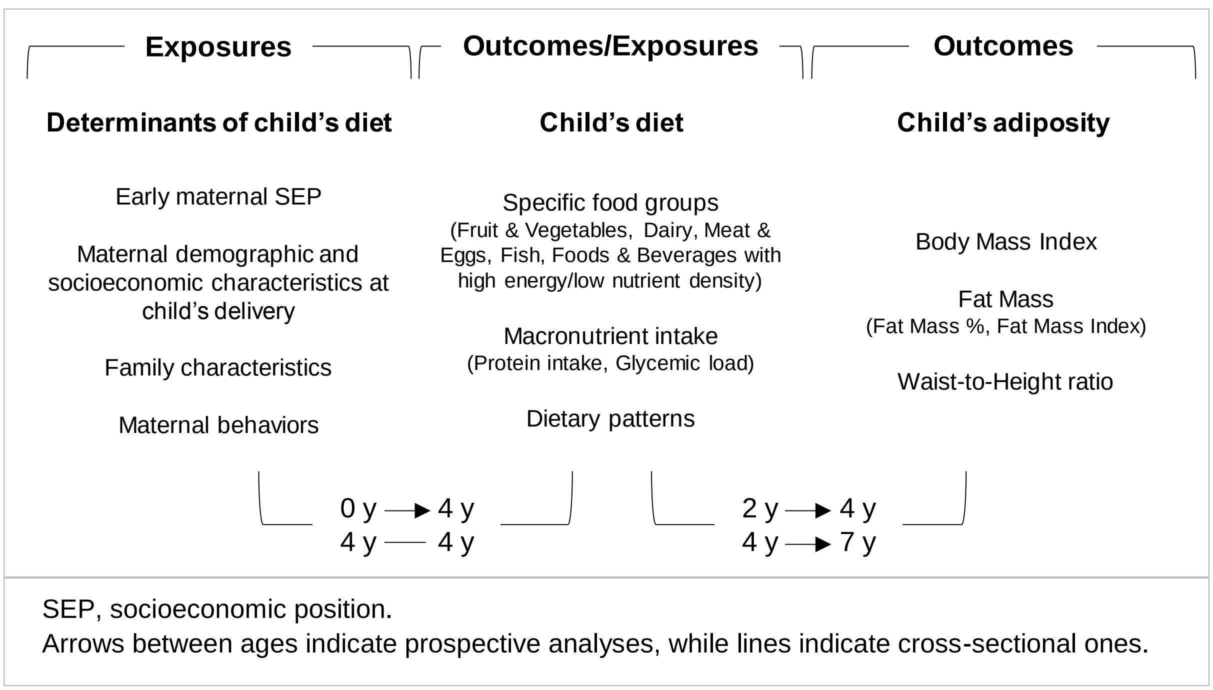


Figure 6. Exposures, outcomes and ages considered for analyses

2. Participants

At baseline, a participation proportion of 92% was achieved with 8647 newborns and their mothers (*n*=8495) being enrolled. Subsamples of the cohort were evaluated at the ages of 6 and 15 months (n=1555 and n=1043, respectively), but information collected during these waves was not used in the current thesis.

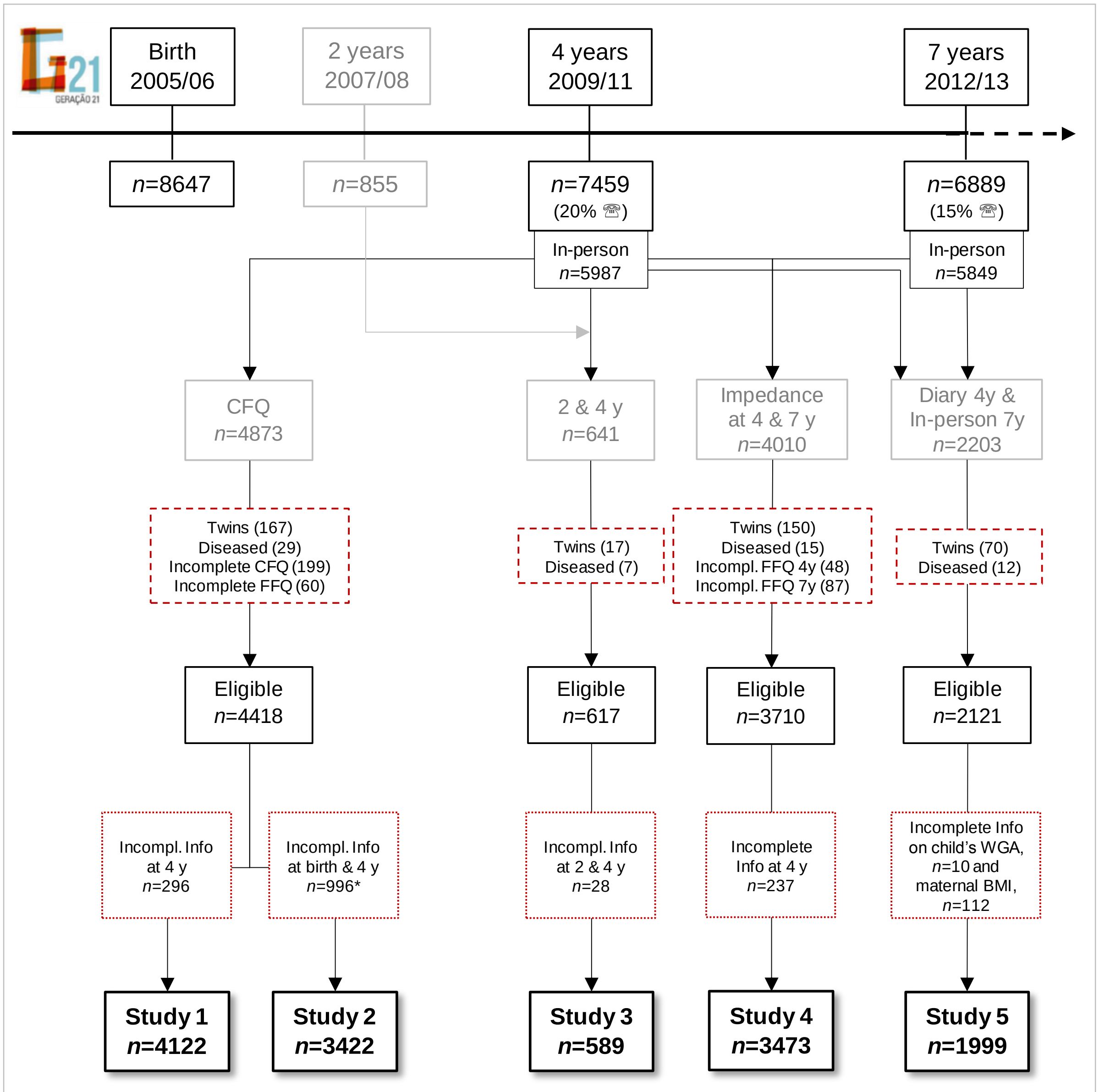
At child's 2 years a subsample of 855 children was reevaluated in two phases: those completing 2 years of age between April and August of 2007 were invited to participate in the first phase; later, all children born in January 2006 were invited to a similar evaluation in January 2008. For the current research, these two groups were combined, totaling 821 children of whom 641 were also evaluated at 4 years.

At both 4 and 7 years of age the entire cohort was invited to be reevaluated achieving respective participation proportions of 86% and 80%. Evaluation was 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 with a shorter version of the questionnaire.

For the first two studies – after exclusion of twins (*n*=167), children with congenital anomalies or diseases that might influence diet (*n*=29), incomplete child-feeding questionnaires (*n*=199), and incomplete information on child's diet (*n*=60) – a subsample of 4418 children was considered. In the first paper, those with incomplete information on BMI (*n*=56) as well as on maternal BMI (*n*=233) and education (*n*=7) at child's 4 years were excluded, 4122 children remaining for analysis. In the second study, after exclusion of subjects with missing information on variables of interest [maternal SEP at 12 years (*n*=77); characteristics at child's birth (family income *n*=481, maternal work status *n*=21, marital status *n*=7 and education *n*=16); characteristics at child's 4 years (siblings *n*=12, day-time caregivers *n*=7, maternal physical exercise *n*=13 and BMI *n*=45); and child's characteristics (screen time *n*=73, exercise *n*=13, BMI *n*=45], 3422 children remained for analysis.

In the prospective studies examining diet and adiposity, different subsamples were considered: i) children evaluated at 2 and 4 years (*n*=641); ii) children evaluated at both 4 and 7 years of age with complete information on body composition (*n*=4010); and those whose diet was evaluated by 3-days food diaries at 4 years (*n*=2493) also evaluated at 7 years (*n*=2203).

The following figure summarizes information on subsamples, exclusions, and participants included in each study.



BMI, Body Mass Index; CFQ, Child-Feeding Questionnaire; FFQ, Food Frequency Questionnaire; WGA, birthweight for gestational age.

Boxes in grey represent subsamples. Dashed red boxes, include information on exclusion criteria. Dotted red boxes present subjects with incomplete information on variables of interest.

*Higher incomplete information due to the substantial number of variables considered for analysis, missing data being particularly high on socioeconomic factors (SEP-12 *n*=77; family income at child's delivery, *n*=481).

Figure 7. Time frame, subsamples and participants included in final analyses

3. Data collection

At baseline, trained interviewers were located in the five hospitals in order to present the project G21 and to invite mothers in the course of the first 24 to 72 hours after delivery. At all evaluations, structured questionnaires designed by a multidisciplinary team, were used. Information was collected by trained interviewers, periodically supervised, responsible for application of the questionnaires to the mothers and for performing the anthropometric evaluation of parents and babies. Data on demographic and social characteristics, lifestyle behaviors (e.g., diet, physical activity), and medical history were collected.

3.1. Social and demographic characteristics

Information on socioeconomic conditions when mothers were 12 years of age was based on retrospective recall at the time of delivery, as described elsewhere (231). In brief, 12 items described SEP; 9 referring to family assets (e.g., house, car, washing machine, television, and telephone ownership), 2 referring to club or association membership (cultural, sports or other); and 1 considering the number of completed years of schooling of the father or mother (grandparents of the child enrolled in the G21 cohort). Based on this information, using models of latent class analysis, three categories of SEP at mothers' 12 years where identified representing low, intermediate, and high SEP (reference category).

Data on maternal demographic and socioeconomic characteristics at child's birth considered for this research were: age in completed years (used as continuous or categorized into <25, 25-29, >29 years); number of completed schooling years (used as continuous or categorized into ≤9, 10-12, >12 years); marital status (not married/cohabiting *vs.* married/cohabiting); work status [not working (unemployed, retired, housewife) or working (part/full-time, student, working-student)]; and family income (€/month) categorized into ≤1000, 1001-1500 and >1500.

Family characteristics at child's 4 years of age were also considered. Siblings' age was used to define a categorical variable (older and younger, only younger, only older, and no siblings), number of persons in the household was categorized into >4, 4 and <4 persons (reference category), and child's main day-time caregiver was defined as family (parent or other family member) or non-family (kindergarten or nanny).

3.2. Maternal behaviors

3.2.1. Smoking

Information on maternal smoking habits was collected during the face-to-face interview at child's 4 years. Mothers were classified as current smokers or non-smokers (including former smokers with at least six-months abstinence, and never-smokers). Besides age at smoking initiation, mothers were asked about number of cigarettes smoked as well as other types of tobacco used. Considering the almost nil contribution of other types of tobacco, smokers were categorized according to cigarette smoking (1-10 or >10 cigarettes/day).

3.2.2. Physical exercise

Maternal physical exercise, as considered in the current research, was also evaluated at child's 4 years of age. Information on type, frequency, duration, and intensity was collected by structured questions during the face-to-face interviews. Considering the proportion of mothers not practicing any regular structured physical exercise (80.5%), a nominal variable of practice of physical exercise was defined (no *vs.* yes).

3.2.3. Diet

Maternal diet was assessed at child's age of 4 years by a food frequency questionnaire (FFQ) adapted from a previous one validated for the general adult population (232) and for pregnant women in a subsample of the G21 cohort (233). The FFQ included 18 items and assessed consumption in the previous year with response options in a nine-point frequency scale ranging from never to ≥4 times/day. Frequencies were converted into daily frequencies (e.g., once a week was converted to 1/7 days, equaling 0.14 times/day).

An *a priori* dietary pattern (i.e., dietary score) was used to summarize maternal diet (234). In study 1 (figure 7), maternal diet was considered as potential confounding factor and the dietary score considered five food items/groups (milk, fish, red meat, fruit and vegetables, plus energy-dense foods and sugar-sweetened beverages). In study 2 (figure 7), maternal diet was a main exposure of interest and the dietary score considered eight food groups; milk, fish, red meat (red and processed meat), bread, fruit, vegetables (vegetable soup, and vegetables on a plate), foods high in energy density (cakes, salty pastry), and sugar-sweetened beverages.

In both studies each item/group was categorized into quartiles and 1 to 4 points were assigned according to increasing (milk, fish, bread, fruit, vegetables) or decreasing (red meat, foods high in energy density, sugar-sweetened beverages) quartiles of consumption. Points assigned were summed up, resulting in a dietary score that reflects maternal dietary quality. The score ranged from 5 to 20 points in study 1 and from 8 to 32 points in study 2, higher values corresponding to better maternal diet. In the two studies, scores were categorized into quartiles and the 4th quartile was considered as reference category.

3.2.4. Child-feeding practices

Maternal perceptions, concerns and child-feeding practices were assessed by a combined version of the Child-Feeding Questionnaire (66) and the scales of overt and covert control (235), self-administered to mothers. This version was previously adapted to Portuguese and validated as detailed elsewhere (236).

Examination of maternal child-feeding practices was performed considering two approaches; evaluation of six isolated maternal child-feeding practices or perceptions, and evaluation of maternal child-feeding patterns.

First, six isolated factors relative to children's diet (perceived responsibility, restriction, monitoring, overt control, covert control, and pressure-to-eat) were considered. Average scores were computed for each factor, ranging from 1 to 5 points with higher scores reflecting higher levels of maternal control.

In a second approach, three maternal child-feeding patterns identified by principal component analysis (93) were considered; 'perceived monitoring', 'restriction', and 'pressure-to-eat'. The pattern 'perceived monitoring' represents mothers with higher degrees of monitoring, perceived responsibility and overt control. The 'restriction' pattern is characterized by mothers with higher restriction, covert control and concerns about the child's weight. Mothers following the pattern 'pressure-to-eat' reported higher practice of pressuring the child to eat and higher overt control.

3.3. Child's characteristics and behaviors

3.3.1. Exact age

At each evaluation wave, child's exact age was computed in days using the birthdate and the date of each follow-up, further converted into exact age (neither rounded nor truncated) in months (age in days / 30.44) or years (age in days / 365.25) and was used as continuous in all analyses.

3.3.2 Physical activity

Physical exercise practice and daily time spent in front of a screen (television, computer, or game devices) were considered as proxies of physical activity at child's 4 years of age. Practice of physical exercise was assessed through a structured question assessing both type and duration (minutes per week) of physical exercise, converted into a nominal variable of regular practice of structured physical exercise (non-practitioners *vs.* practitioners) or into a continuous variable of weekly hours spent in regular practice of structured physical exercise. Daily screen time was also evaluated by a structured question assessing average daily minutes spent in front of a screen at both week and weekend days. Time was converted into hours per day and an average of daily screen time was calculated [(daily hours per day x 5 weekdays) + (daily hours per day x 2 weekend days) / 7 days], used either as continuous or categorized into >2 *vs.* ≤2 hours/day (237).

3.3.3. Diet

Information on breastfeeding as considered in this specific research was collected retrospectively at child's 4 years of age. Type of breastfeeding and duration were recorded and a variable of duration of non-exclusive breastfeeding was defined, being categorized into <6 months and ≥6 months (reference category).

At 2 years of age, diet was assessed by a 17-items FFQ aiming to assess consumption of foods and beverages high in energy density and/or low in nutrient density. Response options were in six-point frequency scale ranging from never to every day that were transformed into average daily frequencies. Energy-dense foods and beverages (EDF) 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 FFQ aiming to assess overall frequency of consumption in the previous six months. Response options were in nine-point frequency scale; ≥ 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. Average daily frequencies were also computed (e.g., 5-6 times/week was converted into a mean of 5.5 that, divided by 7 days, equaled 0.79 times/day).

In a first approach, consumption of specific food groups at child's 4 years was considered. By summing average daily consumption frequencies, five groups were defined based on similarity of food type and nutritional composition: fruit and vegetables (fruit, vegetable soup, raw vegetables, and cooked vegetables eaten on a plate); dairy (milk, yogurts, cheese); meat and eggs (red meat, white meat, and eggs); fish; and EDF (crisps, pizza, hamburgers, cakes, sweet pastry, chocolates, candies, carbonated and non-carbonated sugar-sweetened beverages).

In order to define dietary adequacy intervals, three food-based dietary guidelines for preschool children were selected (35, 36, 238) on the basis of the following criteria: i) same age frame; ii) clearly assessed for adequacy to meet nutritional recommendations for preschool children; and iii) with quantified food recommendations. After conversion of recommendations into comparable frequencies, intervals of adequacy were defined by identification of the lowest and highest frequencies between guidelines: fruit and vegetables, 4-7 times/day; dairy 3-5 times/day; meat and eggs, 5-10 times/week; and fish, 2-4 times/week. Dietary inadequacy was considered as below or above these intervals. As EDF are not recommended, but rather regarded as permissible (35), a single cut-point was defined dichotomizing consumption into ≥6 and <6 times/week (reference category).

In a second approach, empirically-derived overall measures of children's diet (i.e., *a posteriori* dietary patterns) were considered. At both 4 and 7 years of age, average daily consumption frequencies were computed and 16 food items/groups were defined. Five food groups were computed based on similarity of food type and nutritional composition; milk (whole, semi or skimmed), yogurt (sugared and non-sugared), meat and eggs (red meat, white meat, eggs), sweets (cakes, biscuits, chocolates, sugar, candies, and ice-cream), and sugar-sweetened beverages (colas, other carbonated beverages, iced tea, fruit drinks, and other non-carbonated beverages). Eleven foods were considered as isolated items; fruit, vegetable soup, vegetables on a plate, cheese, fish, processed meat, rice-pasta-potatoes, bread, crisps, pizza-burger, and salty pastry.

Both isolated items and groups were further categorized into; 1st quintile, 2nd to 4th quintiles combined, and 5th quintile. For foods eaten only once or twice a day (vegetable soup, and rice-pasta-potatoes) only two categories were defined (1 time/day and 2 times/day).

Macronutrient intake was considered as well. For a subsample of children evaluated in face-to-face interviews at both 4 and 7 years with complete 3-days food diaries at 4 years, energy and nutrient intakes were calculated using the software Food Processor SQL version 2004-5 (ESHA Research, Salem, Oregon, USA). Mean daily intakes of energy (kcal), and absolute mean intakes of macronutrients (g/day) were calculated for each child from the mean of the 3 days (2 weekdays, 1 weekend day) of food recorded by parents and day-time caregivers. Protein intake and dietary GL were main exposures of interest.

Protein intake and dietary GL were computed in g per day. As we were interested in their effect independently of their energy, these variables were energy-adjusted by the residuals method (239). Regression models with total energy intake as independent variable and protein or dietary GL as dependent variables were fitted separately for boys and girls. The predicted protein intake (or dietary GL) for the sex-specific mean energy intake (1581 and 1670 kcal, in girls and boys respectively) was added to the residuals in order to improve interpretability. Energy-adjusted protein intake and dietary GL were further categorized into sex-specific tertiles and the lowest tertile was used as reference category in analyses.

In order to compute dietary GL, foods reported in the diaries were assigned a GI value according to standardized procedure (240). Table A1 of the International Tables of Glycemic Index and Glycemic Load Values (241) was used as reference, and updates released on the University of Sydney website (242) were searched. As depicted in figure 8, foods were either assigned: i) a published GI value; ii) the GI value of a close match; iii) a calculated GI value (e.g., for recipes, the GI of each ingredient and recipe composition were used to calculate a GI value); iv) a nominal GI value (e.g., vegetables with no published GI were assigned a nominal GI value of 40); or no GI value (foods with a carbohydrate content <1g per 100g of food).

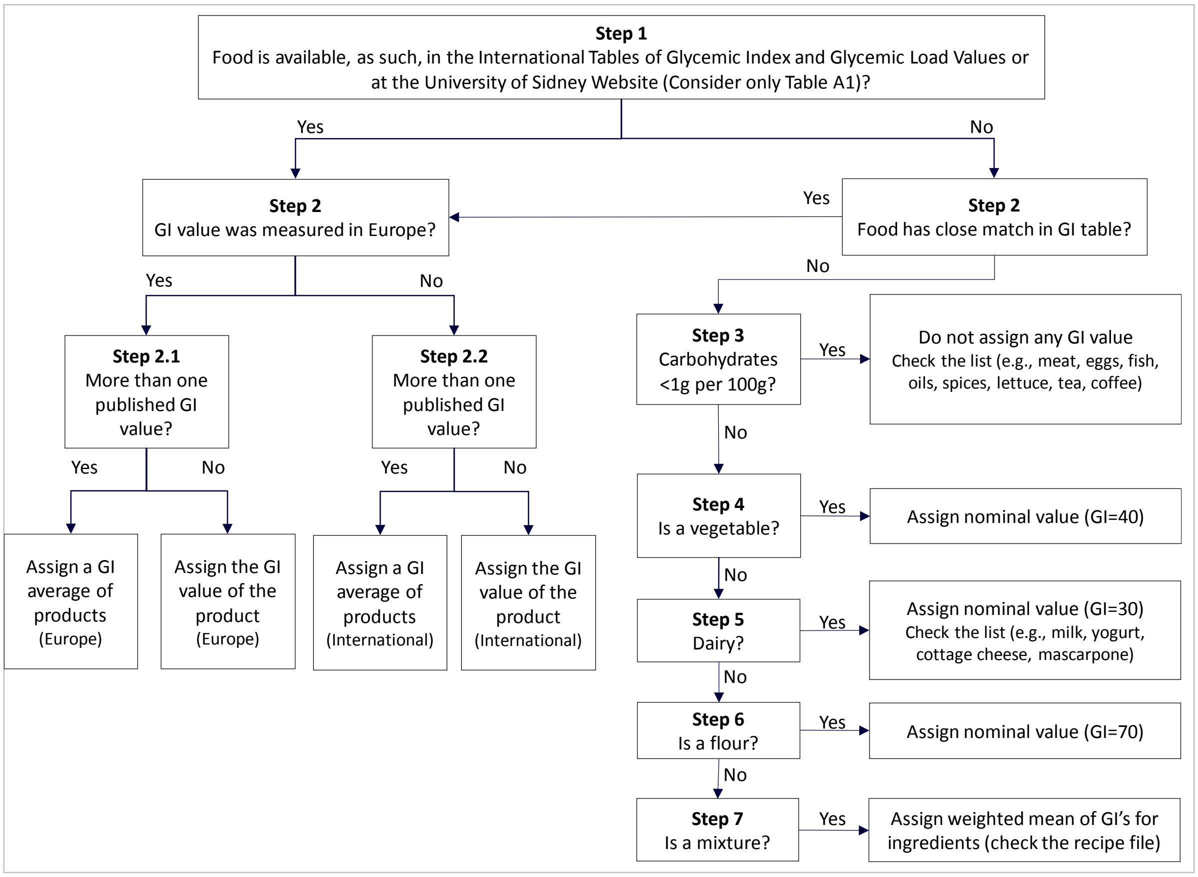


Figure 8. Flow chart for assigning GI values to food items [Adapted from the EPIC study (240)]

Each food's GL was calculated by multiplying the available carbohydrate content (total carbohydrate minus fiber) of one serving by the food's GI. Dietary GL was computed as the sum of these products for all food items consumed. As such, it represents quantity and quality of carbohydrates as well as their interaction (i.e., the multiplication of carbohydrates by GI means that a higher GI will have increased effect at higher intakes of carbohydrates).

3.4. Anthropometrics

Maternal pre-pregnancy BMI was calculated using self-reported weight and measured height at birth (in 72% of mothers) or height as recorded in the identity card. At child's 4 years, maternal 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) by trained staff. BMI was computed as weight in kg divided by height in squared meters and was used either as a continuous or categorical (<25 or ≥25 kg/m^2) variable in analyses.

Data on newborn's anthropometrics were evaluated as described elsewhere (243). In brief, weight at birth was abstracted from medical records by trained interviewers. During hospital stay, trained examiners weighed babies to the nearest 1 g using infant scales. Standard deviation scores (Z-scores) of birthweight adjusted for gestational age were computed as a continuous variable according to the Canadian reference (244).

At child's 2 years of age, either 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), which was also used to measure height at both 4 and 7 years of age. 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). 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 (11, 245).

Waist circumference was measured at the umbilicus level by trained personnel, according to standard procedures (246) and waist-to-height (W/Ht) ratio [$waist_{(cm)}/height_{(cm)}$] was computed for both 4 and 7 years, being further converted into sex-specific z-scores using the means and standard deviations observed in the current sample.

In 85% of children evaluated at 4 years and 98% of those evaluated at 7 years, body composition was assessed by tetrapolar bioelectric impedance (BIA 101 Anniversary®, Akern, Florence, Italy) according to standard procedures (247). Equipment outputs were converted into free-fat mass by Schaeffer's equation (248) and Fat Mass Percentage (FM%) plus Fat Mass Index [FMI ($Fat\ Mass_{(kg)}/Height_{(m)}^2$)] were derived accordingly, being further converted into z-score units using the sex-specific means and standard deviations observed in the present sample.

3.5. Fasting serum insulin

After an overnight fast, a venous blood sample was collected before 11 a.m., after applying a topical analgesic cream (EMLA cream). Fasting Serum Insulin (FSI) was measured using electrochemiluminescence immunoassay, was expressed in μIU/ml and was further converted into z-score units using the sex-specific means and standard deviations observed in the current sample.

4. Statistical analyses

Differences between proportions were tested with the Chi-squared test, considering Bonferroni's correction for *post hoc* multiple comparisons when appropriate. Average scores of maternal child-feeding practices were compared by the t-test for independent samples, and one-way analysis of variance with Bonferroni's correction for *post hoc* multiple comparisons was used to test for differences across dietary patterns.

Children's dietary patterns were identified by models of latent class analysis (162). This methodology focuses on relationships among individuals (person-centered approach) and describes how the probabilities of a set of observed categorical variables (e.g., FFQ's items) vary across groups of individuals (distinguishing clusters of individuals homogeneous within groups) in order to find the smallest number of latent classes by adding classes stepwise until the model fits the data well (162). Probabilities of choosing each item response, conditionally on class membership, can be interpreted based on profiles in each class (249, 250). The number of dietary patterns was defined using the Bayesian Information Criteria. Children were assigned to each pattern according to higher probability of class membership.

Depending on the nature of the dependent variable (continuous, or categorical), different models were fitted. For continuous outcomes, associations were estimated by regression coefficients (β) and respective 95% confidence intervals (95%CI), while for categorical ones they were estimated by odds ratios (OR) and respective 95%CI.

The association between maternal child-feeding practices and children's dietary inadequacy was assessed by multinomial (e.g., fruit and vegetables) or binomial (EDF) logistic regression models, using as reference the category in accordance with dietary recommendations. The association of family and maternal characteristics with children's dietary patterns was also estimated by multinomial logistic regression models with a healthier dietary pattern identified at preschool age used as reference category. The analysis was based in a predefined theoretical framework with four conceptual levels: i) SEP of the mother at 12 years of age; maternal socioeconomic and demographic characteristics at child's delivery; family characteristics at child's 4 years; and maternal behaviors (e.g., diet, child-feeding practices) at child's age of 4 years. Models were compared by the Likelihood Ratio Test for nested models and the coefficient of determination proposed for logistic regression, Nagelkerke's R-squared (251), was used to assess which factors were most important.

The association between consumption of EDF and BMI z-scores from 2 to 4 years was estimated by linear regression models and by cross-lagged panel design models. As depicted in figure 9, these models included three components: i) an analysis of exposure across time; ii) an analysis of the outcome across time; and iii) the cross-lagged analysis [exposure at time 1 on outcome at time 2 (a), and vice-versa (b)].

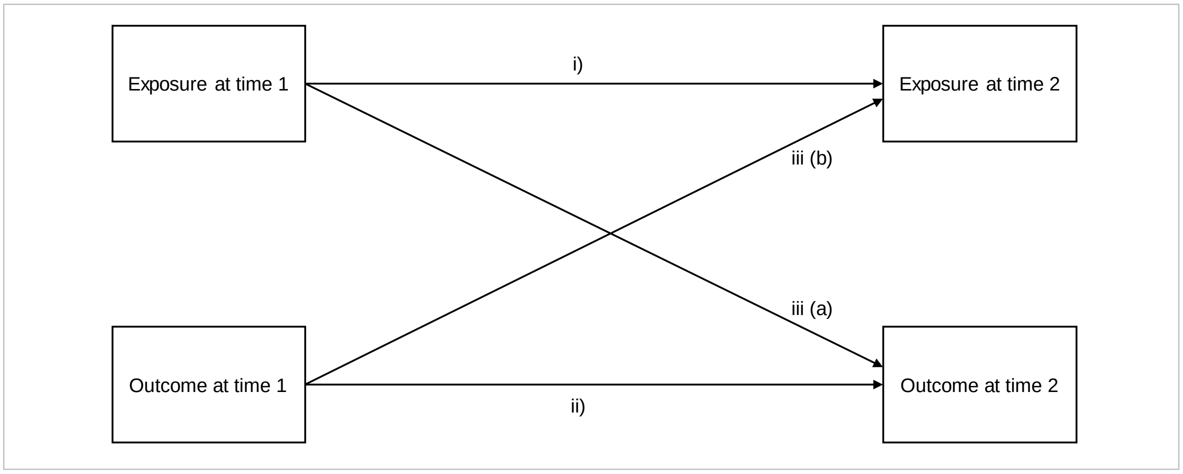


Figure 9. Cross-lagged panel design model

The association of children's dietary patterns with adiposity from 4 to 7 years was estimated in four separate models; BMI z-scores, FM% z-scores, FMI z-scores and W/Ht ratio z-scores. In order to assess temporal relationships of dietary patterns and adiposity across the two ages an analysis based on the cross-lagged concept (figure 9) was also conducted. Association of adiposity measures across the two ages and association of dietary patterns at 4 years with adiposity at 7 years were estimated by linear regression models. Associations of adiposity at 4 years with dietary patterns at 7 years and of dietary patterns across time were estimated by multinomial logistic regression models.

The associations of protein intake and dietary GL with later BMI z-scores, FMI, W/Ht ratio, and FSI were assessed by linear regression models.

In all analyses, potential confounding factors were evaluated on an individual basis and those that did not change the association of interest were not included in final models. Interactions between two variables were studied by addition of interaction terms (i.e., the product of the two variables) into the models. Dose-response relationships of variables were assessed with tests for linear trends, by modelling categorical measures as continuous variables.

Dietary patterns identification and the cross-lagged panel design models were analyzed using the software Mplus version 5.2 (Múthen & Múthen, Los Angeles, California, USA). The remaining analyses were performed using SPSS statistical software package version 21 (SPSS inc., Chicago, Illinois, USA). A significance level of 5% was adopted in all analyses.

5. Ethics

All the phases of this research complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (252). The study was approved by the University of Porto Medical School / S. João Hospital ethics committee and a signed informed consent according to Helsinki was required for all participants.

PAPERS

PAPER I

Maternal child-feeding practices and dietary inadequacy of 4-year-old children

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Research report

Maternal child-feeding practices and dietary inadequacy of 4-year-old children ☆

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A B S T R A C T

This study aimed to evaluate the association between maternal perceived responsibility and child-feeding practices and dietary inadequacy of 4-year-old children. We studied 4122 mothers and children enrolled in the population-based birth cohort – Generation XXI (Porto, Portugal). Mothers self-completed the Child Feeding Questionnaire and a scale on covert and overt control, and answered to a food frequency questionnaire in face-to-face interviews. Using dietary guidelines for preschool children, adequacy intervals were defined: fruit and vegetables (F&V) 4–7 times/day; dairy 3–5 times/day; meat and eggs 5–10 times/week; fish 2–4 times/week. Inadequacy was considered as below or above these cut-points. For energy-dense micronutrient-poor foods and beverages (EDF), a tolerable limit was defined (<6 times/week). Associations between maternal perceived responsibility and child-feeding practices (restriction, monitoring, pressure to eat, overt and covert control) and children's diet were examined by logistic regression models. After adjustment for maternal BMI, education, and diet, and children's characteristics (sex, BMI z-scores), restriction, monitoring, overt and covert control were associated with 11–18% lower odds of F&V consumption below the interval defined as adequate. Overt control was also associated with 24% higher odds of their consumption above it. Higher perceived responsibility was associated with higher odds of children consuming F&V and dairy above recommendations. Pressure to eat was positively associated with consumption of dairy above the adequate interval. Except for pressure to eat, maternal practices were associated with 14–27% lower odds of inadequate consumption of EDF. In conclusion, children whose mothers had higher levels of covert control, monitoring, and restriction were less likely to consume F&V below recommendations and EDF above tolerable limits. Higher overt control and pressure to eat were associated, respectively, with higher possibility of children consuming F&V and dairy above recommendations.

Abbreviations: BMI, body mass index; BMI z-scores, age- and sex-specific body mass index standard deviation scores according to the World Health Organization; CFQ, Child Feeding Questionnaire; 95% CI, 95% Confidence Interval; EDF, energy-dense micronutrient-poor foods and beverages; FFBDG, Flemish food-based dietary guidelines for preschool children; FFQ, Food Frequency Questionnaire; F&V, fruit and vegetables; OMD, Optimized Mixed Diet for German Children and Adolescents; OR, Odds Ratio; SD, Standard Deviation; USA, United States of America; USDA's Guidelines, United States Department of Agriculture's Dietary Guidelines for Americans 2010.

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Introduction

Parents play an important role in the development of children's food preferences, dietary choices and eating habits and, consequently, may contribute to childhood overweight (Davison & Birch, 2001). They may promote childhood overweight through their own lifestyle choices as well as by specific child-feeding practices used to control the child's diet (Davison & Birch, 2001; Scaglioni, Salvioni, & Galimberti, 2008; Ventura & Birch, 2008). Parenting child-feeding practices are defined as specific strategies used by parents to control what and how much their children eat, such as pressuring the child to eat, restricting access to certain foods and monitoring children's diet (Davison & Birch, 2001).

Research on the relationship of child-feeding practices with children's dietary intake has mainly focused on fruit and vegetables (F&V), and energy-dense micronutrient-poor foods and beverages (EDF) such as energy-dense snacks, showing conflicting results for, restriction (Coulthard & Blissett, 2009; Fisher & Birch, 1999; Gregory, Paxton, & Brozovic, 2011; Gribble, Falciglia, Davis, & Couch, 2003; Gubbels et al., 2009; Melbye, Øverby, & Øgaard, 2012; Taylor, Wilson, Slater, & Mohr, 2011; Ystrom, Barker, & Vollrath, 2012), monitoring (Arredondo et al., 2006; Gubbels et al., 2011; Hennessy, Hughes, Goldberg, Hyatt, & Economos, 2012; McGowan, Croker, Wardle, & Cooke, 2012) and pressure to eat (Gregory et al., 2011; Murashima, Hoerr, Hughes, & Kaplowitz, 2011; Taylor et al., 2011; Vereecken, Haerens, De Bourdeaudhuij, & Maes, 2010; Vereecken, Rovner, & Maes, 2010; Ystrom et al., 2012). The complex nature of the parental control construct may be one explanation for these contradictory findings (Ogden, Reynolds, & Smith, 2006), and it is possible that distinct instruments are measuring both beneficial and detrimental aspects of parental control. Ogden, Reynolds and Smith (Ogden et al., 2006) proposed two new dimensions to distinguish different aspects of restriction – 'overt control' and 'covert control'. Overt control consists of limiting the child's intake of unhealthy foods in a way perceived by the child (e.g., being firm about how much the child should eat) and covert control consists of limiting intake in a way undetected by the child (e.g., not buying unhealthy foods) (Ogden et al., 2006). Higher levels of covert control have been associated with lower consumption of unhealthy snacks (Brown, Ogden, Vogele, & Gibson, 2008; Ogden et al., 2006), while higher overt control has been related to higher healthy snacks consumption (Ogden et al., 2006), but also to lower consumption of chocolate candies (Ogden, Cordey, Cutler, & Thomas, 2013). Both forms of control have been positively associated with F&V consumption (Brown et al., 2008). However, the number of studies is limited (Brown et al., 2008; Ogden et al., 2006, 2013; Rodgers et al., 2013) and more research is needed about overt and covert control to better clarify the association of these specific child-feeding practices with children's diet.

The specific focus of research on F&V and EDF may be producing only a partial picture of the association between parenting child-feeding practices and children's diet. It is important to evaluate a broader range of foods and to account for dietary inadequacy of children's diet. Therefore, the objective of the present study was to evaluate the association between maternal perceived responsibility and child-feeding practices (restriction, monitoring, pressure to eat, overt and covert control) and dietary inadequacy of 4 year-old children.

Materials and methods

Participants

This study included participants of the population-based birth cohort Generation XXI, described elsewhere (Larsen et al., 2013). A total of 8647 live newborns and their mothers ($n = 8495$) were enrolled between April 2005 and August 2006 at all five public maternity units that cover the six municipalities of the metropolitan area of Porto (Northern Portugal) which, at enrollment, were responsible for 95% of the deliveries in the whole catchment population. Of those invited, 92% mothers agreed to participate.

When children were 4 years of age, the entire cohort was invited to a re-evaluation and 7458 agreed to participate (86% participation proportion). During this evaluation wave, information was collected in face-to-face interviews from April 2009 until April 2011 and, for those families that were not able to participate in-person (20%), the evaluation was performed by telephone using a shorter version of the questionnaire. A self-administered child-feeding questionnaire was sent by post, with instructions to return it during the face-to-face evaluation. Mothers who forgot to bring the questionnaire were invited to fill in another one during their child's evaluation. Among those participating in-person, 4873 mothers returned the questionnaire on child-feeding practices. After exclusion of twins ($n = 167$), participants with congenital anomalies or diseases that might influence dietary intake (cerebral palsy, celiac disease, food allergy, food intolerance and phenylketonuria) ($n = 29$), and mothers with incomplete information on child-feeding practices ($n = 199$), a total of 4478 participants with complete child-feeding questionnaires remained for analysis. We further excluded children with incomplete information on dietary intake ($n = 60$) and body mass index (BMI) ($n = 56$), as well as mothers with missing information on education ($n = 7$) and incomplete information on BMI ($n = 233$). A total of 4122 participants with complete information were included in the final analysis.

Data collection

Information on socioeconomic and demographic characteristics, health history and lifestyles was collected for both mother and child in face-to-face interviews conducted by trained interviewers, using structured questionnaires applied to the child's caregiver (in the present sample, 99.8% were mothers). Mothers' and children's height and weight were measured by trained staff; body weight was measured to the nearest 0.1 kg using a digital scale (Tanita®, Arlington Heights, IL, USA) and height was measured to the nearest 0.1 cm with a wall stadiometer (SECA®, Hamburg, Germany). Children's age- and sex-specific BMI standard deviation scores (BMI z-scores) were defined according to the World Health Organization (2006).

Maternal perceived responsibility and child-feeding practices

Maternal perceived responsibility and child-feeding practices were assessed by a combined version of the Child Feeding Questionnaire (CFQ) (Birch et al., 2001) and the scales of overt and covert control (Ogden et al., 2006), self-administered to mothers. This version was previously adapted to Portuguese and validated as described elsewhere (Real, Oliveira, Severo, Moreira, & Lopes, 2014).

Six factors, relative to children's dietary intake, were included in the present analysis showing the following Cronbach's alpha in the current sample: perceived responsibility, 0.830; restriction, 0.711; monitoring, 0.893; overt control, 0.800; covert control, 0.800; and pressure to eat, 0.686. All items were measured using a 5-point Likert scale. For each factor, an average score was computed which ranged from 1 to 5, higher scores reflecting higher levels of maternal child-feeding practices. These average scores were introduced into the models as continuous variables.

Maternal diet

Maternal dietary intake was evaluated by Food Frequency Questionnaire (FFQ), applied by trained interviewers. Frequencies of consumption were converted into daily frequencies (e.g., once a week was converted into 1/7 days = 0.14 times/day). In order to classify maternal diet, three items (milk, fish, red meat) and two food groups (F&V: fruit, vegetable soup, vegetables eaten on a plate; and EDF: cakes, sugar-sweetened beverages) were considered. Each food item/group was then categorized into quartiles and a dietary score was computed by assigning 1 to 4 points, according to increasing quartiles of consumption for milk, fish, F&V or decreasing quartiles of consumption for red meat and EDF. Points assigned to each food item/group were then summed up, resulting in a dietary score that could range from 5 to 20 points, higher scores reflecting better dietary habits. This score was further categorized into quartiles.

Children's dietary inadequacy

Children's dietary intake was evaluated by a 35-item FFQ, applied to the child's main caregiver in face-to-face interviews conducted by trained staff. The FFQ was developed to assess overall dietary intake of children in the previous six months. Response options were in a nine-point frequency scale; ≥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. For a subsample of 2373 children, 3-day food diaries were also completed and, in order to assess the validity and reliability of the FFQ, Pearson's correlation coefficients and intraclass correlation coefficients were calculated for key food groups as measured by both methods. For those food groups most frequently consumed, weak-to-moderate correlations and fair-to-moderate agreement were observed. Significant positive moderate Pearson's correlations were found for vegetable soup ($r = 0.54$, $p < 0.001$), fruit ($r = 0.42$, $p < 0.001$), milk ($r = 0.46$, $p < 0.001$), and yogurts ($r = 0.48$, $p < 0.001$), and not so strong for cheese ($r = 0.35$, $p < 0.001$), fish ($r = 0.27$, $p < 0.001$), vegetables eaten on a plate ($r = 0.26$, $p < 0.001$), eggs ($r = 0.21$, $p < 0.001$), and non-carbonated sugar-sweetened beverages (iced tea $r = 0.29$, $p < 0.001$; juices $r = 0.22$, $p < 0.001$). Intraclass correlation coefficients varied from 0.54 [95% Confidence Interval (95% CI); 0.51; 0.56] for vegetable soup to 0.17 (95% CI: 0.11; 0.32) for juices.

Frequencies of consumption were converted into daily or weekly frequencies (e.g., 2–4 times/week was converted into a mean of 3 times/week, equivalent to 3/7 days = 0.43 times/day). Five food groups were defined: F&V (fruit, vegetable soup, raw and cooked vegetables eaten on a plate); dairy (milk, yogurts, cheese); meat and eggs (pork, beef, veal, lamb, rabbit, poultry, eggs); fish (all types of fish, including shellfish and seafood); and EDF (sugar-sweetened beverages, crisps, pizza, burger, cakes, sweet pastry, chocolate and candies).

As food-based dietary guidelines for preschool children are not available in Portugal (Rodrigues, Franchini, Graça, & de Almeida, 2006) and because guidelines vary (European Food Safety Authority, 2010; World Health Organization, 2003) increasing the probability of misclassification if a single guideline is used, we decided to define children's dietary adequacy in intervals, rather than using single cut-points. In order to define these intervals, food-based dietary guidelines providing recommendations for preschool children were chosen on the basis of the following criteria: i) same age frame as children in the present study; ii) clearly assessed for adequacy to meet nutritional recommendations for preschool children; iii) with quantified food recommendations. Three guidelines were selected: the United States Department of Agriculture's Dietary Guidelines for Americans 2010 (USDA's Guidelines) (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2010); the Optimized Mixed Diet for German Children and Adolescents (OMD) (Kersting, Alexy, & Clausen, 2005); and the Flemish Food-Based Dietary Guidelines for Preschool Children (FFBDG) (Huybrechts & De Henauw, 2007; Huybrechts et al., 2008, 2010) (Table 1). These guidelines are not directly comparable, as they are not presented in the same units and types of foods included in groups differ. USDA's guidelines are quantified in cups and ounces (or kcal from solid fats and added sugars), provide portion sizes for preschool children (U.S. Department of Agriculture, 2012), and include potatoes and legumes in the vegetable group. Germany's OMD is quantified in grams and does not present portion sizes. FFBDGs also do not present portion sizes and are quantified in grams or, for milk, in milliliters.

Using appendix 6 of the USDA's Guidelines (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2010) estimated energy requirements for moderately active children aged 4 to 6 years were considered (1400–1600 kcal, 5858–6694 kJ) and, using appendix 7, recommendations were collected for fruit, vegetables (dark green, red and orange, and other vegetables), protein foods (meat, poultry, eggs, seafood) and solid fats and added sugars. Weekly recommendations (vegetables, protein foods) were converted into daily recommendations (weekly amount/7 days). Based on USDA's Healthy Eating for Preschoolers (U.S. Department of Agriculture, 2012), daily amounts were then converted into frequencies (e.g., 1½ cup of fruit per day divided by the portion size, ½ cup, corresponds to a frequency of 3 daily servings). As the other two guidelines (OMD, FFBDG) did not provide portion sizes, after converting milk presented in milliliters into grams (1.04 g/mL), food amounts were converted into frequencies using ranges of portion sizes recently proposed for preschool children aged 1 to 4 years (More & Emmett, 2014). Averages of the upper limits of these ranges were chosen as covering 4 year-old children (e.g., 200 g of fruit recommended in the OMD, were divided by the calculated average portion size, 78 g, being converted into 2.6 times/day). Concerning meat, eggs and fish, this methodology was used for the three guidelines, using the average portion size of 60 g after conversion of ounces into grams (28 g/oz).

Intervals of adequacy were finally defined by identification of the lowest and highest frequencies between the three guidelines. As can be observed, the final intervals considered as adequate in the present study were: 4–7 times/day for F&V, 3–5 times/day for dairy, and 1–2 times/day for meat, fish and eggs (Table 1). This last interval was further converted into weekly frequencies (7–14 times/week) and the weekly interval identified for fish (2 to 4 times/week) was subtracted from the total of 'meat, fish and eggs', an interval of 5 to 10 times/week remaining for 'meat and eggs'. Finally, as EDF are not recommended but rather regarded as permissible, and considering that for some of these foods (e.g., pizza, burger) the tolerable daily amount would not reach one serving, a single cut-point was defined, dichotomizing consumption into: '≥6 times/week' vs. '<6 times/week'.

Statistical analysis

Mean scores of perceived responsibility and child-feeding practices according to maternal and child characteristics were compared by the t-test for independent samples. Associations between maternal perceived responsibility and child-feeding practices and children's dietary inadequacy were estimated by crude and adjusted odds ratios (OR) and respective 95% CI, by multinomial unconditional logistic regression models (in the case of F&V, dairy, meat and eggs, and fish), and binomial unconditional logistic regression models (in the case of EDF), using as reference those categories in accordance with the dietary recommendations (Table 1).

Six separate univariate models were fitted for each factor of maternal perceptions and child-feeding practices (independent variables). Into these models, potential confounders assessed at child's 4 years of age were introduced, one-by-one, by sequential forward selection. For theoretical reasons (Davison & Birch, 2001; Ventura & Birch, 2008), child's sex and BMI z-scores, as well as maternal BMI, were forced to remain in all models. Besides these, other

Table 1
Food-based dietary guidelines for preschool children and frequencies of consumption intervals defined as adequate.

	USDA 4–6 years 5021–6694 kJ	OMD 4–6 years 6067 kJ	FFBDG 3–6 years 5798–6995 kJ	USDA's Portion sizes	ALSPAC's Portion sizes[§§]	USDA[¶¶]	OMD[¶]	FFBDG[¶]	Frequencies of consumption intervals defined as adequate[**]
	Cups,[a] kJ,[b] oz[c]	g	g	Cups,[a] oz[c]	g	Servings	Servings	Servings	Servings
Fruit, day	1.5[a]	200.0	100.0–150.0	0.5[a]	78.0	3.0	2.6	1.6–3.2	4.0–7.0
Vegetables, day	0.9–1.3[a*]	200.0	125.0–250.0	0.5[a]	45.0	1.8–2.8	4.4	2.2–3.3	
Milk products, day	2.5[a]	350.0	520.0–624.0[‡]	0.5[a]	125.0	5.0	2.8	4.0–5.0	3.0–5.0
EDF, day	506[b]	50	30	–	–	<1.0	<1.0	<1.0	<1.0[††]
Meat, fish and eggs, day	3.0–4.0[c]	70.0	75.0–100.0	1.0[c]	60.0	1.4–1.9	1.2	1.3–1.7	1.0–2.0
Meat, fish and eggs, week	–	–	–	–	–	–	–	–	7.0–14.0[‡‡]
Fish, week	6.0–8.0[c]	150.0[†]	137.5[§]	–	60.0	2.8–3.7	2.5	2.3	2.0–4.0[‡‡]
Meat and eggs, week	–	–	–	–	–	–	–	–	5.0–10.0[‡‡]

USDA, United States Department of Agriculture's Dietary Guidelines for Americans 2010 (U.S. Department of Agriculture, 2012; U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2010); OMD, Optimized Mixed Diet for German Children and Adolescents (Kersting et al., 2005); FFBDG, Flemish food-based dietary guidelines for preschool children (Huybrechts & De Henauw, 2007; Huybrechts et al., 2008, 2010); ALSPAC, The Avon Longitudinal Study of Parents and Children (More & Emmett, 2014); EDF, energy-dense micronutrient-poor foods and beverages.

[a] Cups.
[b] Kilojoules.
[c] Ounces.
[*] Potatoes and legumes have been excluded and weekly recommendations for dark-green vegetables (1.0–1.5 cups), red and orange vegetables (3.0–4.0 cups) and other vegetables (2.5–3.5 cups) were summed up giving a range of 6.5 to 9.0 cups/week, which when divided by 7 days resulted in an interval of 0.9–1.3 cups/day.
[†] The recommendation for fish is given as one portion (100–200.0 g), which has been converted to an average of 150.0 g.
[‡] The recommendation for milk is given in milliliters (500.0–600.0 mL), having been converted into grams using the specific mass of milk (1.04 g/mL).
[§] The recommendation for fish is given as once to twice a week (75.0–100.0 g) which ranges from 75.0 to 200.0 g with an average of 137.5 g.
[¶] Servings were calculated through the division of recommended amounts by the average portion sizes calculated from ALSPAC.
[**] The intervals of frequencies defined as adequate were identified through the lowest and highest servings proposed between the three guidelines.
[††] As in ALSPAC average portion sizes vary a lot depending on the EDF consumed (cakes and pastries 47.0 g, sweets and candies 17.0 g, crisps 30.0 g, soft drinks 124.0 g) calculation of an average was deemed inappropriate. Considering that usual portion sizes for some EDF (soft drinks, pizza, burger) are higher than daily allowances, a single cut-point of <6.0 times/week was considered as tolerable.
[‡‡] Multiplying the daily interval by 7 days, a weekly interval of 7.0–14.0 was defined. From this interval, weekly frequencies for fish (2.0–4.0) were subtracted and an interval of 5.0–10.0 times/week remained for meat and eggs.
[§§] Portion sizes for 1 to 4 years of age are given in ranges. The upper limits of these ranges, for foods commonly eaten in Portugal, were used to determine average portion sizes for fruit, vegetables, meat and eggs, and fish for 4-year-old children.
[¶¶] Servings were calculated through the division of recommended amounts by the USDA's portion sizes (fruit, vegetables, milk products). As the portion size for meats, fish and eggs is only one ounce (28 g), in order to have comparable values, recommendations for these groups were converted into servings using the average portion sizes calculated from ALSPAC.

tested variables, also assessed at child's 4 years of age, that did not have a significant effect on the outcome (children's dietary inadequacy) and that did not change the association of interest (based on a change over 10% in the OR) were removed (child's age, practice of physical exercise, daily screen time, siblings, day-time caregivers, doctor used for routine medical visits, breastfeeding, maternal practice of exercise, and maternal smoking). The final model included as covariates: maternal BMI in kg/m², maternal education as the number of completed schooling years, and child's BMI z-scores as continuous variables. Categorical variables included were child's sex and maternal dietary score categorized into quartiles. Interactions between maternal BMI, child's BMI, child's sex and maternal child-feeding practices on children's dietary inadequacy were assessed by interaction terms added into the models.

Statistical analysis was conducted using SPSS statistical software package version 21 (SPSS inc., Chicago IL., USA) and a significance level of 5% was adopted.

Ethics

All the phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (World Medical Association, 2013). The study was approved by the University of Porto Medical School/S. João Hospital Centre ethics committee and a signed informed consent according to the Declaration of Helsinki was required for all participants.

Results

In the present sample children had a mean age of 4.3 years (SD = 0.39), a mean BMI z-score of 0.6 (SD = 1.08), and 51.1% were boys. The mean age of mothers was 34.1 years (SD = 5.19), their mean BMI was 26.3 kg/m² (SD = 5.12) and their mean education was 11.4 complete schooling years (SD = 4.20). Comparison of baseline characteristics between this sample and the remaining cohort, showed that mothers in the present sample were slightly older (mean = 30.2 years; SD = 5.22 vs. mean = 29.2 years; SD = 5.70; $p < 0.001$) and more educated (mean = 11.2 complete schooling years; SD = 4.25 vs. mean = 10.0 complete schooling years; SD = 4.20; $p < 0.001$). No significant differences were found for child's sex (51.1% vs. 50.6% boys; $p = 0.625$).

Mean scores of maternal perceived responsibility and child-feeding practices according to maternal and child's characteristics are shown in Table 2. Mothers reported high mean scores of child-feeding practices and perceived responsibility, ranging from 3.2 (covert control) to 4.4 (perceived responsibility). More educated mothers exhibited lower mean scores of perceived responsibility, overt control and pressure to eat, and higher mean scores of restriction and covert control than less educated mothers. Mothers with children at risk of overweight, overweight or obese reported lower mean scores in monitoring and pressure to eat, while mean scores of covert control were higher (Table 2).

As shown in Table 3, children below the lower boundary of the defined adequacy intervals were 33.5% for F&V, 13.4% for dairy, 7.0% for meat and eggs, and 6.4% for fish, while children above the upper boundary were 15.4% for F&V, 39.3% for dairy, 22.2% for meat and eggs, 34.3% for fish and 84.4% for EDF.

Crude and adjusted associations of maternal perceived responsibility and child-feeding practices with children's dietary inadequacy are also shown in Table 3. In multivariate analysis, children of mothers reporting higher levels of overt and covert control were significantly less likely to consume F&V below the interval defined as adequate. However, concerning perceived responsibility and overt control, children were also significantly more likely to consume F&V above it. Higher levels of maternal restriction and monitoring were significantly negatively associated with F&V consumption below recommendations. Children whose mothers reported higher levels of pressure to eat were significantly more likely to consume dairy above the interval defined as adequate. Perceived responsibility was also significantly positively associated with the odds of children consuming dairy above this interval. None of the factors showed significant associations with children's consumption of meat and eggs or fish. In contrast, maternal perceived responsibility and child-feeding practices (except for pressure to eat) were significantly and negatively associated with consumption of EDF above the tolerable limit.

Discussion

The present study, conducted in 4 year-old children, showed that maternal restriction, monitoring, overt and covert control were associated with lower consumption of EDF and with lower odds of children consuming F&V below the recommendations. Additionally, it also showed that overt control and pressure to eat were, respectively, associated with consumption of F&V and dairy above recommendations, suggesting that it is possible that these

Table 2
Mean scores of maternal perceived responsibility and child-feeding practices according to maternal and child's characteristics ($n = 4122$).

	Perceived responsibility		Restriction		Monitoring		Overt control		Covert control		Pressure to eat	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total mean	4.4	0.61	4.3	0.72	4.2	0.77	4.1	0.58	3.2	0.86	3.7	0.97
Maternal education												
<12 years	4.4	0.61	4.3	0.77	4.2	0.82	4.2	0.64	3.1	0.87	3.9	0.92
≥12 years	4.3	0.61	4.4	0.68	4.3	0.72	4.1	0.53	3.2	0.84	3.5	0.99
p-value	<0.001		0.011		0.067		<0.001		0.002		<0.001	
Maternal BMI												
Underweight/normal	4.3	0.61	4.3	0.72	4.3	0.73	4.1	0.55	3.2	0.86	3.7	0.97
Overweight/obese	4.4	0.61	4.3	0.73	4.2	0.80	4.1	0.61	3.2	0.86	3.7	0.98
p-value	0.047		0.702		0.076		0.359		0.437		0.862	
Child's sex												
Girls	4.4	0.62	4.3	0.75	4.2	0.76	4.1	0.59	3.2	0.86	3.6	0.98
Boys	4.4	0.61	4.4	0.70	4.2	0.78	4.2	0.58	3.2	0.85	3.7	0.97
p-value	0.154		0.124		0.842		0.292		0.860		0.077	
Child's BMI z-score												
Underweight/normal	4.4	0.61	4.3	0.72	4.3	0.76	4.1	0.58	3.1	0.86	3.8	0.92
At risk overweight/overweight/obese	4.3	0.61	4.4	0.73	4.2	0.78	4.1	0.58	3.2	0.84	3.4	1.03
p-value	0.050		0.256		0.018		0.737		0.018		<0.001	

SD, standard deviation; BMI, body mass index; BMI z-scores, body mass index standard deviation scores according to the World Health Organization (2006).

Table 3
Crude and adjusted odds ratios for the association between maternal perceived responsibility and child-feeding practices and children's dietary inadequacy (n = 4122).

	F&V[a]				Dairy[a]				Meat and eggs[a]				Fish[a]				EDF[a]	
	<4/day		>7/day		<3/day		>5/day		<4/week		>10/week		<2/week		>4/week		≥6/week	
	n = 1382 (33.5%)		n = 634 (15.4%)		n = 552 (13.4%)		n = 1622 (39.3%)		n = 289 (7.0%)		n = 915 (22.2%)		n = 264 (6.4%)		n = 1413 (34.3%)		n = 3478 (84.4%)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Crude[b]																		
Perceived responsibility	0.90	0.81–1.01	1.21	1.04–1.41	1.00	0.86–1.16	1.16	1.04–1.29	1.11	0.91–1.36	1.02	0.91–1.16	0.95	0.78–1.17	1.07	0.96–1.19	0.89	0.77–1.02
Restriction	0.85	0.78–0.93	1.04	0.91–1.18	1.02	0.90–1.17	0.95	0.86–1.03	0.97	0.83–1.15	1.02	0.92–1.13	0.90	0.76–1.06	1.10	1.01–1.21	0.78	0.69–0.89
Monitoring	0.85	0.78–0.93	1.04	0.92–1.17	0.94	0.84–1.06	0.99	0.91–1.08	0.98	0.84–1.15	1.07	0.97–1.18	0.97	0.83–1.15	1.03	0.95–1.12	0.84	0.74–0.94
Overt control	0.85	0.76–0.96	1.20	1.02–1.40	0.91	0.78–1.07	1.14	1.02–1.28	0.91	0.74–1.11	0.90	0.80–1.02	0.85	0.69–1.05	1.11	0.99–1.25	0.80	0.69–0.93
Covert control	0.82	0.76–0.89	1.16	1.04–1.29	1.02	0.91–1.14	0.91	0.84–0.99	1.11	0.96–1.28	0.96	0.88–1.04	0.85	0.74–0.99	1.20	1.11–1.30	0.73	0.66–0.81
Pressure to eat	1.11	1.03–1.19	0.85	0.78–0.92	0.95	0.86–1.04	1.13	1.06–1.21	1.03	0.91–1.16	0.99	0.92–1.07	1.11	0.97–1.27	0.85	0.80–0.91	1.13	1.04–1.23
Adjusted[c]																		
Perceived responsibility	0.89	0.79–1.00	1.24	1.06–1.46	1.01	0.86–1.18	1.12	1.00–1.25	1.06	0.87–1.31	1.05	0.93–1.19	0.94	0.77–1.15	1.05	0.94–1.18	0.85	0.73–0.99
Restriction	0.89	0.81–0.98	0.99	0.87–1.13	1.03	0.90–1.18	0.96	0.87–1.05	0.95	0.81–1.12	1.02	0.92–1.13	0.96	0.81–1.13	1.04	0.94–1.14	0.81	0.72–0.93
Monitoring	0.88	0.80–0.96	1.03	0.91–1.17	0.94	0.84–1.07	1.00	0.92–1.10	0.97	0.83–1.13	1.06	0.96–1.18	1.01	0.86–1.19	0.99	0.91–1.09	0.86	0.76–0.97
Overt control	0.82	0.73–0.92	1.24	1.05–1.47	0.92	0.78–1.08	1.09	0.97–1.22	0.86	0.70–1.05	0.93	0.82–1.06	0.83	0.68–1.02	1.12	0.99–1.27	0.73	0.62–0.86
Covert control	0.89	0.82–0.96	1.07	0.96–1.19	1.03	0.92–1.15	0.93	0.86–1.00	1.07	0.92–1.23	0.96	0.88–1.05	0.92	0.79–1.07	1.08	0.99–1.17	0.80	0.72–0.89
Pressure to eat	1.00	0.93–1.09	0.91	0.83–1.00	0.95	0.86–1.05	1.08	1.01–1.17	1.05	0.92–1.19	0.99	0.91–1.07	0.97	0.84–1.12	0.93	0.87–1.00	1.01	0.92–1.11

F&V, fruit and vegetables; EDF, energy-dense micronutrient-poor foods and beverages; OR, odds ratio; 95% CI, 95% confidence interval.
[a] Children within the range of intervals defined as adequate were used as reference categories and odds ratios for these categories are not shown to avoid redundancy. Reference categories; 4–7 times/day for F&V, 3–5 times/day for dairy, 5–10 times/week for meat and eggs, 2–4 times/week for fish, <6 times/week for EDF.
[b] Univariate models, one for each practice.
[c] Multivariate models, one for each practice, adjusted for children's (sex, body mass index standard deviation scores according to the World Health Organization (2006)) and maternal (body mass index, education, and diet) characteristics.

practices may be associated with overconsumption of food groups usually regarded as healthy.

In European samples, restrictive parental practices have shown comparable results to the findings of this study, being associated with lower consumption of EDF and higher consumption of F&V in 2-year-old children (Gubbels et al., 2009), as well as with a more wholesome diet and a less unhealthy diet among 3-year-old children (Ystrom et al., 2012). Although in the present sample restriction seems to be beneficial at 4 years of age, this result should be regarded with care. Longitudinal studies in preschool children (Birch, Fisher, & Davison, 2003; Francis & Birch, 2005) have shown restriction to be associated with subsequent higher consumption of palatable snacks in the absence of hunger due to higher preference for the restricted food and higher responsiveness to external cues such as the presence of freely available palatable foods. Therefore, this practice may have detrimental effects later on. Nevertheless, there is also the possibility that some level of parental control, probably a moderate level (Blissett, 2011), may be beneficial. Concerning monitoring, overt control, covert control, and pressure to eat, the present study is partly comparable to other studies. In a sample of preschool children from the United Kingdom (McGowan et al., 2012) monitoring was associated with higher F&V consumption and lower non-core food consumption, and covert and overt control were both associated with higher consumption of F&V in Southern England samples (Brown et al., 2008), while – in school-aged children from Flanders (Vereecken, Haerens et al., 2010) – pressure to eat was not associated with consumption of EDF or F&V.

Using the current perspective of non-compliance with dietary guidelines, comparison of present results with other studies is difficult. The findings that maternal perceived responsibility and overt control were associated with consumption of F&V above recommended frequencies and that pressure to eat was similarly associated with consumption of dairy, may be explained by maternal perceptions about what constitutes a healthy diet. Maternal overestimation of the quality of children's diet has been shown to be high (Kourlaba, Kondaki, Grammatikaki, Roma-Giannikou, & Manios, 2009) and it is possible that an overestimation of consumption of foods usually regarded as healthy by parents (Brewis & Gartin, 2006) may have occurred in the present study. If overestimation of healthy foods occurred, it would also be expected to have underestimation of foods considered less healthy by parents (Brewis & Gartin, 2006) such as EDF. However, the prevalence of children reported as consuming these foods above the tolerable limit was very high in the current sample (84%). Keeping this in mind, there is another possible explanation related with maternal perceptions which is that mothers included in this study may believe that there is no upper limit for consumption of healthy foods.

The observation that overt control and pressure to eat were associated with higher odds of children consuming, respectively, F&V and dairy above the interval defined as adequate may be related with the overt nature of these practices, both being therefore perceived by the child. Other studies support this hypothesis. Pressure to eat has been shown to be positively associated with overt control (Nowicka, Flodmark, Hales, & Faith, 2014; Ogden et al., 2006) and, in a study previously conducted in the present sample (Moreira et al., 2014), a similar correlation was observed. The association between pressure to eat and consumption of dairy may have an additional explanation. It has been suggested that children play an active role in response to parenting practices (Birch, 2006; Brewis & Gartin, 2006), and it is possible that, although parents pressure for both food groups, dairy may be more easily accepted by children than F&V, depending on children's enjoyment of food as results of other authors (Cooke, Wardle, & Gibson, 2003) suggest. Furthermore, pressure to eat has a distinct nature when compared to other practices being related with parents' tendency to push the child to eat more food, typically at meals (Birch et al., 2001), and parents have reported to use more pressure when they perceive their child to be thin or a picky eater, being usually associated with reduced intake by children (Ventura & Birch, 2008).

The present study showed that overt and covert control may be slightly different with respect to children's consumption of F&V, as overt control was associated with consumption of this food group above recommended levels. This suggests that parental firmness is indeed a distinct theoretical concept from covert control (Ogden et al., 2006). It is possible that mothers in this sample use overt control particularly at meals, similarly to pressure to eat (Birch et al., 2001), which could explain the association between overt control and consumption of F&V that, in Portugal, are usually consumed at main meals (lunch and dinner).

Mothers included in this study showed high mean scores of perceived responsibility when compared to scores reported in the United States of America (USA) (Birch et al., 2001; Spruijt-Metz, Lindquist, Birch, Fisher, & Goran, 2002). Maternal scores for restriction and pressure to eat were also higher than those reported in samples from the USA (Birch et al., 2001; Spruijt-Metz et al., 2002) and Europe (Melbye et al., 2012; Webber, Cooke, Hill, & Wardle, 2010), while monitoring showed similar scores to other European samples (Gubbels et al., 2011; Melbye et al., 2012; Webber et al., 2010), but higher than those reported in the USA (Birch et al., 2001; Spruijt-Metz et al., 2002). Overt control mean scores were higher than those reported among young children from Southern England (Ogden et al., 2013), while covert control had a moderate mean score with 42% of mothers in a middle level (mean score 3.0), a prevalence higher than described in another Southern England sample (Brown et al., 2008).

When compared to Flemish children aged ≥4 years (Huybrechts et al., 2008), children in the present sample had lower prevalence of F&V consumption below recommendations and higher prevalence of their consumption above them. A lower prevalence of consumption of dairy below recommendations was also observed. Prevalence of dietary inadequacy for meat and fish was lower than observed in the Flemish sample (Huybrechts et al., 2008) for both consumption below and above recommendations. The prevalence of children consuming EDF above the tolerable limit seems higher in Portuguese children, but results are only partially comparable to the Flemish sample (Huybrechts et al., 2008) as in the current study sugar-sweetened beverages were considered together with sweet snacks and cakes. When sugar-sweetened beverages were excluded from the EDF group, the proportion of children above the tolerable limit was lower (65.6%) – a prevalence similar to the one reported for Flemish girls (Huybrechts et al., 2008).

Some methodological decisions taken in the present study deserve further discussion. Generally, dietary guidelines vary considerably across countries (European Food Safety Authority, 2010; World Health Organization, 2003). Many reference nutrient intakes for children have additional limitations (for instance, those related with extrapolation from other age groups (Atkinson & Koletzko, 2007) contributing to the disparities in pediatric dietary guidelines. Yet, food-based dietary guidelines are useful as they are more closely linked to the diet–health relationship and may enable an interpretation closer to nutritional recommendations. Taking these aspects into account, and considering that guidelines could be harmonized (Smitasiri & Uauy, 2007), that some recommendations for macronutrients are moving toward ranges (King, Vorster, & Tome, 2007), and that single cut-points could increase the possibility of misclassification, definition of intervals of adequacy, based on different dietary guidelines, was deemed appropriate.

The relationship between an essential dietary factor and a given health-related outcome has a hypothetical spectrum in which low intakes are detrimental, intakes within a certain interval are beneficial, and intakes above that level may also be detrimental (Willett, 1998a). Published evidence suggests that high consumption of vegetables and whole grain foods may, in particular circumstances, be detrimental to children (Bushnell, 1992; Dagnelie, van Dusseldorp, van Staveren, & Hautvast, 1994; Sanders, 1988). A safe range of fiber has been previously suggested (Williams, Bollella, & Wynder, 1995), but current recommendations for ages above 1 year are extrapolated from adults (Atkinson & Koletzko, 2007). Hence, it is possible that exaggerated consumption of F&V in early childhood may be unnecessary and/or imprudent, particularly if high food volumes (e.g., bulky foods such as F&V) are not balanced with other foods resulting in low energy density (Kersting et al., 2005). High intakes of animal protein in infancy (Koletzko et al., 2009) and childhood (Günther, Remer, Kroke, & Buyken, 2007) have been associated with subsequent weight gain and adiposity, high intake of protein sources, especially milk (Günther et al., 2007), being considered imprudent. Furthermore, particularly in children, high consumption of some foods may be associated to low consumption of other food groups as published evidence on self-regulation of energy intake in young children suggests (Birch, McPhee, & Sullivan, 1989; Cecil et al., 2005). In light of this evidence, we consider that the theoretical concept used to define dietary inadequacy is appropriate.

The use of a FFQ to assess children's dietary intake must be discussed. It can be argued that inadequate consumption frequencies (below or above recommendations) as defined in the present study may not correspond, respectively, to insufficient or excessive consumption as it would depend on the amount eaten at each consumption occasion. The collection of additional data on portion sizes is controversial as the inclusion of questions on portion-sizes in FFQ has shown to provide little additional information and portions inaccurately reported can reduce validity (Willett, 1998b). The use of usual portion sizes for preschool children (More & Emmett, 2014) to convert food amounts into frequencies was nevertheless necessary. Considering that these portions were proposed for children from the United Kingdom, it is possible that they are not adequate for Portuguese children. However, the proposed portions were shown as adequate regarding nutritional recommendations for preschool children (More & Emmett, 2014), supporting their use in the present sample.

The correlation coefficients found for key groups as measured by the FFQ or the food diary also deserve discussion. Considering that at 4 years of age a 3-day food diary may not be the ideal method to represent consumption of foods eaten less frequently, the week-to-moderate correlations and fair-to-moderate agreement found for foods most frequently consumed support an acceptable validity and reliability of the FFQ data. Social desirability bias is a possible limitation that should also be considered. Dietary intake was assessed in face-to-face interviews conducted by trained professionals and, if over-reporting of healthy foods and/or under-reporting of unhealthy ones occurred, associations may have been underestimated.

The cross-sectional nature of this analysis is another limitation that must be acknowledged. We could not assess if child-feeding practices are affecting dietary inadequacy or if children's dietary inadequacy is prompting maternal child-feeding practices. It has been suggested that this association is bidirectional (Birch, 2006), but more longitudinal studies are needed to clarify this hypothesis. Differences between the included sample and the remaining cohort also deserve discussion. As the CFQ was self-completed by mothers it would be expected to have participants with higher education level. Still, taking into account that Cohen's effect size values indicate that the magnitude of the differences in maternal age and education are not high (Husted, Cook, Farewell, & Gladman, 2000), it is not expected that these differences affected our results.

Strengths of this study are the use of children and mothers enrolled in a population-based cohort, its sample size, the study of two concepts of restriction for which research is still very scarce and the use of dietary guidelines for children for defining intervals of dietary adequacy, using a perspective of inadequacy below or above these intervals which may be particularly important in young children.

Conclusions

Maternal child-feeding practices such as covert control, monitoring and restriction may be beneficial to children's diet since they were associated with lower prevalence of inadequate consumption of F&V and EDF. However, child-feeding practices such as overt control and pressure to eat may be detrimental as they may be respectively associated with overconsumption of F&V and dairy.

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PAPER II

Association of maternal characteristics and behaviours with 4 year-old children’s dietary patterns

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Original Article

Association of maternal characteristics and behaviours with 4-year-old children's dietary patterns

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Abstract

This study examined the association of family and maternal characteristics with preschool children's dietary patterns. Trained interviewers evaluated subsample 3422 mothers and children enrolled in the population-based birth cohort Generation XXI (Porto, Portugal, 2005–2006). Maternal characteristics and behaviours (exercise, smoking habits, diet and child-feeding practices) and family characteristics were evaluated. Maternal diet was classified by a dietary score, and children's dietary patterns were identified by latent class analysis. Odds ratios (OR) and confidence intervals (95% CI) were estimated by multinomial regression models. The analysis was based on a framework with four conceptual levels: maternal socio-economic position (SEP) at 12 years, maternal socio-economic and demographic characteristics at child's delivery, family characteristics and maternal behaviours at child's 4 years. Three dietary patterns were identified in children: high in energy-dense foods (EDF); low in foods typically consumed at main meals and intermediate in snacks (Snacking); higher in healthy foods; and lower in unhealthy ones (Healthier, reference). Lower maternal SEP had an overall effect on children's diet (low vs. high SEP; EDF, OR = 1.76, 95% CI: 1.42–2.18; Snacking, OR = 1.73, 95% CI: 1.27–2.35), while maternal education was directly associated with it (≤9 vs. >12 schooling years, EDF, OR = 2.19, 95% CI: 1.70–2.81; Snacking, OR = 2.22, 95% CI: 1.82–3.55). Children whose mothers had worse dietary score were significantly more likely to follow unhealthier patterns (first vs. fourth quartile; EDF, OR = 9.94, 95% CI: 7.35–13.44, *P*-trend < 0.001; Snacking, OR = 4.21, 95% CI: 2.94–6.05, *P*-trend < 0.001). Maternal diet was the key factor associated with children's diet, above and beyond socio-economic and demographic characteristics, accounting for one-third of the determination coefficient of the fully adjusted model. At preschool age, interventions should give a particular focus on maternal diet and low SEP groups.

Keywords: socio-economic factors, demographic factors, family characteristics, maternal behaviours, preschool children, dietary habits.

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Introduction

Eating habits develop during the first years of life within a family context, being shaped by parental behaviours (e.g. child-feeding practices or parental diet) and family characteristics. In turn, the family is embedded in a larger social context where characteristics such as socio-economic position (SEP) influence children's diet through the influence exerted on parental behaviours (Davison & Birch, 2001).

Considering that early diet tracks into later ages and influences current and future health (Mikkila *et al.* 2005; Northstone & Emmett, 2008; Ambrosini *et al.* 2013), prevention of unhealthy dietary habits in early childhood might be a major step in the promotion of health. However, evidence base for interventions in preschool children is limited (Waters *et al.* 2011).

Research on determinants of preschool children's diet is particularly scarce (De Craemer *et al.* 2012; Lakshman *et al.* 2013), and studies based on conceptual frameworks are especially warranted (de Vet *et al.* 2011; Zarnowiecki *et al.* 2014). Also, research seems somewhat disconnected between studies assessing socio-economic factors and those mostly focused on parental behaviours such as child-feeding practices and/or parental diet (Savage *et al.* 2007; Scaglioni *et al.* 2008; De Craemer *et al.* 2012; Lakshman *et al.* 2013).

Childhood SEP is correlated with subsequent socio-economic status and is strongly associated with adult health, but the behavioural pathways that link SEP to health, as well as the length needed for socio-economic exposures to exert their influence, warrant particular attention (Cohen *et al.* 2010). Hence, studies examining parental determinants of children's diet should take into account both distal and proximal indicators of SEP.

Relatively few studies have examined broader ranges of potential determinants of empirically derived dietary patterns among preschool children (North & Emmett, 2000; Northstone & Emmett, 2005; Leventakou *et al.* 2015; Lioret *et al.* 2015), but parenting child-feeding practices were not included. Also, studies examining overall measures of maternal diet are practically non-existent (Fisk *et al.* 2011). Moreover, to the best of our knowledge, only one study (Moreira *et al.* 2014) identified patterns of maternal child-feeding practices, and none examined their association with *a posteriori* dietary patterns of preschool children.

Thus, the aim of the present study was to evaluate a broad range of potential determinants of dietary patterns among children aged 4 years and – in order to better understand upstream and downstream factors, overall and 'direct effects', as well as factors most relevant to the child's diet – to support the analysis on a framework considering plausible pathways between the several determinants. As such, a theoretical framework (Fig. 1) based on previous models (UNICEF, 1990; Victora *et al.* 1997; Davison & Birch, 2001; Solar & Irwin, 2010), ensuring time precedence between blocks of variables, was defined including four conceptual levels: (i) SEP of the mother at 12 years of age; (ii) maternal socio-economic and demographic characteristics at child's delivery; (iii) family characteristics at child's 4 years; and (iv) maternal characteristics and behaviours (physical exercise practice, smoking habits, child-feeding patterns and diet) at child's 4 years of age.

Participants and methods

Participants

Participants are part of the population-based birth cohort Generation XXI, described elsewhere (Larsen *et al.* 2013). Briefly, a total of 8647 live newborns and their mothers ($n = 8495$) were enrolled between April 2005 and August 2006 at all five public maternity units from the metropolitan area of Porto, Portugal. At 4 years of age, the entire cohort was invited to a re-evaluation, and 7458 subjects participated (86% participation proportion). Data were collected by face-to-face interviews or, for those families that were not able to be present during the face-to-face interview (20%), by telephone with a shorter version of the questionnaire.

Of those evaluated in person, 82% returned Child-Feeding Questionnaires (CFQ), of which 99% were completed by mothers. After exclusion of twins, and children with congenital anomalies or diseases that

Key messages

- The prevalence of unhealthy diets in Portuguese preschool children is high: Energy-Dense Foods dietary pattern (41%) and Snacking dietary pattern (14%).
- This study clarifies that early maternal socio-economic position is indirectly associated with 4-year-old children's dietary patterns and that maternal education is directly associated with these patterns.
- Maternal behaviours (dietary quality and child-feeding practices) are directly associated with children's diet, above and beyond socio-economic and demographic factors.
- Maternal diet is the most important factor associated with children's diet. Hence, interventions aimed at promoting healthy diets among preschool children should include mothers and give particular focus to the quality of their diet.

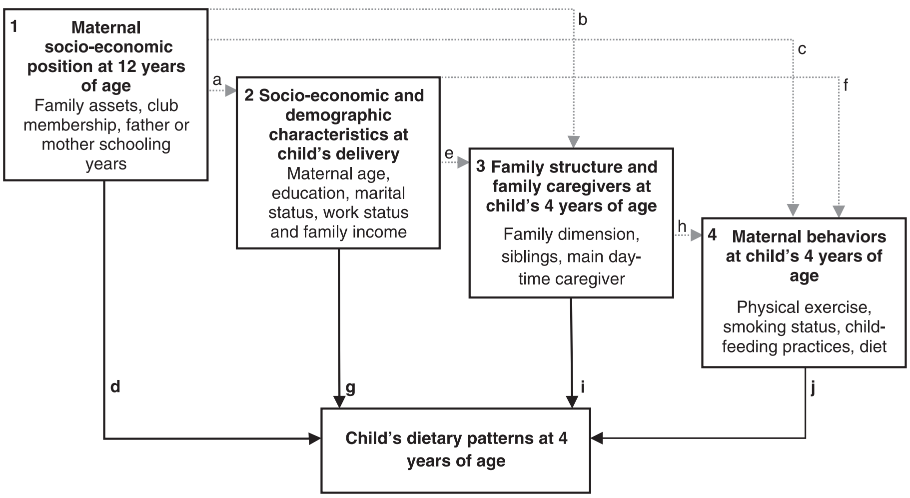

Fig. 1. Theoretical framework of family and maternal determinants of children's diet. This figure depicts the theoretical framework defined for analysis in the present study adapted prom previously published models (UNICEF, 1990; Victora *et al.* 1997; Davison & Birch, 2001; Solar & Irwin, 2010). Arrows represent theoretical causal relationships between determinants of children's dietary patterns. Dashed grey lines represent possible indirect effects in the pathway between levels of determinants. Solid black lines represent the direct effects of factors, after adjustment for determinants in preceding levels that is not mediated by subsequent ones, but that may be explained by other factors (unknown or unmeasured). 1. Socio-economic position at mothers' 12 years may exert an effect on children's diet through socio-economic and demographic characteristics at child's delivery (a), through its influence on subsequent family characteristics (b), through maternal behaviours (c) and/or through unknown or unmeasured determinants (d). 2. Socio-economic and demographic characteristics at child's delivery may have an effect on children's diet through subsequent family characteristics (e), through maternal behaviours (f) and/or through unknown or unmeasured factors (g). 3. Family characteristics at child's 4 years of age may have an effect on children's dietary patterns through their influence on maternal behaviours (h) and/or through unknown or unmeasured determinants (i). 4. In this conceptual framework, maternal behaviours would then influence children's dietary patterns directly and/or through other unknown or unmeasured factors (j). In the present study, we were particularly interested in the overall effects and the direct effects (highlighted in bold, d, g, i and j).

might influence dietary intake (e.g. cerebral palsy or food allergy), a subsample of 4341 subjects with complete CFQ and Food Frequency Questionnaire (FFQ) was considered eligible for the present study. From these, we excluded subjects with incomplete information on variables of interest: maternal SEP at 12 years ($n = 76$); characteristics at child's delivery [family income ($n = 481$), maternal work status ($n = 21$), maternal education ($n = 16$) and maternal marital status ($n = 7$)]; family characteristics at child's 4 years [siblings ($n = 12$), daytime caregiver ($n = 7$)]; maternal characteristics at child's 4 years [exercise ($n = 44$), body mass index (BMI) ($n = 124$)]; and child's characteristics [screen time ($n = 73$), physical exercise ($n = 13$) and BMI ($n = 45$)]. A total of 3422 mothers and children remained for final analysis.

Comparison of baseline characteristics between this sample ($n = 3422$) and the remaining cohort (5225) showed that mothers in the present sample were slightly older (mean = 30.4, $SD = 4.99$ vs. mean = 29.0 years, SD = 5.86; $P < 0.001$) and slightly more educated (mean = 11.3, $SD = 4.22$ vs. mean = 10.0 complete schooling years, $SD = 4.16$; $P < 0.001$). Cohen's effect size values were not high (<0.35) (Husted *et al.* 2000).

All the phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (World Medical Association, 2013). The study was approved by the University of Porto Medical School/S. João Hospital Centre ethics committee, and a signed informed consent according to Helsinki was required for all participants.

Data collection

With the exception of child-feeding practices (self-completed by mothers), information was collected through structured questionnaires applied by trained staff during face-to-face interviews to the child primary caregiver (in the present sample, 100% were mothers) at baseline [maternal SEP at the age of 12 years; and family or maternal characteristics at child's birth (age, work status, marital status and family income)] and at the 4-year follow-up [family characteristics (size, siblings and main daytime caregiver); maternal characteristics (BMI) and behaviours (physical exercise practice, smoking status, diet and child-feeding practices); and child's characteristics (sex, age and BMI) and behaviours (physical exercise and daily screen time)].

Family and maternal demographic and socio-economic characteristics

As described elsewhere (Teixeira *et al.* 2015), latent class analysis (LCA) models were used to identify distinct categories of maternal SEP when the mother was 12 years of age (SEP-12). Three latent classes were identified representing low, intermediate and high SEP. Maternal age was categorized into <25, 25–29 or >29 years. Maternal education corresponded to the number of completed schooling years, categorized into ≤9, 10–12 or 12 years. Maternal work status was assessed as a nominal variable, converted into not working (unemployed, retired and housewife) vs. working (part or full-time, student and working student). Maternal marital status was defined as not married or cohabiting vs. married or cohabiting. Family income was measured as an ordinal variable, categorized as ≤1000, 1001–1500 or >1500 euros/month. Siblings' age was used to define a categorical variable (older and younger, only younger, only older or no siblings); the number of persons in the household was categorized into >4, 4 or <4 persons, and child's main daytime caregiver was defined as family (parent or other family member) vs. non-family (kindergarten and nannies).

Maternal behaviours

Physical exercise was assessed as to type, frequency and duration, and, considering the proportion of mothers not practicing any physical exercise (80.5%), a nominal variable of practice of physical exercise was defined (no vs. yes). Maternal smoking status was categorized into smoking (1–10 or >10 cigarettes/day) vs. non-smoker.

Maternal dietary intake was evaluated by a FFQ adapted from a previous questionnaire validated for the general adult population (Lopes *et al.* 2007) and for pregnant women in a subsample of this cohort (Pinto *et al.* 2010). This FFQ assessed consumption in the previous year and included 18 items with response options in 9-point frequency scale, ranging from 'never' to '≥4 times per day'. Frequencies of consumption were converted into daily frequencies (e.g. 1 time/week was converted into 1/7 days = 0.14 times/day). A dietary score adapted from a previous one (González *et al.* 2002) was defined considering eight food items/groups: milk, fish, red meat (red and processed meat), bread, fruit, vegetables (vegetable soup and vegetables on a plate), energy-dense micronutrient-poor foods (EDF) consisting of cakes and salty pastry, and sugar-sweetened beverages (carbonated and non carbonated). Each item/group was categorized into quartiles, and 1 to 4 points were assigned according to increasing quartiles of consumption (milk, fish, bread, fruit and vegetables) or decreasing quartiles of consumption (red meat, EDF and sugar-sweetened beverages). Points assigned were summed up resulting in a dietary score ranging from 8 to 32 points, where higher scores reflect better maternal diet. This score was further converted into quartiles (fourth quartile considered as reference category in analyses).

Child-feeding practices were assessed through a combined version of the CFQ (Birch *et al.* 2001) and the scales of overt and covert control (Ogden *et al.* 2006), self-completed by mothers. This questionnaire was previously adapted to Portuguese and validated in a subsample of the Generation XXI cohort (Real *et al.* 2014). The dimensions of this questionnaire showed acceptable reliability in a previous study (Durão *et al.* 2015) conducted in the same sample frame. As described previously (Moreira *et al.* 2014), participants received mean scores for each dimension with at least 50% of answers. Scores ranged from 1 to 5, higher scores reflecting higher levels of practices. Three patterns of maternal child-feeding practices were identified by principal component analysis; 'perceived monitoring', 'restriction' and 'pressure to eat'. These factors' scores are in a standard deviation scale, ranging from −3 to 3. A score of 0 represents mothers' ratings close to the average of the sample, while a score of −2 corresponds to maternal ratings two standard deviations below the mean of the sample.

Child's age and physical activity

Exact age was computed in days using birthdate and the date of the 4-year follow-up and was further converted into years. Daily screen time was assessed by a structured question assessing average daily minutes spent in front of a screen (television, computer or game devices) at both week and weekend days. Time was converted into hours per day, and an average of daily screen time was calculated [(daily hours per day × 5 week days) + (daily hours per day × 2 weekend days)/7 days]. Physical exercise practice was also collected using a structured question assessing both type and time (minutes per week) of exercise. Time spent practicing each type of exercise was converted into hours and summed up to define weekly hours of physical exercise.

Child's dietary intake

Dietary intake was evaluated by a 35-item FFQ, assessing food consumption in the previous 6 months. As previously described (Durão *et al.* 2015), nine response options were available ranging from never to ≥4 times/day further converted into daily frequencies (e.g. ≥4 times/day was converted into 4 times/day, and 1–3 times/month was considered as 2 times/month, which, divided by 30 days, corresponds to 0.07 times/day). Five items were excluded from analysis because of either low frequency of consumption (coffee, tea and herbal tea) or low between-subject variability (crackers and butter). Hence, 30 items were considered for identification of dietary patterns. A total of 16 foods items/groups were defined: five groups based on similarity of food type and nutritional composition [milk (whole, semi or skimmed), yoghurt (sugared and non-sugared), meat and eggs (red meat, white meat and eggs), sweets (cakes, biscuits, chocolate, sugar, candies and ice cream) and sugar-sweetened beverages (colas, other carbonated beverages, iced tea, fruit drinks and other non-carbonated beverages)] and 11 isolated items (fruit, vegetable soup, vegetables on a plate, cheese, fish, processed meat, rice–pasta–potatoes, bread, crisps, pizza–burger and salty pastry).

In a subsample of 2493 children with complete 3-day food diaries, the validity and reliability of the FFQ were assessed by calculation of Pearson's correlation coefficients and intraclass correlation coefficients for key food groups as measured by both methods. For food groups most frequently consumed, weak-to-moderate correlations and fair-to-moderate agreement were observed. Significant positive moderate Pearson's correlations were found for vegetable soup ($r = 0.54$, $P < 0.001$), fruit ($r = 0.42$, $P < 0.001$), milk ($r = 0.46$, $P < 0.001$) and yoghurts ($r = 0.48$, $P < 0.001$). Intraclass correlation coefficients varied from 0.54 [95% confidence interval (95% CI) 0.51–0.56] for vegetable soup to 0.17 (95% CI 0.11–0.32) for juices.

Anthropometric measures

Mothers' and children's height and weight were measured by trained professionals; body weight was measured to the nearest 0.1 kg with a digital scale (Tanita®, Arlington Heights, IL, USA), and height was measured to the nearest 0.1 cm with a wall stadiometer (SECA®, Hamburg, Germany). Maternal and child BMI were computed as weight in kilograms divided by height in squared metres. Maternal BMI was used as a continuous variable. Children's BMI was further converted into age and sex-specific standard deviation scores (BMI *z*-scores) according to the standards of the World Health Organization (WHO, 2006), also used as continuous.

Statistical analysis

Considering the categorical nature of the response items in the FFQ and their asymmetric distribution, children's dietary patterns were identified by LCA. This methodology focuses on relationships among individuals (person-centred approach) rather than relationships among variables and describes how the probabilities of a set of observed categorical variables (e.g. FFQ's items) vary across groups of individuals (distinguishing clusters of individuals homogeneous within groups) in order to find the smallest number of latent classes (e.g. dietary patterns) by adding classes stepwise until the model fits the data well (Muthén & Muthén, 2000). Probabilities of choosing each item response, conditionally on class membership, can be interpreted based on item profiles in each class (Vermunt & Magidson, 2002; Severo *et al.* 2012). In the present study, the number of dietary patterns was defined according to the Bayesian information criteria (BIC). The analysis used the 16 food items/groups, categorized into: first quintile, second to fourth quintiles combined, and fifth quintile. For foods eaten only once or twice a day (vegetable soup and rice–pasta–potatoes), two categories were defined: once a day, and twice a day. Children were assigned to each pattern according to the highest probability of class membership, and profiles of probabilities in each item category, conditionally on pattern membership, were used to interpret dietary patterns.

Chi-square tests or one-way analysis of variance with Bonferroni's correction for *post hoc* multiple comparisons were used, as appropriate, to test for differences across dietary patterns. Associations between exposures (family and maternal characteristics) and the outcome (children's diet) were estimated by crude and adjusted odds ratios (OR) and respective 95% CI, using multinomial logistic regression models. The main aim was to assess overall and 'direct effects' between exposures and the outcome. A step-by-step approach was used: first, univariate models were fitted; second, conceptually predefined blocks of variables were fitted separately (variables within each block mutually adjusted); and third, blocks including significant variables were introduced cumulatively into the analysis, with a fixed order based on a predefined theoretical framework (Fig. 1): (i) SEP of the mother at 12 years of age; (ii) maternal socio-economic and demographic characteristics at child's delivery; (iii) family characteristics at child's 4 years; and (iv) maternal characteristics and behaviours at child's 4 years of age. Models were adjusted for child's characteristics (sex, daily screen time, weekly time spent practicing physical exercise and exact age) and were compared by likelihood ratio test (LRT) for nested models. The coefficient of determination proposed for logistic regression (Nagelkerke, 1991), Nagelkerke's R^2, was used to assess which factors were most relevant to the child diet.

Latent class analysis for dietary patterns identification was conducted using MPLUS (V5.2; Muthén & Muthén, Los Angeles, CA, USA), and the remaining statistical analysis were conducted using SPSS statistical software package version 21 (SPSS Inc., Chicago, IL, USA). A significance level of 5% was adopted in all analyses.

Results

At child's 4 years of age, three dietary patterns were identified (two patterns, BIC = 147 384; three patterns, BIC = 146 698; four patterns, BIC = 146 656). Comparison between patterns (Table 1) showed that class 1 (41% children) had higher percentage of children within the fifth quintile of sweets, sugar-sweetened beverages, pizza-burguer and processed meat, being named 'EDF dietary pattern'. Class 2 (14% children) showed high proportion of children in the first quintile (or once a day) of foods typically eaten at main meals (vegetables on a plate, fish, meat and eggs, rice–pasta–potatoes and fruit) and intermediate/high proportions of children within the fifth quintile of foods usually eaten as snacks (bread, milk, yoghurt, crisps, pizza–burger, salty pastry and sugar-sweetened beverages) and was named 'Snacking dietary pattern'. Class 3 (45% children), named 'Healthier dietary pattern' (reference category in multinomial logistic regression models), was characterized by higher proportion of children within the fifth quintile (or twice a day) of healthy foods (vegetable soup, vegetables on the plate and fish), as well as lower proportion of children in the fifth quintile of unhealthy foods (processed meat, crisps, pizza–burger, salty pastry, sweets and sugar-sweetened beverages).

As presented in Table 2, children following the 'Healthier' dietary pattern had mothers with higher SEP-12, higher education, older age, married or cohabiting, working and from higher income families. At 4 years, these children belonged to less-crowded families, had younger siblings more often and attended kindergartens. Also, they had more mothers practicing physical exercise, not smoking, with better dietary scores, higher scores of monitoring and restriction, and lower scores of 'pressure to eat'.

In the analysis of separate blocks (not shown), with variables mutually adjusted within each block, maternal age, education and work status remained significant, while family income and marital status did not, being therefore excluded from posterior analysis. Other variables retained statistical significance.

In multivariate analysis (Table 3), the predefined theoretical framework depicted in Fig. 1 was followed by consecutive addition of blocks into the model. When

Table 1. Proportion of subjects within consumption categories in each latent class (dietary pattern), n = 3422*

	Total sample	Latent class (dietary pattern)		
		Class 1 (EDF)	Class 2 (Snacking)	Class 3 (Healthier)
	n = 3422	n = 1400 (41%)	n = 484 (14%)	n = 1538 (45%)
Fruit				
1st quintile, %	38.3	51.9^{a}	62.6^{b}	18.4^{c}
5th quintile, %	2.4	2.6^{a}	1.0^{a}	2.6^{a}
Vegetable soup				
Once a day, %	23.3	34.1^{a}	27.5^{b}	12.2^{c}
Twice a day, %	76.7	65.9^{a}	72.5^{b}	87.8^{c}
Vegetables on a plate				
1st quintile, %	30.1	29.2^{a}	79.5^{b}	15.4^{c}
5th quintile, %	20.8	16.6^{a}	3.3^{b}	30.1^{c}
Milk				
1st quintile, %	19.8	19.4^{a}	20.9^{a}	19.8^{a}
5th quintile, %	9.3	9.6^{a}	14.3^{b}	7.4^{a}
Yoghurt				
1st quintile, %	55.2	47.1^{a}	45.0^{a}	65.9^{b}
5th quintile, %	4.5	5.0^{a}	6.0^{a}	3.6^{a}
Cheese				
1st quintile, %	24.4	17.0^{a}	56.2^{b}	21.1^{c}
5th quintile, %	7.4	10.4^{a}	6.8^{b}	4.8^{b}
Fish				
1st quintile, %	65.4	77.2^{a}	84.3^{b}	48.6^{c}
5th quintile, %	9.4	4.1^{a}	6.2^{a}	15.1^{b}
Meat and eggs				
1st quintile, %	39.0	37.2^{a}	50.8^{b}	37.0^{a}
5th quintile, %	17.3	21.1^{a}	8.7^{b}	16.4^{c}
Processed meat				
1st quintile, %	20.8	6.1^{a}	49.2^{b}	25.3^{c}
5th quintile, %	18.8	28.8^{a}	13.6^{b}	11.2^{b}
Rice–pasta–potatoes				
Once a day, %	8.1	6.9^{a}	18.6^{b}	5.9^{a}
Twice a day, %	91.9	93.1^{a}	81.4^{b}	94.1^{a}
Bread				
1st quintile, %	50.9	46.4^{a}	55.6^{b}	53.4^{b}
5th quintile, %	1.5	2.1^{a}	1.7^{a}	1.0^{a}
Crisps				
1st quintile, %	23.7	7.4^{a}	24.0^{b}	38.4^{c}
5th quintile, %	8.3	15.6^{a}	12.8^{a}	0.3^{b}
Pizza–burger				
1st quintile, %	24.5	7.6^{a}	59.7^{b}	28.9^{c}
5th quintile, %	16.0	29.4^{a}	9.3^{b}	6.0^{c}
Salty pastry				
1st quintile, %	19.2	9.3^{a}	47.7^{b}	19.3^{c}
5th quintile, %	3.2	5.5^{a}	4.8^{a}	0.7^{b}
Sweets				
1st quintile, %	20.8	5.4^{a}	20.5^{b}	34.9^{c}
5th quintile, %	18.7	31.8^{a}	24.6^{b}	4.9^{c}
Sugar-sweetened beverages				
1st quintile, %	20.8	2.7^{a}	27.9^{b}	35.0^{c}
5th quintile, %	18.1	39.4^{a}	20.7^{b}	2.6^{c}

EDF, energy-dense foods dietary pattern. *Intermediate category (2nd, 3rd and 4th quintiles combined) is not shown in order to avoid redundancy. [†]Proportions were compared by chi-square test considering Bonferroni's correction for *post hoc* multiple comparisons. Different superscript letters indicate significant differences between dietary patterns at a significance level of 5%.

compared with children following the Healthier dietary pattern, lower maternal SEP-12 (Model 1) had the overall significant effect of increasing the odds of children following both unhealthier dietary patterns. When maternal socio-economic and demographic characteristics at child's delivery were added (Model 2), maternal SEP-12 ceased to be significantly associated with children's dietary patterns, and both maternal age and education were significantly associated with children's dietary patterns. After accounting for maternal socio-economic and demographic characteristics, having only older siblings significantly increased the odds of children following the EDF dietary pattern, while children with only younger siblings were significantly more likely to follow the Snacking pattern.

Maternal behaviours were added in Model 4, resulting in a significant increase in Nagelkerke's R^2 (from 0.15 to 0.27, $P < 0.001$). In this final model, several maternal characteristics were directly associated with both unhealthier dietary patterns (education, diet and child-feeding practices). Lower maternal education increased the odds of children following both the EDF and Snacking dietary patterns (≤9 vs. >12 years, OR = 2.19, 95% CI: 1.70–2.81; OR = 2.55, 95% CI: 1.82–3.55, respectively). Also, the worse the maternal dietary score, the higher the odds of children following unhealthier patterns (first vs. fourth quartile; EDF, OR = 9.94, 95% CI: 7.35–13.44, *P*-trend < 0.001; Snacking, OR = 4.21, 95% CI: 2.94–6.05, *P*-trend < 0.001). Higher levels of maternal perceived monitoring and restriction were inversely associated with children's practice of unhealthier dietary patterns.

Some factors were significantly directly associated with only one of the unhealthier dietary patterns. Younger age of the mother at child's delivery was positively associated with the EDF (<25 vs. >29 years, OR = 1.80, 95% CI: 1.34–2.44) but not with the Snacking dietary pattern. Likewise, children with only older siblings were more likely to follow the EDF pattern (only older vs. no siblings, OR = 1.67, 95% CI: 1.21–2.30). In contrast, having only younger siblings and being mainly cared by a parent (95% were mothers) were both associated only with the Snacking dietary pattern (OR = 1.67, 95% CI: 1.01–2.73 and OR = 1.84, 95% CI: 1.09–3.10, respectively).

Table 2. Maternal, family and child's characteristics according to children's dietary patterns, $n = 3422$

	EDF	Snacking	Healthier	*P*-value*
	$n = 1400$	$n = 484$	$n = 1538$	
Socio-economic position at mothers' 12 years[†]				
Low	364 (26.0)[a]	122 (25.2)[a]	307 (20.0)[b]	
Intermediate	742 (52.0)[a]	260 (53.7)[a,b]	733 (47.7)[b]	
High	294 (21.0)[a]	102 (21.1)[a]	498 (32.4)[b]	<0.001
Socio-economic and demographic characteristics at child's delivery				
Maternal age[†]				
<25 years	259 (18.5)[a]	68 (14.0)[a]	105 (6.8)[b]	
25–29 years	424 (30.3)[a]	152 (31.4)[a]	417 (27.1)[a]	
>29 years	717 (51.2)[a]	264 (54.5)[a]	1016 (66.1)[b]	<0.001
Maternal education[†]				
≤9 years	704 (50.3)[a]	242 (50.0)[a]	436 (28.3)[b]	
10–12 years	424 (30.3)[a]	136 (28.1)[a]	430 (28.0)[a]	
>12 years	272 (19.4)[a]	106 (21.9)[a]	672 (43.7)[b]	<0.001
Maternal marital status[†]				
Married or cohabiting	1329 (94.9)[a]	465 (96.1)[a,b]	1489 (96.8)[b]	
Not married or cohabiting	71 (5.1)[a]	19 (3.9)[a,b]	49 (3.2)[b]	0.035
Maternal work status[†]				
Working	1067 (76.2)[a]	380 (78.5)[a]	1295 (84.2)[b]	
Not working	333 (23.8)[a]	104 (21.5)[a]	243 (15.8)[b]	<0.001
Family income[†]				
Lower (≤1000 euros/month)	582 (41.6)[a]	188 (38.8)[a]	416 (27.0)[b]	
Intermediate (1001–1500 euros/month)	445 (31.8)[a]	150 (31.0)[a,b]	425 (27.6)[b]	
Higher (>1500 euros/month)	373 (26.6)[a]	146 (30.2)[a]	697 (45.3)[b]	<0.001
Family at child's 4 years				
Family dimension[†]				
>4 persons	235 (16.8)[a]	70 (14.5)[a]	227 (14.8)[a]	
4 persons	615 (43.9)[a]	176 (36.4)[b]	665 (43.2)[a]	
<4 persons	550 (39.3)[a]	238 (49.2)[b]	646 (42.0)[a]	0.003
Child's siblings[†]				
Older and younger	34 (2.4)[a]	9 (1.9)[a]	41 (2.7)[a]	
Only younger	154 (11.0)[a]	63 (13.0)[a]	202 (13.1)[a]	
Only older	582 (41.6)[a]	149 (30.8)[b]	575 (37.4)[a]	
No siblings	630 (45.0)[a]	263 (54.3)[b]	720 (46.8)[a]	0.001
Main daytime caregiver[†]				
Parent (95% mothers)	66 (4.7)[a]	34 (7.0)[a]	40 (2.6)[b]	
Other family member (96% grandparents)	151 (10.8)[a]	60 (12.4)[a]	120 (7.8)[b]	
Not family (kindergarten, nannies)	1183 (84.5)[a]	390 (80.6)[a]	1378 (89.6)[b]	<0.001
Maternal characteristics at child's 4 years				
Physical exercise practice[†]				
Practitioners	217 (15.5)[a]	74 (15.3)[a]	376 (24.4)[b]	
Non-practitioners	1183 (84.5)[a]	410 (84.7)[a]	1162 (75.6)[b]	<0.001
Smoking status[†]				
Non-smoker	1046 (74.7)[a]	382 (78.9)[a,b]	1277 (83.0)[b]	
1–10 cigarettes/day	221 (15.8)[a]	69 (14.3)[a,b]	169 (11.0)[b]	
>10 cigarettes/day	133 (9.5)[a]	33 (6.8)[a,b]	92 (6.0)[b]	<0.001
Dietary score[†]				
1st quartile (<16 points)	551 (39.4)[a]	165 (34.1)[a]	208 (13.5)[b]	
2nd quartile (17–19 points)	485 (34.6)[a]	142 (29.3)[a]	365 (23.7)[b]	
3rd quartile (20–22 points)	280 (20.0)[a]	116 (24.0)[a]	503 (32.7)[b]	
4th quartile (>22 points)	84 (6.0)[a]	61 (12.6)[b]	462 (30.0)[c]	<0.001
BMI[†]				
<25.0 kg/m²	627 (44.8)[a]	237 (49.0)[a,b]	786 (51.1)[b]	
≥25.0 kg/m²	773 (55.2)[a]	247 (51.0)[a,b]	752 (48.9)[b]	0.003

(*Continues*)

Table 2. (Continued)

	EDF	Snacking	Healthier	*P*-value*
	n = 1400	*n* = 484	*n* = 1538	
Patterns of child-feeding practices‡				
Perceived monitoring	−0.1 (1.04)[a]	0.0 (1.04)[a,b]	0.1 (0.92)[b]	<0.001
Restriction	−0.1 (0.97)[a]	0.0 (1.03)[a,b]	0.1 (0.97)[b]	<0.001
Pressure to eat	0.0 (1.00)[a]	0.1 (1.01)[a,b]	−0.1 (1.00)[a]	0.003
Child's characteristics				
Sex†				
Girls	662 (47.3)[a]	228 (47.1)[a]	783 (50.9)[a]	
Boys	738 (52.7)[a]	256 (52.9)[a]	755 (49.1)[a]	0.102
Screen time, h/day‡	1.9 (1.17)[a]	1.9 (1.19)[a]	1.5 (0.90)[b]	<0.001
Time practicing physical exercise, h/week‡	1.1 (1.18)[a]	1.0 (1.07)[a]	1.3 (1.18)[b]	<0.001
BMI *z*-score‡	0.6 (1.11)[a]	0.6 (1.16)[a]	0.6 (1.05)[a]	0.276
Exact age, years‡	4.4 (0.41)[a]	4.3 (0.35)[b]	4.3 (0.35)[b]	<0.001

BMI, body mass index; BMI *z*-score, age and sex-specific BMI standard deviation scores according to the World Health Organization (WHO, 2006); EDF, energy-dense foods dietary pattern. *For categorical variables, comparison between dietary patterns was performed by the chi-square test. For continuous variables, comparison was performed by one-way analysis of variance. For both tests, Bonferroni's correction for *post hoc* multiple comparisons was considered. Different superscript letters indicate significant differences between dietary patterns at a significance level of 5%. †*n* (%). ‡Mean (standard deviation).

In order to assess which factor was key to children's diet, variables significantly associated with both unhealthier patterns were removed one by one from the final model (Model 4). Comparison between models was performed by both comparison of Nagelkerke's R^2 and LRT for nested models. Removal of maternal child-feeding practices did not produce a substantial reduction in Nagelkerke's R^2 (full vs. reduced model; $R^2 = 0.27$ vs. $R^2 = 0.26$, $\chi^2_6 = 35.4$, $P < 0.001$), and the same was observed after removal of maternal education (full vs. reduced model; $R^2 = 0.27$ vs. $R^2 = 0.25, \chi^2_4 = 52.0$, $P < 0.001$). Maternal dietary score was the key factor associated with children's dietary patterns as its removal from the final model produced a drop of 9% in Nagelkerke's R^2 (full vs. reduced model; $R^2 = 0.27$ vs. $R^2 = 0.18, \chi^2_6 = 323.2$, $P < 0.001$).

Discussion

The present study showed that several maternal and family characteristics were associated with preschool children's dietary patterns and that maternal diet was the factor most strongly associated with them, above and beyond socio-economic and demographic characteristics, accounting for one-third of the determination coefficient in the fully adjusted model.

Using LCA to identify dietary patterns is an advantage as it is especially well suited for categorical variables asymmetrically distributed, common in data from FFQ, resulting in attenuation of correlations between items. LCA uses Pearson's correlation coefficients and de-attenuates these correlations. Additionally, as highlighted by Muthén & Muthén (2000), this methodology avoids subjective choice of cut-points on underlying dimensions, because the classification is provided directly by the model. Finally, it can be compared with factor analysis (Muthén & Muthén, 2000), enabling comparison with studies that use principal component analysis.

Two studies conducted in the UK identified three dietary patterns practised at both 3 (North & Emmett, 2000) and 4 years of age (Northstone & Emmett, 2005): a 'traditional' pattern characterized by meat and vegetables; a 'junk' pattern characterized by high-fat processed foods and snacks (comparable with the present EDF pattern); and a 'health-conscious' pattern rich in vegetarian style foods, rice, pasta, fruit, cheese and fish (comparable with the current Healthier pattern). Additionally, in the study examining 3-year-olds (North & Emmett, 2000), a 'snack' dietary pattern – partly comparable with the current Snacking pattern – was identified. Two other studies identified dietary patterns comparable with the current EDF pattern: a

Table 3. Multivariate analysis of the associations of maternal and family characteristics with dietary patterns of 4-year-old children, $n = 3422$*

	n	Model 1†		Model 2†		Model 3†		Model 4†	
		EDF‡	Snacking‡	EDF‡	Snacking‡	EDF‡	Snacking‡	EDF‡	Snacking‡
		n = 1400	*n* = 484	*n* = 1400	*n* = 484	*n* = 1400	*n* = 484	*n* = 1400	*n* = 484
Socio-economic position at mothers' 12 years									
High	894	1	1	1	1	1	1	1	1
Intermediate	1735	**1.52 (1.27–1.83)**	**1.56 (1.20–2.02)**	1.04 (0.85–1.26)	1.09 (0.82–1.44)	1.04 (0.86–1.26)	1.09 (0.82–1.44)	1.03 (0.83–1.27)	1.08 (0.81–1.45)
Low	793	**1.76 (1.42–2.18)**	**1.73 (1.27–2.35)**	1.10 (0.86–1.41)	1.06 (0.82–1.49)	1.10 (0.86–1.41)	1.07 (0.75–1.51)	1.19 (0.91–1.55)	1.12 (0.79–1.60)
Socio-economic and demographic characteristics at child's delivery									
Maternal age									
>29 years	1997			1	1	1	1	1	1
25–29 years	993			**1.28 (1.07–1.52)**	1.26 (0.99–1.60)	**1.38 (1.15–1.66)**	1.14 (0.88–1.46)	1.14 (0.93–1.39)	0.97 (0.75–1.25)
<25 years	432			**2.17 (1.67–2.84)**	**1.63 (1.14–2.33)**	**2.47 (1.87–3.28)**	1.40 (0.96–2.04)	**1.80 (1.34–2.44)**	1.07 (0.73–1.58)
Maternal education									
>12 years	1050			1	1	1	1	1	1
10–12 years	990			**1.91 (1.54–2.35)**	**1.61 (1.19–2.18)**	**1.87 (1.51–2.31)**	**1.67 (1.23–2.27)**	**1.51 (1.20–1.90)**	**1.44 (1.05–1.98)**
≤9 years	1382			**2.87 (2.29–3.59)**	**2.81 (2.06–3.83)**	**2.76 (2.19–3.47)**	**3.02 (2.20–4.15)**	**2.19 (1.70–2.81)**	**2.55 (1.82–3.55)**
Maternal work status									
Working	2742			1	1	1	1	1	1
Not working	680			1.07 (0.87–1.30)	0.99 (0.76–1.30)	1.06 (0.86–1.29)	1.03 (0.78–1.35)	1.00 (0.80–1.24)	0.99 (0.74–1.31)
Family at child's 4 years									
Family dimension									
<4 persons	1434					1	1	1	1
4 persons	1456					0.96 (0.71–1.29)	0.74 (0.49–1.13)	0.91 (0.66–1.25)	0.71 (0.46–1.09)
>4 persons	532					0.83 (0.59–1.15)	0.74 (0.47–1.17)	0.80 (0.56–1.15)	0.72 (0.45–1.16)
Child's siblings									
No siblings	1613					1	1	1	1
Older and younger	84					1.09 (0.61–1.95)	0.77 (0.32–1.81)	1.40 (0.75–2.61)	0.92 (0.38–2.21)
Only younger	419					1.10 (0.77–1.57)	1.43 (0.88–2.32)	1.38 (0.95–2.03)	**1.67 (1.01–2.73)**
Only older	1306					**1.40 (1.04–1.89)**	0.91 (0.60–1.38)	**1.67 (1.21–2.30)**	1.02 (0.66–1.58)
Main daytime caregiver									
Not family§	2951					1	1	1	1
Parent§	140					1.12 (0.73–1.73)	1.63 (0.98–2.72)	1.36 (0.86–2.16)	**1.84 (1.09–3.10)**
Other family member§	331					1.23 (0.93–1.62)	1.29 (0.91–1.84)	1.14 (0.85–1.53)	1.23 (0.86–1.77)
Maternal characteristics at child's 4 years									
Physical exercise									
Practitioners	667							1	1
Non-practitioners	2755							1.10 (0.89–1.36)	1.21 (0.91–1.62)
Smoking status									
Non-smokers	2705							1	1
1–10 cigarettes/day	459							1.22 (0.95–1.56)	1.11 (0.80–1.54)
>10 cigarettes/day	258							1.09 (0.79–1.50)	0.86 (0.55–1.34)

(*Continues*)

Table 3. (Continued)

	n	Model 1†		Model 2†		Model 3†		Model 4†	
		EDF‡	Snacking‡	EDF‡	Snacking‡	EDF‡	Snacking‡	EDF‡	Snacking‡
		$n = 1400$	$n = 484$	$n = 1400$	$n = 484$	$n = 1400$	$n = 484$	$n = 1400$	$n = 484$
Dietary score									
4th quartile (>22 Pt)	607							1	1
3rd quartile (20–22 Pt)	899							**2.69 (2.02–3.58)**	**1.59 (1.13–2.25)**
2nd quartile (17–19 Pt)	992							**6.13 (4.62–8.12)**	**2.41 (1.71–3.40)**
1st quartile (<16 Pt)	924							**9.94 (7.35–13.44)**	**4.21 (2.94–6.05)**
BMI									
<25.0 kg/m^2	1650							1	1
≥25.0 kg/m^2	1772							1.16 (0.98–1.38)	0.95 (0.76–1.19)
Child-feeding patterns									
Perceived monitoring	3422							**0.84 (0.77–0.91)**	**0.89 (0.80–0.99)**
Restriction	3422							**0.85 (0.78–0.93)**	**0.88 (0.78–0.98)**
Pressure to eat	3422							0.95 (0.87–1.04)	1.07 (0.95–1.20)
Nagelkerke's R^2		**0.08**		**0.14**		**0.15**		**0.27**	
LRT P-value		**<0.001**		**<0.001**		**<0.001**		**<0.001**	

BMI, body mass index; EDF, energy-dense foods dietary pattern; Nagelkerke's R^2, Nagelkerke's R-squared (Nagelkerke, 1991); Pt, points; LRT, likelihood ratio test. *Statistically significant associations are highlighted in bold. †Blocks of variables (socio-economic position at mothers' 12 years of age; maternal socio-economic and demographic characteristics at child's delivery; family characteristics at child's 4 years; and maternal characteristics at child's 4 years) were added sequentially into the analysis. Models are adjusted for child's characteristics (sex; daily screen time; weekly time spent practicing physical exercise; and exact age). ‡Reference category is the Healthier dietary pattern ($n = 1538$), not shown to avoid redundancy. §Not family, kindergarten and nannies; parent, mostly mothers (95%); other family member, mostly grandparents (96%).

'Processed and fast foods' pattern identified in multi-time points (2, 3 and 5 years) in French children (Lioret *et al.* 2015) and a 'Snacky' pattern identified in Greek 4-year-olds (Leventakou *et al.* 2015). Similar associations with maternal age and education were found in all samples. In contrast, maternal work status was significantly associated with children's dietary patterns in the UK sample but not in the Portuguese or Greek samples. As for maternal smoking status, present results are consistent with both the UK and Greek samples.

We only found one study including an overall measure of maternal diet (Fisk *et al.* 2011). In 1640 3-year-olds from the UK, results were consistent with present findings, with maternal diet being shown as the most important factor associated with children's diet. Despite not assessing children's or mothers' dietary patterns, other studies support these findings with consistent positive associations between children's and parents' consumption of specific foods (Fisher *et al.* 2000; Fisher *et al.* 2002; Cooke *et al.* 2003; Vereecken *et al.* 2004; Wyse *et al.* 2011; McGowan *et al.* 2012).

Finding that different factors within the family context were associated with distinct dietary patterns deserves to be discussed. The EDF pattern was more likely among children with only older siblings. In a previous study conducted in a subsample of 2-year-olds enrolled in this cohort (Vilela *et al.* 2014), a similar association was found, consistent with findings from both the UK and French samples (Northstone & Emmett, 2005; Lioret *et al.* 2015). Older children have been reported to be more exposed to energy-dense micronutrient-poor foods and beverages (Rangan *et al.* 2008), and it is possible that older children influence younger siblings. In contrast, children mainly cared by a parent during daytime, as well as those with only younger siblings, were significantly more likely to follow the Snacking pattern. Regarding parents as main daytime caregivers and keeping in mind that children model behaviours from their peers (Schunk, 1987), lower exposure to other children at the kindergarten may explain this finding. Concerning siblings, it is possible that mothers having to attend to younger children are overburdened and may have less time to prepare healthy meals.

In respect to maternal control over the child's diet, we did not find studies examining patterns of both child-feeding practices and children's diet. However, comparable findings have been reported. Monitoring was negatively associated with unhealthier eating (Arredondo *et al.* 2006), and restriction (Ystrom *et al.* 2012) was positively associated with a wholesome dietary pattern.

Possible limitations inherent to the use of FFQ to assess maternal and children's diet deserve discussion. Estimation of dietary intake using frequency and not quantities could be considered a limitation, but inclusion of additional questions on portion sizes may reduce validity (Willett, 1998). Taking into account that at 4 years of age a 3-day food diary may not be the ideal method to represent consumption of foods eaten less frequently, the week-to-moderate correlations and fair-to-moderate agreement found for foods most frequently consumed support an acceptable validity and reliability of the FFQ. Also, comparison of the dietary patterns with 3-day food diaries showed that the EDF pattern was significantly higher in energy when compared with the other two patterns and that the Snacking pattern was significantly lower in fibre, supporting the methodology used to asses children's diet. Using an adapted FFQ for assessment of maternal diet could be regarded as a limitation, but, as it was adapted from a previously validated questionnaire (Lopes *et al.* 2007; Pinto *et al.* 2010), its use was considered acceptable.

Inclusion of a subsample and exclusion of participants owing to missing data could introduce some bias into the analysis. We conducted sensitivity-analyses fitting models with the highest possible number of subjects for each variable and compared them with models considering complete-subject analysis (not shown), and results were very similar. A previous study (Teixeira *et al.* 2015) managed missing data using full-information maximum likelihood estimation, and comparison of this model with the model excluding missing values suggested that substantial bias was not expected (Cohen's $\kappa = 0.85$). Considering that this analysis was performed in the same sample frame, we do not expect that alternative methods to manage missing data would result in different conclusions. In addition, given that Cohen's effect size values were not high, the slight differences between this sample and the remaining cohort are likely due to large sample size, rather than to large differences between participants (Husted *et al.* 2000). As such, assuming 'missing completely at random', the complete-subject analysis was considered valid within the limits of this study (Greenland & Rothman, 2008).

In order to assess social desirability bias, we stratified the analysis by maternal weight status. As overweight mothers reported higher consumption of crisps and soft drinks and lower consumption of fruit and vegetables, this bias seems unlikely. Still, mothers may have incorrectly reported foods eaten out of home. Considering that a previous study (Moreira *et al.* 2015) conducted in this cohort at child's 4 years of age showed that most meals were taken at home, we do not expect that out-of-home consumption would result in different findings. However, associations between maternal and child's diet may have been overestimated, because assessment was performed at the same time by the same individual. Considering that limitations in general cognitive competencies are an obstacle to self-reporting among young children (Bevans *et al.* 2010) and that information was collected by trained interviewers, the approach used was deemed adequate.

Not including paternal characteristics in the present study must be discussed. Fathers from different SEP appear to be increasingly involved in child feeding (Mallan *et al.* 2013), and two-parent families have shown to be associated with some dietary behaviours (Patrick & Nicklas, 2005; de Vet *et al.* 2011) in children. Maternal marital status was considered in order to clarify possible differences between two-parent and one-parent families, but no differences were observed. Given this, and taking into account that mothers spend more time caring and feeding young children (Scaglioni *et al.* 2008; Bauer *et al.* 2012), not including the father was considered acceptable.

A major strength of the current study was basing the analysis on a predefined theoretical framework. Besides sample size and measurement of several potential determinants, as participants were part of a population-based birth cohort regularly followed up, we were able to ensure temporal sequence between blocks of variables, allowing for better interpretation of the associations. Additionally, the use of composite measures to assess early maternal SEP, maternal child-feeding practices, maternal diet and children's diet was also an advantage as it enabled us to better capture their inherent complexity.

In conclusion, early maternal lower SEP indirectly increases the odds of exposing their children to unhealthier dietary patterns, while lower maternal education at child's delivery directly increases these odds. These findings may reflect a transgenerational influence of less-favourable socio-economic conditions on children's diet. Specific maternal behaviours are associated with children's dietary patterns, above and beyond socio-economic and demographic factors, and maternal diet is a key factor to the child's diet.

Future research should further study the mechanisms by which SEP influences children's diet, including the effect of life-course changes in SEP. Also, special attention must be given to the identification of those determinants with higher impact on preschool children's diet, because this period of life may be a window of opportunity for action. Finally, interventions developed with the aim of preventing unhealthy dietary behaviours among preschool children should involve mothers with particular focus on their diet and pay special attention to lower socio-economic status groups.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Contributions

CD contributed to the design of the study, performed statistical analyses, drafted the initial manuscript and contributed to the interpretation of data. MS carried out statistical analysis, contributed to the interpretation of data and critically revised the manuscript. AO contributed to the design of data collection instruments, contributed to the interpretation of data and critically reviewed the manuscript. PM contributed to the interpretation of data and critically reviewed the manuscript. AG contributed to the interpretation of data and discussion of the results and critically reviewed the manuscript. HB conceptualized and designed the study, coordinated and supervised data collection, contributed to the discussion of results and critically reviewed the manuscript. CL contributed to the design of study, contributed to the design of data collection instruments, contributed to the discussion of results and critically reviewed the manuscript. All authors approved the final manuscript as submitted.

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PAPER III

Evaluating the effect of energy-dense foods consumption on preschool children's body mass index: a prospective analysis from 2 to 4 years of age

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ORIGINAL CONTRIBUTION

Evaluating the effect of energy-dense foods consumption on preschool children's body mass index: a prospective analysis from 2 to 4 years of age

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Abstract
Purpose The aim of this study was to study the association between the consumption of energy-dense foods at 2 years and body mass index (BMI) at 4 years, using a cross-lagged panel design.
Methods The present study included 589 children evaluated at 2 and 4 years of age, as part of the birth cohort generation XXI. Information was obtained by face-to-face interviews. Consumption of energy-dense foods (salty snacks, soft drinks, cakes, and sweets) was measured using a food frequency questionnaire. Children's weight and height were measured by standard procedures, and BMI standard deviation scores (BMI z-scores) were calculated according to the World Health Organization. Linear regression and cross-lagged panel design models were fitted to estimate the associations between the consumption of energy-dense foods and BMI z-scores (controlled for maternal age, education and prepregnancy BMI, and children's exact age at 2 years).
Results The consumption of energy-dense foods at 2 years was significantly associated with their consumption at 4 years ($\beta = 0.522$, 95 % CI 0.432–0.612). Children's BMI z-scores at 2 years were associated with posterior BMI z-scores ($\beta = 0.747$, 95 % CI 0.688–0.806). In the cross-lagged analysis, consumption of energy-dense foods at 2 years had no effect on subsequent BMI z-scores ($\beta = -0.030$, 95 % CI -0.095 to 0.035) and BMI z-scores at 2 years were not significantly associated with the consumption of energy-dense foods at 4 years ($\beta = -0.012$, 95 % CI -0.086 to 0.062).
Conclusions Consumption of energy-dense foods and BMI tracked over time, but the consumption of energy-dense foods at 2 years was not associated with BMI z-scores at 4 years.

Keywords Energy-dense foods · Sugar-sweetened beverages · Preschool children · Weight · Cohort studies · Cross-lagged panel design

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Introduction

The worldwide prevalence of overweight in school-aged children [1] and the prevalence of overweight among preschool children [2–4], with its short- and long-term health consequences and health-related economic costs, place childhood overweight as a major public health priority [1, 5]. In Europe, about 20 % of children and adolescents are overweight [6], southern countries showing higher prevalence [4]. In Portugal, a subnational sample of preschool children had a prevalence of overweight (including obesity) of 20.5 and 23.1 % in 3- and 4-year-old children, respectively [3, 7].

The increased consumption of energy-dense, micronutrient-poor foods and beverages [energy-dense foods (EDF)]—particularly sugar-sweetened beverages (SSB) [8–13] and foods high in fat and/or sugar [9, 12]—among children and adolescents, followed by increased prevalence of overweight, emphasized the potential role of these major contributors to the daily energy intake of children and adolescents [12, 14] as key environmental determinants of childhood overweight.

Several systematic reviews have evaluated the association between SSB consumption and weight status, reaching distinct conclusions. A systematic review published in 2006 [15] concluded that epidemiologic and experimental research provided strong evidence for the independent role of the intake of SSB in the promotion of weight gain and obesity in children and adolescents, but another review of the same year [16] concluded that a variety of inconsistencies and methodological difficulties precluded definite conclusions. In 2007, a systematic review and meta-analysis [17], including nearly the same studies, concluded that there was a clear association between soft drink intake and body weight, while a posterior meta-analysis addressing only longitudinal studies and randomized controlled trials among children and adolescents [18] reported that there was practically no association between SSB consumption and body mass index (BMI), raising controversy for several reasons [19]. Also, in 2011, a meta-analysis of randomized experiments found that evidence did not demonstrate conclusively that SSB contributed to obesity [20]. Some of these reviews were criticized as to its quality [21], and a posterior revision emphasized that the relation between SSB intake and obesity was convincing [22]. In a recent meta-analysis [23], randomized controlled trials did not show such an association, while prospective cohort studies seem to support it.

Studies on the effect of SSB on BMI are particularly scarce in preschool children and may be giving only a partial picture of the effect of EDF in childhood overweight. SSB have been associated with the consumption of other EDF [24–27], but there are few studies on EDF as a whole. In longitudinal studies, food purchased away from home, consumption of fast food, and consumption of fried foods away from home have been positively associated with weight status in children [28–30], but in other studies fast food was not associated with weight status [31, 32]. Additionally, longitudinal studies have not shown an association between energy-dense snacks and weight gain in children and adolescents [29].

Keeping in mind that dietary habits tend to persist over time [33–35] and that childhood overweight tracks into adulthood [36], it may be important to evaluate the effect of early consumption of EDF on weight among preschool children, taking into account the change of EDF consumption and BMI over time simultaneously in a single model. Therefore, our objective was to study the associations between the consumption of EDF at 2 years of age and BMI at 4 years of age, using a cross-lagged panel design [37].

Methods

Participants

This study was based on the population-based birth cohort generation XXI, described elsewhere [38]. A total of 8,647 live born infants and their mothers were enrolled between April 2005 and August 2006 at all the five public maternity units that cover six municipalities of the metropolitan area of Porto (Northern Portugal). These maternity units were responsible, at enrollment, for 91.6 % of the deliveries in the whole catchment population.

At 2 years of age, a subsample of 855 children was re-evaluated. Follow-up took place in two phases: children completing their second anniversary between April and August of 2007 were invited to participate in the first follow-up; later, all children born in January 2006 were invited to a similar evaluation during January 2008. In the present study, these two groups were combined and 821 children with complete information on food consumption were included. Of these, 641 were also re-evaluated at 4 years, when a follow-up of the entire cohort took place. From the 641 children evaluated at both 2 and 4 years, we excluded 17 twins, seven children with congenital anomalies or diseases that might influence dietary intake (cerebral palsy, celiac disease, food allergy, food intolerance, and phenylketonuria) and 28 subjects without complete information on co-variables, 589 participants remaining for the final analysis.

Data collection

Information was collected using structured questionnaires during face-to-face interviews conducted by trained interviewers at birth, at 2 years, and at 4 years.

Consumption of energy-dense foods

Children's EDF consumption was evaluated using a food frequency questionnaire (FFQ) answered by the primary caregiver (97 % mothers). The FFQ used at the age of two comprised 17 items aiming to assess consumption of EDF. The FFQ used at 4 years comprised 35 items and was developed with the aim of assessing overall food consumption. Both FFQ used frequency response options ranging from never to daily. At 2 years, 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, <once a month, and never). At 4 years, nine response options were available (≥4 times 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, and never). EDF items common to both evaluations were carbonated soft drinks, non-carbonated sugar-sweetened beverages, crisps, pizzas, hamburgers, cakes, chocolate, sweets, and candies.

Responses were transformed into average daily frequencies (e.g., 2–4 times per week was converted into a mean of 3 times per week, equivalent to 3/7 days = 0.43 times per day). One new variable of daily consumption of EDF was created for each evaluation moment by summing up these frequencies. This variable was used as continuous for analysis.

At 2 and 4 years, 2- and 3-day food diaries were completed, respectively. At both ages, correlations between key groups measured by the FFQ and the food diaries were calculated to assess the validity of the FFQ. Pearson's correlation coefficients showed a weak-to-moderate correlation for the EDF food groups at 2 and 4 years. At 2 years, sweets showed the strongest coefficient (r = 0.53) and weaker correlations were found for the other EDF groups. At 4 years, moderate correlation coefficients were found for cakes ($r = 0.57$) and sweets ($r = 0.49$), and not so strong for soft drinks ($r = 0.23$) and salty snacks ($r = 0.14$). Both at 2 and 4 years, when compared to food diaries, the FFQ seems to overestimate consumption of EDF. In particular, at 2 years, a 2-day food diary may not be the ideal method to validate as it may not be sufficient for representing foods with relatively low intakes. Nevertheless, the magnitudes of the Pearson correlation coefficients for the EDF foods more frequently consumed support an acceptable validity of the FFQ data.

Children's weight and height

Anthropometrics were measured by trained professionals. At 2 years, either recumbent length was measured to the nearest centimeter with a length measuring board or height was measured to nearest centimeter using a wall stadiometer when children were able to stand alone. At 4 years, height was measured to the nearest centimeter with a wall stadiometer. At both 2 and 4 years, weight was measured in light clothing to the nearest 0.1 kg using a digital scale. BMI was defined as weight in kg divided by height in squared meters. Age- and sex-specific standard deviation scores (BMI z-scores) were computed according to the World Health Organization Child Growth Standards [39].

Co-variables

Maternal education corresponded to the number of completed schooling years at delivery. Maternal prepregnancy BMI was calculated using the self-reported weight and measured height at birth (in 72 % of mothers) or height as recorded in the identity card. Breastfeeding duration was recorded in weeks, and a dichotomous variable was defined (non-exclusive breastfeeding <6 vs. ≥6 months). Practice of structured exercise (planned, structured, and repetitive physical activity) was measured at 4 years as a qualitative variable (non-practitioners vs. practitioners). Child's age was defined in exact months at both follow-ups. Screen time (television, computer, and games devices) was assessed at the 4-year follow-up as average hours per day.

Statistical analysis

To estimate the association of participants' characteristics with the exposure (children's consumption of EDF) and the outcome (children's BMI z-scores), linear regression models were used to calculate β regression coefficients and respective 95 % confidence intervals (β, 95 % CI).

Interaction terms between child's age, maternal age, education and prepregnancy BMI, and EDF consumption at 2 years on BMI z-score at 4 years were tested.

A cross-lagged panel design analysis [37] was performed. This model includes two components: longitudinal analysis for EDF consumption and BMI z-scores across the two time periods and the cross-lagged analysis of EDF consumption at 2 years on BMI z-scores at 4 years, as well as of BMI z-scores at 2 years on EDF consumption at 4 years. Models were adjusted for maternal education, age, and prepregnancy BMI and for the child exact age in months at the 2-years evaluation. These variables were used as continuous. Cross-lagged analysis enables to use the time inherent to longitudinal data to answer to temporality questions, providing a global measure of the model likelihood with all tests of model fit in a single regression.

For linear regression models, homoscedasticity and normality of errors distribution were evaluated. To assess the cross-lagged model fit, chi-square test, root mean square error of approximation, and the comparative fit index were used. Final models only include those variables that were significantly associated with the exposures or outcomes. In all analyses, we considered a p value <0.05 as statistically significant. Statistical analysis was conducted using SPSS statistical software package version 21 (SPSS Inc., Chicago, IL, USA) and Mplus version 5.2 (Muthen & Muthen, Los Angeles, California, USA).

Results

Table 1 presents participants' characteristics at birth and at both follow-up evaluations. At the evaluations of 2 and 4 years, children had, respectively, a mean BMI *z*-score of 0.7 (SD = 0.99) and 0.6 (SD = 1.06) and a mean consumption of EDF of 0.9 times per day (SD = 0.90) and 2.0 times per day (SD = 1.05).

The associations of maternal and child characteristics with the consumption of EDF and with children's BMI *z*-scores at 2 and 4 years (Table 2) were estimated in order to identify potential confounding variables to be included in the final cross-lagged model. After mutual adjustment, maternal education was significantly negatively associated with EDF consumption at both 2 and 4 years ($\beta = -0.045$; 95 % CI −0.065 to −0.029 and $\beta = -0.057$; 95 % CI −0.077 to −0.036, respectively) and maternal age showed similar associations at both ages. Maternal prepregnancy BMI was significantly positively associated with BMI *z*-scores at 2 ($\beta = 0.057$; 95 % CI 0.038 to 0.007) and 4 years ($\beta = 0.065$; 95 % CI 0.045 to 0.086). Current child's age was also significantly associated with EDF consumption and with BMI *z*-scores at 2 and 4 years.

As no other variables were significantly associated with the exposures (EDF consumption at 2 and 4 years) or the outcomes (BMI *z*-scores at 2 and 4 years), the cross-lagged model was adjusted for maternal age, education, and prepregnancy BMI, as well as for children's exact age at 2 years. No significant interactions were found between child's age or maternal age, prepregnancy BMI or education, and EDF consumption at 2 years.

As can be observed in Fig. 1, consumption of EDF at 2 years of age was associated with consumption at 4 years ($\beta = 0.522$; 95 % CI 0.432 to 0.612) and children's BMI *z*-scores at 2 years were significantly positively associated with posterior BMI *z*-scores ($\beta = 0.747$; 95 % CI 0.688 to 0.806). The cross-lagged association between consumption of EDF at 2 years and BMI *z*-scores at 4 years was not significant, as well as there was no association between BMI *z*-scores at 2 years and consumption of EDF at 4 years.

Separate models for salty snacks (crisps, pizza, and burger), soft drinks (sweetened carbonated drinks and other sweetened drinks), cakes (creamy, non-creamy, and sweet pastry), and sweets (chocolate and candies) were tested, and results were similar (data not shown).

Discussion

In this study, although consumption of EDF and BMI tracked from 2 to 4 years, no association was observed between the consumption of EDF at 2 years and BMI at 4 years.

Evidence on the longitudinal association between EDF, as a single food group, and weight status in preschool children is scarce, hampering the comparison of our results with those of other studies. Nevertheless, considering that SSB have been linked to consumption other EDF including fast food [24–27], we compare our results with studies concerned with either SSB, fast food or snacks.

Results of the present study are in accordance with other prospective studies in which consumption of SSB [40–42], snacks [43, 44], and fast food [31, 32] was not associated with weight status in children. Also, a recent study [45] suggested a positive association between soft drinks consumption at 3–6 years of age and overweight at 18 and 30 months of follow-up, but after adjustment for sociodemographic and behavioral variables, no association between soft drink consumption and overweight was found.

In contrast, results of other studies among preschool children differ from results of the present study. In a study including 10,904 children from low social economic status

Table 1 Maternal and child's characteristics ($n = 589$)

		At birth
Maternal age (years)[a]		30.4 (5.07)
Maternal prepregnancy BMI (kg/m^2)[a]		23.8 (4.24)
Maternal education (years)[a]		10.9 (4.27)
Sex, boys[b]		295 (50.1)
	At 2 years	At 4 years
Age (months)[a]	25.4 (1.06)	50.0 (3.41)
BMI *z*-score[a]	0.7 (0.99)	0.6 (1.06)
Non-exclusive breastfeeding, <6 months[b]	312 (53.0)	
EDF (frequency/day)[a]	0.9 (0.90)	2.0 (1.05)
Screen time (h/day)[a]		1.6 (1.23)
Structured physical activity, non-practitioners[b]		187 (31.8)

BMI body mass index; BMI *z*-score, BMI standard deviation scores according to the World Health Organization [39]

[a] Mean (SD)

[b] *n* (%)

Table 2 Association between maternal and child's characteristics and frequency of consumption of energy-dense foods and BMI *z*-scores[a]

	Energy-dense foods consumption at 2 years		BMI *z*-score at 2 years	
	Crude β (95 % CI)	Adjusted[b] β (95 % CI)	Crude β (95 % CI)	Adjusted[b] β (95 % CI)
At birth				
Maternal age (years)	−0.032 (−0.046 to 0.018)	**−0.030 (−0.043 to −0.016)**	−0.009 (−0.025 to 0.007)	−0.015 (−0.031 to 0.001)
Prepregnancy BMI (kg/m^2)	**0.025 (0.008 to 0.042)**	0.016 (−0.001 to 0.033)	**0.055 (0.036 to 0.073)**	**0.057 (0.038 to 0.077)**
Maternal education (years)	**−0.058 (−0.074 to −0.042)**	**−0.045 (−0.062 to −0.029)**	−0.019 (−0.038 to 0.000)	−0.001 (−0.021 to 0.019)
Child sex				
Girls	Ref	Ref	Ref	Ref
Boys	0.075 (−0.071 to 0.220)	0.015 (−0.121 to 0.150)	−0.083 (−0.244 to 0.078)	−0.090 (−0.248 to 0.067)
Non-exclusive breastfeeding				
≥6 months	Ref	Ref	Ref	Ref
<6 months	**0. 154 (0.003 to 0.306)**	0.058 (−0.085 to 0.200)	−0.103 (−0.271 to 0.065)	−0.128 (−0.293 to 0.038)
At child's 2 years				
Age (months)	**0.093 (0.024 to 0.161)**	**0.081 (0.017 to 0.145)**	**−0.092 (−0.168 to −0.016)**	**−0.079 (−0.154 to −0.005)**
BMI *z*-score	0.050 (−0.023 to 0.123)	0.010 (−0.060 to 0.081)	–	–
EDF (freq/day)	–	–	0.062 (−0.028 to 0.152)	0.014 (−0.081 to 0.109)

	Energy-dense foods consumption at 4 years		BMI *z*-score at 4 years	
	Crude β (95 % CI)	Adjusted[b] β (95 % CI)	Crude β (95 % CI)	Adjusted[b] β (95 % CI)
At birth				
Maternal age (years)	**−0.031 (−0.048 to −0.015)**	**−0.021 (−0.038 to −0.005)**	−0.001 (−0.018 to 0.016)	−0.006 (−0.023 to 0.011)
Prepregnancy BMI (kg/m^2)	0.016 (−0.004 to 0.036)	0.007 (−0.014 to 0.027)	**0.069 (0.050 to 0.089)**	**0.065 (0.045 to 0.086)**
Maternal education (years)	**−0.067 (−0.086 to −0.048)**	**−0.057 (−0.077 to −0.036)**	**−0.028 (−0.048 to −0.008)**	−0.014 (−0.035 to 0.008)
Child sex				
Girls	Ref	Ref	Ref	Ref
Boys	0.043 (−0.127 to 0.214)	−0.026 (−0.188 to 0.136)	0.029 (−0.143 to 0.201)	0.000 (−0.168 to 0.167)
Non-exclusive breastfeeding				
≥6 months	Ref	Ref	Ref	Ref
<6 months	0.131 (−0.047 to 0.309)	0.061 (−0.109 to 0.231)	−0.021 (−0.201 to 0.158)	−0.064 (−0.240 to 0.112)
At child's 4 years				
Age (months)	**0.052 (0.027 to 0.077)**	**0.043 (0.019 to 0.066)**	−0.010 (−0.036 to 0.015)	−0.007 (−0.032 to 0.018)
BMI *z*-score	−0.013 (−0.094 to 0.067)	−0.048 (−0.127 to 0.032)	–	–
EDF (freq/day)	–	–	−0.014 (−0.095 to 0.068)	−0.051 (−0.135 to 0.034)
Screen time (h)	**0.160 (0.092 to 0.229)**	**0.119 (0.051 to 0.187)**	0.052 (−0.018 to 0.122)	0.062 (−0.008 to 0.133)
Structured physical activity				
Practitioners	Ref	Ref	Ref	Ref
Non-practitioners	**0.195 (0.013 to 0.378)**	0.055 (−0.125 to 0.236)	−0.113 (−0.297 to 0.072)	−0.133 (−0.320 to 0.054)

Ref, reference class; BMI *z*-score, BMI standard deviation scores according to the World Health Organization [39]

BMI body mass index, *EDF* energy-dense foods

[a] Significant associations are highlighted in bold

[b] Mutually adjusted for all the other variables

in the United States of America (USA)[46], after adjustment for the consumption of sweet and high-fat foods, a positive association between consumption of SSB in young children (2–3 years) and development of overweight after one year was observed. Additionally, also in the USA, a recent longitudinal study [47] including a large sample of preschool children evaluated at 2, 4, and 5 years observed that children drinking ≥1 SSB daily at 2 years of age had a greater increase in BMI *z*-score by 4 years and that those drinking SSB daily at 4 years had a similar change in BMI *z*-score at 5 years.

Prospective studies including consumption of EDF other than SSB by younger children are particularly scarce [32, 45, 48] and, similarly to the present study results, found no association between EDF consumption and weight status. However, in older children, distinct results have been reported. In a large cohort of children from the USA, aged 9–14 years, an association between high consumption of fried food away from home and higher BMI one year later was reported [49] and another large study with adolescents from the USA observed that frequency of fast-food consumption predicted BMI *z*-score at young adulthood [50]. Additionally, a study with adolescents from the United Kingdom (UK) observed that consumption of fast food at 13 years was associated with BMI *z*-score at 15 years [30].

In the present analysis, not finding evidence of an association between EDF consumption at 2 years and BMI at 4 years could be explained by children's age frame. Although BMI showed tracking from 2 to 4 years, since adiposity rebound usually occurs between 5 and 7 years [51], it is probable that most children are still decreasing toward a nadir of BMI. In fact, 53.7 % of children included in this study decreased BMI *z*-score from 2 to 4 years. In contrast, in studies among preschool children from the USA, prevalence of risk of overweight/overweight increased between 2–3 and 3–4 years [46] and BMI *z*-scores increased between 2 and 4 years [47]. This fact could be justified by an association between increased energy intake and early adiposity rebound [51].

Another possible explanation for the present results could be that young children show better compensation for extra energy and this ability seems to reduce with age [52–54]. Children aged 2–4 years old exposed to EDF may still be able to self-regulate energy intake and avoid an impact on their weight at such early ages.

The relatively short follow-up period could also explain present findings. The study in USA among preschool children that did not find an association between SSB and weight gain also had short follow-up time [40].

Additionally, a low frequency of both the exposure and the outcome in this population at these ages could, at some extent, justify our results. Although children showed an average consumption of EDF of 0.9 times per day at 2 years, this level of consumption may not be enough to observe significant associations with BMI. Most of the previous studies were developed in populations from the USA or UK [30, 46, 49, 50], where levels of exposure were higher. For instance, in the USA, a mean daily consumption of SSB of 2.9 drinks per day was reported in preschool children [46] and a mean consumption of fast food of 2 days per week was observed in adolescents [50], while in the present sample mean consumption of SSB was 0.2 times per day and 0.7 times per day, at 2 and 4 years, respectively, and salty snacks consumption (fast food and crisps) was 0.2 times per day at both ages. A previous study [55] in this same sample reported that, at 2 years, the proportion of children consuming soft drinks and salty snacks at least once a week was 35.0 and 16.9 %, respectively. Results from a study in the USA [40] give strength to the possibility of a low frequency of exposure, as levels of SSB consumption were also lower and no association with weight change was found. The higher prevalence of overweight and obesity in studies conducted in the USA [45, 49, 50], as well as in a study in the UK [30], may also explain the different results as the prevalence of overweight (including obesity) was much lower in our sample (8.6 % at 2 and 9.0 % at 4 years of age).

Not including other energy-dense foods in the present analysis could also explain our findings. The inclusion of foods such as butter, oils, or nuts could eventually produce different results, as parents regard some foods with relatively higher energy density, such as cheese or whole milk [56], as healthy and could tend to be less restrictive with these foods. Inclusion of other foods contributing to energy density was not possible in the present study because the FFQ applied at 2 years only assessed energy-dense, micronutrient-poor foods.

Low statistical power to detect significant differences between distinct levels of EDF consumption could not be discarded; however, the present study showed to have enough power to detect significant associations of EDF consumption and BMI *z*-scores across the two ages.

Dietary assessment by FFQ could be also pointed as a possible limitation as we only assessed frequencies of consumption and not quantity. The variation of intake in a group is more likely explained by frequency than portion sizes that vary less among individuals, and for most foods, portion sizes vary considerably more within a person over time than between persons [57]. As our main objective was to discriminate children's consumption of EDF (between-person variability), the use of frequency was deemed appropriate.

The validity of both FFQ deserves discussion. The weak-to-moderate correlation with food diaries for most food groups at 4 years and, excepting sweets, the weak correlations found at 2 years may be a limitation. At both ages, when compared to food diaries, the FFQ seemed to overestimate consumption of EDF. In particular, at 2 years, a 2-day food diary may not be the ideal method to validate measurement of less frequently consumed foods. Nevertheless, the magnitudes of the correlation coefficients observed for the EDF foods more frequently consumed support and acceptable validity of the FFQ.

We could not guarantee that information on consumption of EDF was complete, as consumption may not be thoroughly accounted for, if mothers were not aware of all out-of-home consumption. Considering that Portuguese children usually take snacks from home to school and that preschoolers consume high proportions of their daily

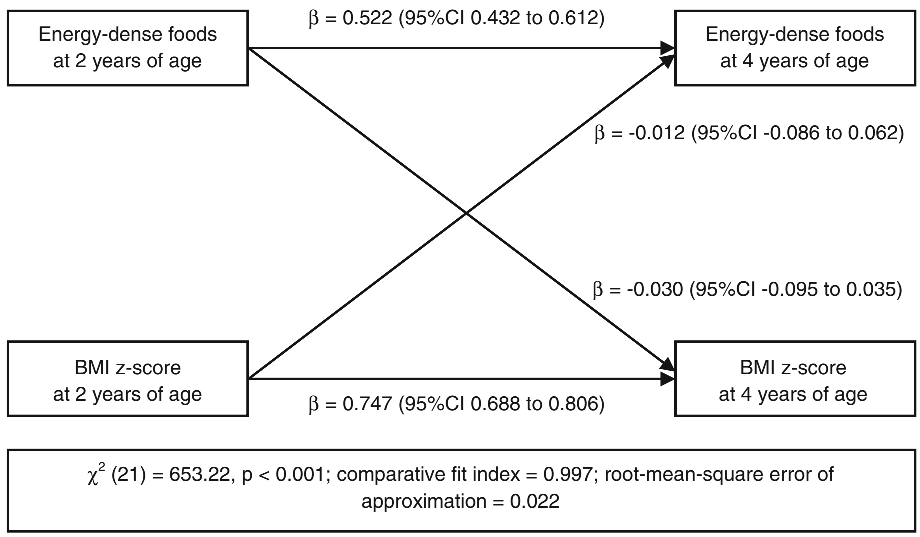

Fig. 1 Cross-lagged analysis of frequency of consumption of energy-dense foods and BMI *z*-scores. After adjustment for maternal (education, age, and prepregnancy BMI) and child's characteristics (exact age in months at 2 years), energy-dense foods consumption at 2 years of age was significantly positively associated with their consumption at 4 years of age. Children's BMI *z*-scores at 2 years were also significantly positively associated with BMI *z*-scores at 4 years. In the cross-lagged analysis, energy-dense foods consumption at 2 years of age was not associated with BMI *z*-scores at 4 years and BMI *z*-scores at 2 years had no significant effect on children's consumption of energy-dense foods at 4 years

energy intake at home [58], we consider that a bias is less likely at such early ages. Social desirability bias is also a possible limitation. In order to test this possibility, we tested whether consumption of EDF differed by maternal weight status and found that it did not differ between normal and overweight mothers. Also, considering that, when compared to their normal weight counterparts, overweight mothers actually reported slightly higher frequencies of consumption of EDF by their children, it is very unlikely that misreporting due to social desirability related with maternal weight status occurred.

The inclusion of several energy-dense, micronutrient-poor foods, and beverages is an advantage, although it could be argued that beverages should be analyzed separately from solid foods as intervention laboratory studies suggest different post-ingestive effects on satiety [59]. In order to assess whether beverages and energy-dense foods showed different associations with weight status, we performed a sensibility analysis with separate groups (salty snacks, soft drinks, cakes, and sweets), but results were similar. It is possible that not finding different results between solid EDF and sugar-sweetened beverages is due to the infrequent consumption of these beverages in the present sample. Yet, as the sensitivity analysis did not show any difference between foods and beverages, we consider acceptable to join these energy-dense, micronutrient-poor foods, and beverages in one single group of EDF.

The use of BMI as measure of children's adiposity may also be argued as a limitation, as the accuracy of this measure may vary with body fat proportions [60]. However, this variation is more important in older children [60] and BMI is a widely used indicator of body fatness that has been significantly associated with relative fatness in children [61] being a good indicator of excessive adiposity in overweight/obese children [60].

Since physical activity is very difficult to measure in the first years of life, we have no information on structured physical activity at 2 years of age. So, trying to evaluate this possible limitation, we tested practice of structured physical activity and screen time as potential confounding factors at 4 years, but these variables did not change the estimates and, after adjustment for other potential confounding factors, they were not associated with BMI. Considering this, as well as that physical activity and screen time show tracking in children [62–64], we do not expect that these variables measured at 2 years would change our results.

Longitudinal studies at such early age are very scarce [40, 45–47]; thus, the longitudinal approach and the ages of children considered are strengths of the present study. Also, the possibility of evaluating many potential confounding variables constitutes an advantage.

Conclusions

Consumption of EDF at 2 years did not show an association with BMI at 4 years, in a population with relatively low levels of exposure. Nevertheless, the tracking over time of EDF consumption observed in this study supports the need of avoiding an early overconsumption of these foods and beverages with little nutritional benefit and previously associated with decreased dietary quality.

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Conflict of interest The authors have no conflict of interest to disclose.

Ethical standards The Generation XXI birth cohort was approved by the Ethical Committee of São João Hospital/University of Porto Medical School and by the Portuguese Authority of Data Protection. Legal representatives of each participant were informed about the benefits and potential discomfort, and written informed consent was obtained prior to the collection of information at baseline and follow-up evaluations.

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PAPER IV

Association between dietary patterns and adiposity from 4 to 7 years of age

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[Submitted]

Association between dietary patterns and adiposity from 4 to 7 years of age

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ABSTRACT

This study aimed to evaluated the association of 4-year-old children's dietary patterns (DP) with adiposity at 7 years of age, and to examine if child's sex modifies this association. A total of 3473 children were evaluated at 4 and 7 years, as part of the population-based birth cohort Generation XXI (Porto-Portugal, 2005-2006). Diet was assessed by food frequency questionnaire. Age- and sex-specific Body Mass Index (BMI) standard deviation scores (z-scores) were defined according to the WHO. Fat Mass Percent (FM%), Fat Mass Index (FMI), and Waist-to-Height (W/Ht) ratio were also considered, converted into z-scores using sex-specific means and standard deviations of the current sample. DP were identified by Latent Class Analysis and their association with each adiposity measure was estimated by linear regression models. Three DP were identified: high in energy-dense foods (EDF); low in foods typically consumed at main meals and intermediate in snacks (Snacking); higher in vegetables and fish and lower in EDF (Healthier, reference). In fully adjusted models, the Snacking DP was not significantly associated with later adiposity. The EDF DP at 4 years was positively associated with later BMI only in girls (β=0·075, 95%CI 0·009-0·140, *P*-interaction=0·046). This DP was also associated with other adiposity measures only in girls (FMI, β=0·071, 95%CI 0·000-0·142; W/Ht ratio, β=0·094, 95%CI 0·023-0·164). In conclusion, an EDF DP at 4 years increased adiposity at 7 years only in girls. Future research should further examine the interaction of child's sex on the association between diet and adiposity.

INTRODUCTION

Despite reports suggesting that prevalence of childhood obesity may be reaching a plateau in some countries[1, 2], paediatric overweight has generally increased in the last four decades and remains high[3]. In Europe, prevalence of overweight and obesity in children below 10 years is higher in Southern countries and differences by sex are dependent of the classification system considered[4]. In Portugal[5], both considering Cole and Lobstein's[6] or the World Health Organization's (WHO)[7] criteria, prevalence of overweight among 7-year-olds is higher in girls than in boys; 30·5% *vs.* 22·7% and 36·2% *vs.* 31·5%, respectively.

Sexual dimorphism in body composition has been extensively described in adults and women are considered at higher risk of obesity[8]. The higher propensity of females to gain fat has been suggested[9] to be a possible reproductive strategy reflecting evolved adaptive differences which – in the past – may have benefited female reproduction. In current obesity-promoting environments, this evolved adaptive pattern may contribute to higher adiposity in females. The association between adiposity and female reproductive success has also been suggested to start at very early ages[9].

In children, sexual dimorphism has been observed since early ages in both total and regional adiposity[8-10]. In addition, girls may develop complex social interaction skills (e.g., peer influences) earlier than boys[11-13], and – hypothetically – temporal relationships between diet and adiposity could differ between girls and boys. However, prospective studies on the association between preschool children's dietary patterns and adiposity[14, 15] have not reported formal examination of an interaction of child's sex on this association.

We hypothesized that child's sex could modify the association between diet and adiposity. Therefore, the aim of the present study was to evaluate if dietary patterns of 4-year-old children were associated with adiposity at 7 years of age and to examine the possible interaction of child's sex on this association.

METHODS

Subjects

This study was based on the population-based birth cohort Generation XXI (Porto, Portugal, 2005-2006), described elsewhere[16]. At 4 years of age, the entire cohort was invited to a re-evaluation conducted as previously outlined[17] and, at 7 years, a new follow-up of the cohort took place (81% participation). Body composition assessment was performed in 85% of children at 4 years, and in 98% of children at 7 years. Children with valid information on adiposity measured at both ages totalled 4010. After excluding subjects with incomplete information on dietary intake (*n* 48 and *n* 87, at 4 and 7 years respectively), a subsample of 3875 children was considered eligible. We further excluded twins (*n* 150), children with congenital anomalies or diseases that might affect diet (e.g., cerebral palsy or food allergies, *n* 15), and those with missing values on variables of interest (child's physical exercise practice, *n* 12; maternal weight or height, *n* 225). A total of 3473 children (49·6% girls) were considered for analysis.

Data collection

At both 4 and 7 years of age, data were collected by trained interviewers in face-to-face interviews, through structured questionnaires collecting information on parents' sociodemographic characteristics, and children's health status and behavioural characteristics.

Dietary intake

Child's dietary intake was evaluated by Food Frequency Questionnaire (FFQ), assessing the previous six months. Details on response options, conversion into daily frequencies, validity and reliability are described elsewhere[17]. As detailed earlier[18], a total of 16 food groups were used to identify dietary patterns at child's 4 years of age. The same food groups and the cut-points observed at 4 years were considered in order to predict in which dietary pattern children would be at 7 years.

Anthropometric data

Child's weight and height were measured at both ages by standard procedures as previously described[17]. Age- and sex-specific Body Mass Index (BMI) standard deviation scores (BMI z-scores) were computed according to the WHO[7, 19].

Fat mass was assessed by tetra-polar bioelectric impedance (BIA 101 Anniversary®, Akern, Florence, Italy), according to standard procedures[20] and equipment outputs were converted into fat-free mass[21] used to calculate Fat Mass Percent (FM%) and Fat Mass Index (FMI). Waist circumference was measured at the umbilicus level[22] and waist-to-height (W/Ht) ratio was calculated. To improve comparability, these measures were converted into z-scores using the sex-specific means and standard deviations (SD) presented in table 3.

Potential confounders

Maternal BMI and completed schooling years at child's 4 years were included as continuous in all analyses. Maternal work status was defined as not-working *vs.* working and maternal smoking status was categorized into smoking *vs.* no. Child's exact age was considered in years. Non-exclusive breastfeeding was defined as <6 *vs.* ≥6 months. Regular practice of structured physical exercise was considered as a dichotomous variable (no *vs.* yes). Screen time was considered as >2 *vs*. ≤2 hours/day.

Ethics

All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki[23]. It was approved by the University of Porto Medical School / S. João Hospital ethics committee and signed informed consent according to the Declaration of Helsinki was required for all participants.

Statistical analysis

Children's dietary patterns were identified by Latent Class Analysis (LCA)[24]. This methodology is a person-centred approach, used to distinguish homogeneous clusters of individuals from a sample[24, 25]. In the present study, the number of dietary patterns was defined according to the Bayesian Information Criteria (BIC) and individuals are assigned to each pattern according to the highest probability of class membership. Probabilities of choosing each item response (e.g., consumption categories), conditionally on class membership, can be interpreted based on item profiles in each cluster[25, 26].

The association of dietary patterns with adiposity was assessed by linear regression coefficients (β) and respective 95% confidence intervals (95%CI). Four separate models (BMI, FM%, FMI, and W/Ht ratio) were fitted.

Confounding factors were assessed in each model and those not associated with the outcome or that did not change the association of interest were not considered in the final models (maternal age, work status, and smoking habits). For theoretical reasons, children's practice of physical exercise, daily screen time, and breastfeeding were included in all models. The hypothesized interaction of child's sex on the association with adiposity was examined by a product term added into fully adjusted models.

In order to assess temporal relationships of diet and adiposity across time – hypothesized to differ between girls and boys – an analysis based on a theoretical concept (figure 1) was also conducted, including three components: i) an analysis of the outcome across time [letter (a) in figure 1], ii) an analysis of exposure across time [letter (b) in figure 1]; and iii) crossed analysis of outcome at time 1 on exposure at time 2 [letter (c) in figure 1] as well as of the main association of interest [i.e., exposure at time 1 on outcome at time 2 [letter (d) in figure 1].

Association of adiposity measures across time were assessed by linear regression models. Association of adiposity at 4 years with later dietary pattern, as well as of patterns across the two ages, were estimated by odds ratios (OR) and respective 95%CI by multinomial logistic regression models.

Statistical analyses were conducted using SPSS statistical software package version 22 (SPSS Inc., Chicago, IL, USA) and Mplus version 5.2 (Muthén & Muthén, Los Angeles, California, USA), and a significance level of 5% was considered.

RESULTS

In the present sample, child's exact age did not differ between sexes, showing a mean of 4·4 years (SD 0·40) and 7·1 years (SD 0·21) at each follow-up. The proportion of children breastfed ≥6 months (non-exclusive breastfeeding) was similar in girls and boys (46%), as was the proportion of children spending ≤2 hours/day in front of a screen [television, computer or game devices (73%)]. Proportion of practice of structured physical exercise was slightly higher in girls (72%) when compared to boys (68%). At child's 4 years of age, mothers had a mean age of 34·3 years (SD 5·30), a mean BMI of 26·5 kg/m^2 (SD 5·19), and a mean of 11·3 complete schooling years (SD 4·20), with no statistically significant differences according to child's sex. Comparison of characteristics between this sample and the remaining cohort, showed that mothers in the remaining cohort were slightly younger [33·6 (SD 5·53) years, *P*<0·001] and less educated [10·2 (SD 4·17) years, *P*<0·001].

At child's 4 years of age, three dietary patterns were identified (two patterns, BIC 147,384; 3 patterns, BIC 146,698; 4 patterns, BIC 146,656). In both sexes (Table 1), in comparison to each respective total sample, latent class 1 had higher proportion of children in the 5th quintile of sweets, soft drinks, salty pastry, and processed meat, being named 'Energy-Dense Foods (EDF)' dietary pattern (43·7% girls, 44·5% boys). Latent class 2 had higher proportion of children in the 1st quintile (or once a day) of foods typically eaten at main meals (e.g., vegetables, fish, meat-eggs, rice-pasta-potatoes), as well as intermediate percentage of children in the 5th quintile of foods usually consumed at snacking occasions (e.g., bread, milk, yogurt, salty pastry), being named the 'Snacking' dietary pattern (12·5% girls, 14·3% boys). Latent class 3 was named 'Healthier' dietary pattern (43·8% girls, 41·2% boys) as it was characterized by higher consumption of healthy foods (e.g., vegetable soup, vegetables, and fish) as well as lower of unhealthy ones (e.g., sweets, soft drinks, processed meat), used as reference category in analyses.

Taking into account the proportion of children in lower and higher categories of consumption at 4 years of age, proportions of children in each dietary pattern at 7 years (Table 2) were: EDF (48·8% girls and 52·7% boys), Snacking (10·1% girls and 9·7% boys), and Healthier (41·1% girls and 37·6% boys). Median probabilities of class membership at child's 4 years were: 83% (EDF pattern); 73% (Snacking pattern); and 83% (Healthier pattern). At child's 7 years, these probabilities were respectively; 86%, 68%, and 86%.

As expected, at 4 years of age, higher adiposity was found in girls when compared to boys (table 3): FM% 18·8 (SD 8·02) *vs.* 15·1 (SD 7·13), P<0·001; and FMI 3·2 (SD 1·72) kg/m^2 *vs.* 2·5 (SD 1·41) kg/m^2, P<0.001. Likewise, at 7 years of age, girls had higher adiposity than boys: FM% 19·4 (SD 9·79) *vs.* 15·6 (SD 9·27), P=0·005; and FMI 3·6 (SD 2·31) kg/m^2 *vs.* 2·8 (SD 2·08) kg/m^2, P<0·001.

Temporal relationships between dietary patterns and adiposity are presented according to the conceptual model depicted in figure 1. After accounting for adjustment for maternal and child's characteristics, adiposity measures tracked across the two ages in both sexes [letter (a) in figure 1, results in table 3].

Proportion of children remaining in the same dietary pattern from 4 to 7 years was higher for the EDF (61% girls and 59% boys) and the Healthier (68% girls and 72% boys) patterns. In contrast, children following the Snacking pattern at 4 years mainly changed to the EDF (42% girls and 45% boys).

Dietary patterns tracked across the two ages [letter (b) in figure 1]. As shown in table 4, when compared to the healthier dietary pattern, the EDF pattern at 4 years significantly increased the odds of children following the same pattern at 7 years in both girls (OR=4·48, 95%CI 3·51-5·70) and boys (OR=4·70, 95%CI 3·68-5·98); likewise, the snacking pattern persisted in time (girls, OR=5·94, 95%CI 3·73-9·46; boys, OR=8·39, 95%CI 5·27-13·31).

After considering adjustment for maternal (BMI, education) and child's (non-exclusive breastfeeding, physical exercise, daily screen time, exact age and dietary patterns at 4 years) characteristics, adiposity measures at 4 years of age were not associated with later dietary patterns [letter (c) in figure 1]. In girls, adiposity measures at 4 years were not associated with the EDF pattern (BMI, OR=0·96, 95%CI 0·86-1·07; FM%, OR=0·94, 95%CI 0·84-1·05; FMI, OR=0·93 95%CI 0·83-1·04; and W/Ht ratio OR=0·93 95%CI 0·83-1·04) or the Snacking pattern (BMI, OR=0·94, 95%CI 0·80-1·11; FM%, OR=0·94, 95%CI 0·78-1·12; FMI, OR=0·91, 95%CI 0·76-1·09; and W/Ht ratio, OR=0·91, 95%CI 0·76-1·09) at 7 years of age.

In boys, adiposity measures at 4 years of age were also not associated with later dietary patterns: EDF (BMI, OR=1·03, 95%CI 0·93-1·15; FM%, OR=1·05, 95%CI 0·94-1·18; FMI OR=1·06, 95%CI 0·94-1·19; and W/Ht ratio, OR=1·01, 95%CI 0·90-1·14); Snacking (BMI, OR=0·99, 95%CI 0·83-1·18; FM%, OR=1·02, 95%CI 0·84-1·22; FMI, OR=1·02, 95%CI 0·84-1·22; and W/Ht ratio OR=1·03; 95%CI 0·85-1·23).

The association between dietary patterns at 4 years and adiposity at 7 years [letter (d) in figure 1] is presented in table 5. As hypothesized, this association was modified by child's sex. The interaction term was statistically significant in the BMI z-score model (*P*=0·046) and, although it did not reach statistical significance at a 5% level, was consistent in other models (FMI, *P*=0·053; and W/Ht ratio, *P*=0·063). In final multivariate models (model 2, table 5), girls following the EDF dietary pattern at 4 years, when compared to those following the Healthier pattern, had significantly higher z-scores of BMI (β=0·075, 95%CI 0·009-0·140), FMI (β=0·071, 95%CI 0·000-0·142), and W/Ht ratio (β=0·094, 95%CI 0·023-0·164), while in boys these associations were not observed. The snacking dietary pattern was not associated with any adiposity indicator in girls or boys.

DISCUSSION

Present findings indicate that an EDF dietary pattern at preschool age is significantly positively associated with measures of total and central adiposity in 7-year-old girls, but not in boys. To the best of our knowledge, this is the first prospective study during early childhood finding an association of *a posteriori* dietary patterns with adiposity and a modifying effect of child's sex on this association.

In a previous study conducted in this cohort[27] in younger children (from 2 to 4 years), sex did not modify the association between consumption of EDF and BMI z-scores. Comparisons with other studies starting at preschool age are difficult, as sex-specific associations of dietary patterns with later adiposity were not reported[14, 15]. Nevertheless, dietary patterns partly comparable to the current EDF pattern have been described. A 'junk' dietary pattern identified in 3-year-olds from the United Kingdom[14] was not associated with obesity 4 years later. In a study conducted in the same cohort[15], an 'energy-dense, low-fibre, high-fat' pattern practiced at 5 years was not significantly associated with fat mass at 7 years, although this pattern practiced at 7 years was significantly positively associated with adiposity at 9 years of age. In older children, some prospective studies reported an association of dietary patterns high in EDF with obesity[28] and one study[29] showed slight sex-differences on the association of a 'starchy foods' pattern with overweight.

Tracking of dietary patterns is also comparable to reports from other studies[15, 30]. In the current sample, most children remained in the Healthier and EDF patterns. In contrast, concerning the Snaking pattern, children mainly switched to the EDF dietary pattern from 4 to 7 years of age. A study conducted in the United Kingdom[31] also identified a 'snack' pattern among 3-year-olds that was not identified at 4, 7 or 9 years of age. It is possible that these 'snack'/'snacking' patterns are still reflecting the transition to the family diet and that, with growing age, children tend to change into other patterns.

The use of LCA to identify dietary patterns is an advantage, as this methodology is especially well-suited for categorical variables asymmetrically distributed, common in data from FFQ. LCA focuses on relationships between individuals and describes how the probabilities of a set of observed categorical variables (e.g., FFQ's items) vary across individuals finding the smallest number of unobserved variables (i.e., latent classes) that distinguish homogeneous clusters of individuals (e.g., dietary patterns) with the advantage of avoiding subjective choices on cut-points on underlying dimensions, as it adds classes stepwise until the model fits the data well and the classification is provided directly by the model[24].

Restricting the analysis of dietary patterns at 7 years to the consumption categories observed at 4 years deserves to be discussed. This approach was defined *a priori* in order to assess if children switched between dietary patterns from 4 to 7 years of age, rather than to identify new patterns at 7 years. Considering that median probabilities of class membership support an acceptable classification of individuals into each latent class at both ages, this approach was deemed appropriate.

Besides the prospective design and children's age frame, another strength of this study was enough statistical power to assess the possible interaction of child´s sex on the association between diet and adiposity. In addition, as children were part of a large population-based cohort regularly followed, several potential confounding factors could be assessed.

As well, evaluation of distinct measures of adiposity was essential to understand the differences observed, once BMI is not able to distinguish between lean and fat mass, the accuracy of FM% depends on height and cannot be assessed independently of free-fat mass, being important to use an index adjusted for height (i.e., FMI)[(32)]. Since these measures do not indicate regional fat distribution, it was also crucial to assess a measure of central fatness (i.e., W/Ht ratio) as abdominal adiposity is associated with increased metabolic complications in children[(33)].

Girls have higher relative body fat and lower visceral adipose tissue when compared to boys and these differences emerge before puberty[(10, 34)]. If sexual dimorphism in regional adiposity was the main explanation for differences between sexes on the association between diet and adiposity, it would be expected that – when measures of total adiposity were used – significant associations were only observed in girls, and – when measures of central adiposity were considered – significant associations could also be observed in boys.

Yet, the EDF pattern was positively associated with W/Ht ratio only in girls. It is possible that, in this population, the early age considered is too soon to observe substantial sex-differences in W/Ht ratio. Keeping in mind that waist circumference and BMI have been reported to be more strongly correlated with abdominal subcutaneous adipose tissue[(35)], the association of the EDF dietary pattern with W/Ht ratio may be reflecting the higher abdominal subcutaneous adiposity at a given W/Ht ratio that has been reported in girls[(35)].

Hypothetically, temporal relationships of dietary patterns and adiposity could differ between girls and boys. For instance, as girls appear to be more influenced by peers and earlier than boys[11-13], tracking of dietary patterns from 4 to 7 years of age could differ according to sex. Yet, the hypothesized distinct temporal associations between girls and boys were not observed.

The strong tracking of adiposity and dietary patterns across the two ages could have hampered assessment of temporal associations between diet and adiposity. Considering that the association between the EDF pattern at 4 years with later adiposity observed in girls was statistically significant in crude models as well as in those adjusted for adiposity at 4 years, it is unlikely that tracking of adiposity in time overcame the association between diet at 4 years and later adiposity. Additionally, considering that cross-sectional Spearman's correlations between diet and adiposity at 4 years in girls were very low and non-significant (not shown), it is unlikely that adiposity measures at 4 years were intermediate steps in the pathway between diet at 4 years and adiposity at 7 years of age. Furthermore, keeping in mind that in children current dietary behaviour could be more strongly associated with adiposity than baseline behaviour[36], adjustment for dietary patterns at 7 years was assessed but results did not change substantially in girls or boys (not shown).

Taken together, these considerations, suggest that sex differences may have other underlying explanations. If the observed effect-modification by sex is true, the evolutionary biology perspective could account for our results. As previously highlighted[8], females have higher propensity to store body fat than males and are considered at higher risk of obesity. Women's metabolic predisposition to store more fat has been suggested to be an evolved adaptive advantage for reproductive success, and this adaptive pattern may start at birth[9]. Considering that the association of the EDF dietary pattern with adiposity was consistent in several measures of adiposity among girls, it is possible that the interaction observed is reflecting a higher propensity of girls to gain fat.

Distinct BMI trajectories could be another explanation for the differences observed in the present study. Sex-differences in BMI trajectories have been reported, with girls showing earlier adiposity rebound[37]. Keeping this in mind, and considering that males and females are both susceptible to obesity, it is possible that the EDF pattern is positively associated with adiposity in boys at later stages.

In addition, it is important to emphasise that – in a public health perspective – consumption of EDF should be avoided in both girls and boys as they have little nutritional interest, have been associated with lower dietary quality[38], are consumed in large portions that contribute to higher energy intake and may promote obesity in the long-term[39].

Assessment of dietary intake by FFQ could be argued as a limitation, since only frequency was assessed. Inclusion of portion sizes in FFQ is controversial as it may reduce validity[40]. Additionally, as published earlier[17], comparison with 3-days food diaries supported a reasonable validity and reliability of the FFQ data. Also, the EDF pattern was significantly higher in energy than the Snacking and Healthier patterns (girls, 1616 *vs.* 1506 and 1571 kcal; boys, 1714 *vs.* 1609 and 1650 kcal).

Inclusion of a subsample could have introduced some bias into the analysis. Comparison of baseline characteristics with the remaining cohort showed that mothers in this sample were slightly older and more educated. Given that Cohen's effect size values are not high (<0.35), differences are likely due to large sample size rather than to substantial differences between participants (41).

In conclusion, a dietary pattern high in EDF, practiced by Portuguese preschool children, is consistently positively associated with adiposity 3 years later in girls but not in boys. Future studies should further examine if sex modifies the association between diet and adiposity in distinct populations at different ages, with particular focus on underlying metabolic pathways. Present findings suggest that interventions to prevent unhealthy dietary habits should focus on early dietary patterns.

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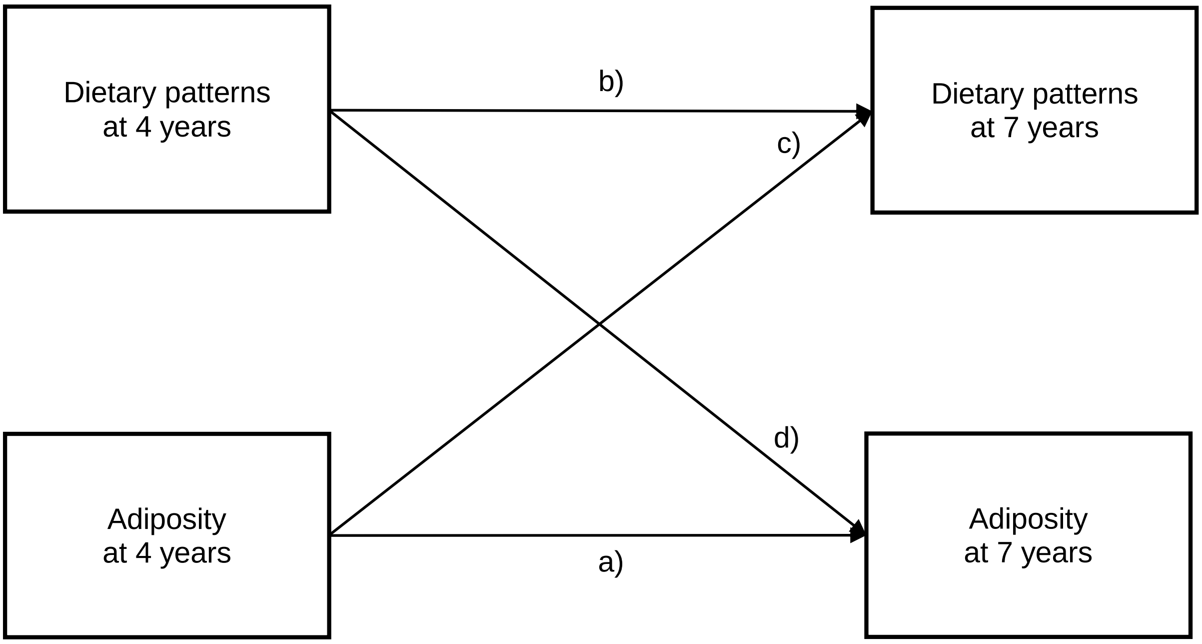

Figure 1. Conceptual model for the temporal relationship between dietary patterns and adiposity

Temporal relationships were analyzed considering associations of: (a) adiposity across ages (results, table 3); (b) dietary patterns across ages (results, table 4); (c) adiposity at 4 years with later dietary pattern; and (d) dietary patterns at 4 years with adiposity at 7 years (results, table 5).

Table 1. Dietary patterns at 4 years of age - proportion of children in lower and higher consumption categories by sex, *n* = 3,473

		Girls								Boys							
		Total sample		LC1		LC2		LC3		Total sample		LC1		LC2		LC3	
		n = 1722		EDF *n* = 753		Snacking *n* = 215		Healthier *n* = 754		*n* = 1751		EDF *n* = 780		Snacking *n* = 250		Healthier *n* = 721	
Food item/group	Category	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Fruit	1st Q	687	39·9	412	54·7	129	60·0	146	19·4	690	39·4	391	50·1	170	68·0	129	18·0
	5th Q	46	2·7	18	2·4	2	0·9	26	3·4	41	2·3	20	2·6	2	0·8	19	2·6
Vegetable soup	≤ 1/day	437	25·4	271	36·1	65	30·2	100	13·3	429	24·5	279	35·8	64	25·6	86	11·9
	≥ 2/day	1285	74·6	481	63·9	150	69·8	654	86·7	1322	75·5	501	64·2	186	74·4	635	88·1
Vegetables on a plate	1st Q	498	28·9	217	28·8	172	80·0	109	14·5	574	32·8	239	30·6	204	81·6	131	18·2
	5th Q	367	21·3	133	17·7	9	4·2	225	29·8	353	20·2	119	15·3	9	3·6	225	31·2
Milk	1st Q	366	21·3	160	21·2	50	23·3	156	20·7	327	18·7	153	19·6	43	17·2	131	18·2
	5th Q	137	8·0	52	6·9	31	14·4	54	7·2	171	9·9	81	10·4	34	13·6	59	8·2
Yogurt	1st Q	994	57·7	381	50·6	100	46·5	513	68·0	885	50·5	337	43·2	100	40·0	448	62·1
	5th Q	78	4·5	39	5·2	10	4·7	29	3·8	98	5·6	55	7·1	13	5·2	30	4·2
Cheese	1st Q	422	24·5	123	16·3	139	64·7	160	21·2	409	23·4	131	16·8	122	48·8	1563	21·6
	5th Q	140	8·1	79	10·5	15	7·0	46	6·1	124	7·1	82	10·5	19	7·6	23	3·2
Fish	1st Q	1162	67·5	591	78·5	177	81·3	394	52·3	1174	67·0	606	77·7	225	90·0	343	47·6
	5th Q	132	7·7	25	3·3	11	5·1	96	12·7	160	9·1	33	4·2	12	4·8	115	16·0
Meat and eggs	1st Q	686	39·8	306	40·6	111	51·6	269	35·7	652	37·2	271	34·7	117	46·8	264	36·6
	5th Q	305	17·7	151	20·1	18	8·4	136	18·0	315	18·0	154	19·7	32	12·8	129	17·9
Processed meat	1st Q	300	17·4	33	4·4	85	39·5	182	24·1	379	21·6	50	6·4	146	58·4	183	25·4
	5th Q	344	20·0	220	29·2	34	15·8	90	11·9	329	18·8	215	27·6	27	10·8	87	12·1
Rice, potatoes, pasta	≤ 1/day	128	7·4	48	6·4	36	16·7	44	5·8	146	8·3	52	6·7	49	19·6	45	6·2
	≥ 2/day	1594	92·6	705	93·6	179	83·3	710	94·2	1605	91·7	728	93·3	201	80·4	676	93·8
Bread	1st Q	885	51·4	358	47·5	111	51·6	416	55·2	812	46·4	340	43·6	123	49·2	349	48·4
	5th Q	24	1·4	13	1·7	4	1·9	7	0·9	31	1·8	17	2·2	4	1·6	10	1·4
Crisps	1st Q	403	23·4	55	7·3	53	24·7	295	39·1	404	23·1	70	9·0	56	22·4	278	38·6
	5th Q	154	8·9	124	16·5	27	12·6	3	0·4	153	8·7	129	16·5	24	9·6	0	0·0
Pizza, burger	1st Q	417	24·2	60	8·0	135	62·8	222	29·4	387	22·1	53	6·8	139	55·6	195	27·0
	5th Q	251	14·6	198	26·3	13	6·0	40	5·3	334	19·1	266	34·1	27	10·8	41	5·7
Sweets	1st Q	333	19·3	29	3·9	43	20·0	261	34·6	363	20·7	54	6·9	53	21·2	256	35·5
	5th Q	340	19·7	249	33·1	53	24·7	38	5·0	312	17·8	225	28·8	51	20·4	36	5·0
Soft drinks	1st Q	362	21·0	30	4·0	57	26·5	275	36·5	323	18·4	22	2·8	68	27·2	233	32·3
	5th Q	310	18·0	252	33·5	37	17·2	21	2·8	358	20·4	290	37·2	51	20·4	17	2·4
Salty pastry	1st Q	313	18·2	65	8·6	107	49·8	141	18·7	354	20·2	85	10·9	119	47·6	150	20·8
	5th Q	66	3·8	49	6·5	11	5·1	6	0·8	70	4·0	52	6·7	14	5·6	4	0·6

LC, latent class; EDF, energy-dense foods dietary pattern; Q, quintile.

Table 2. Dietary patterns at 7 years of age - proportion of children in lower and higher consumption categories by sex, *n* = 3,473

		Girls								Boys							
		Total sample		LC1		LC2		LC3		Total sample		LC1		LC2		LC3	
		n = 1722		EDF *n* = 840		Snacking *n* = 174		Healthier *n* = 708		*n* = 1751		EDF *n* = 922		Snacking *n* = 170		Healthier *n* = 659	
Food item/group	Category	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Fruit	1st Q	697	40·5	421	50·1	129	74·1	147	20·8	695	39·7	454	49·2	115	67·6	126	19·1
	5th Q	48	2·8	19	2·3	3	1·7	26	3·7	78	4·5	35	3·8	3	1·8	40	6·1
Vegetable soup	≤ 1/day	716	41·6	468	55·7	83	47·7	165	23·3	747	42·7	498	54·0	78	45·9	171	25·9
	≥ 2/day	1006	58·4	372	44·3	91	52·3	543	76·7	1004	57·3	424	46·0	92	54·1	488	74·1
Vegetables on a plate	1st Q	403	23·4	192	22·9	121	69·5	90	12·7	471	26·9	251	27·2	129	75·9	91	13·8
	5th Q	416	24·2	180	21·4	8	4·6	229	32·2	387	22·1	160	17·4	9	5·3	222	33·7
Milk	1st Q	1092	63·4	532	63·3	119	68·4	441	62·3	1033	59·0	560	60·7	98	57·6	375	56·9
	5th Q	52	3·0	26	3·1	8	4·6	18	2·5	39	2·2	22	2·4	1	0·6	16	2·4
Yogurt	1st Q	1197	69·5	530	63·1	120	69·0	547	77·3	1134	64·8	572	62·0	92	54·1	470	71·3
	5th Q	22	1·3	14	1·7	2	1·1	6	0·8	33	1·9	20	2·2	5	2·9	8	1·2
Cheese	1st Q	514	29·8	205	24·4	125	71·8	184	26·0	533	30·4	238	25·8	114	67·1	181	27·5
	5th Q	104	6·9	74	8·8	3	1·7	27	3·8	109	6·2	74	8·0	9	5·3	26	3·9
Fish	1st Q	891	51·7	532	63·3	128	73·6	231	32·6	895	51·1	578	62·7	122	71·8	195	29·6
	5th Q	455	26·4	151	19·0	34	19·5	270	33·1	513	29·3	197	21·4	34	20·0	282	42·8
Meat and eggs	1st Q	594	34·5	289	34·4	82	47·1	223	31·5	545	31·1	269	29·2	78	45·9	198	30·0
	5th Q	116	6·7	74	8·8	13	7·5	29	4·1	114	6·5	87	9·4	11	6·5	16	2·4
Processed meat	1st Q	394	22·9	61	7·3	114	65·5	219	30·9	396	22·6	81	8·8	115	67·6	200	30·3
	5th Q	494	28·7	320	38·1	23	13·2	151	21·3	488	27·9	317	34·4	24	14·1	147	22·3
Rice, potatoes, pasta	≤ 1/day	319	18·5	163	19·4	65	37·4	91	12·9	289	16·5	145	15·7	65	38·2	79	12·0
	≥ 2/day	1403	81·5	677	80·6	109	62·6	617	87·1	1462	83·5	777	84·3	105	61·8	659	88·0
Bread	1st Q	676	39·3	311	37·0	95	54·6	270	38·1	540	30·8	260	28·2	68	40·0	212	32·2
	5th Q	43	2·5	28	3·3	2	1·1	13	1·8	60	3·4	41	4·4	8	4·7	11	1·7
Crisps	1st Q	447	26·0	67	8·0	43	24·7	337	47·6	399	22·8	77	8·4	41	24·1	281	42·6
	5th Q	140	8·1	117	13·9	18	10·3	5	0·7	136	7·8	118	12·8	18	10·6	0	0·0
Pizza, burger	1st Q	126	7·3	13	1·5	53	30·5	60	8·5	102	5·8	18	2·0	49	28·8	35	5·3
	5th Q	341	19·8	267	31·8	26	14·9	48	6·8	413	23·6	330	35·8	27	15·9	56	8·5
Sweets	1st Q	615	35·7	138	16·4	61	31·1	416	58·8	634	36·1	175	19·0	60	35·3	399	60·5
	5th Q	125	7·3	101	12·0	16	9·2	8	1·1	145	8·3	115	12·5	22	12·9	8	1·2
Soft drinks	1st Q	236	13·7	17	2·0	30	17·2	189	26·7	204	11·7	14	1·5	29	17·1	161	24·4
	5th Q	406	23·6	334	39·8	46	26·4	26	3·7	485	27·7	392	42·6	48	28·2	45	6·8
Salty pastry	1st Q	83	4·8	21	2·5	24	13·8	38	5·4	76	4·3	19	2·1	29	17·1	28	4·2
	5th Q	632	36·7	466	55·5	67	38·5	99	14·0	379	36·5	500	54·2	58	34·1	81	12·3

LC, latent class; EDF, energy-dense foods dietary pattern; Q, quintile.

Table 3. Adiposity measures at 4 and 7 years of age and associations across the two ages, *n* = 3,473*

	Girls, *n* = 1722						Boys, *n* = 1751					
	Adiposity				Association with adiposity at 7 years		Adiposity				Association with adiposity at 7 years	
	4 years		7 years				4 years		7 years			
	Mean	SD	Mean	SD	β	95%CI	Mean	SD	Mean	SD	β	95%CI
Adiposity at 4 years †												
BMI z-score ‡	0·7	1·10	0·8	1·13			0·6	1·05	0·8	1·20		
Model 1					**0·831**	**0·803, 0·858**					**0·852**	**0·817, 0·886**
Model 2					**0·829**	**0·802, 0·857**					**0·851**	**0·817, 0·885**
FM%§	18·8	8·02	19·4	9·79			15·1	7·13	15·6	9·27		
Model 1					**0·640**	**0·604, 0·676**					**0·573**	**0·534, 0·611**
Model 2					**0·640**	**0·604, 0·676**					**0·573**	**0·535, 0·611**
FMI, kg/m^2 §	3·2	1·72	3·6	2·31			2·5	1·41	2·8	2·08		
Model 1					**0·709**	**0·677; 0·741**					**0·639**	**0·603, 0·674**
Model 2					**0·709**	**0·676, 0·741**					**0·639**	**0·604, 0·675**
W/Ht ratio §	0·5	0·04	0·5	0·05			0·5	0·03	0·5	0·04		
Model 1					**0·711**	**0·679, 0·744**					**0·661**	**0·627, 0·696**
Model 2					**0·711**	**0·678, 0·743**					**0·663**	**0·628, 0·698**

SD, standard deviation; β, regression coefficient; 95%CI, 95% confidence interval; BMI z-score, body mass index standard deviation scores; FM%, fat mass percent; FMI, fat mass index; W/Ht, waist-to-height.

***** Statistically significant associations are highlighted in bold.

† Models correspond to letter (a) in figure 1. Model 1 is adjusted for maternal (education, BMI) and child's (non-exclusive breastfeeding, physical exercise practice, daily screen time, and exact age at the 4 years' follow-up) characteristics. Model 2 is further adjusted for dietary pattern at child's 4 years of age.

‡ Age- and sex-specific BMI z-scores defined according to the World Health Organization's criteria (7,21).

§ Adiposity measures converted into z-scores using the sex-specific mean and standard deviation observed in the study sample.

Table 4. Association of dietary patterns at 4 years of age with dietary patterns at 7 years of age, *n* = 3,473[*,†]

	Dietary pattern at 7 years in girls, *n* = 1722							Dietary pattern at 7 years in boys, *n* = 1751					
	EDF, *n* = 840			Snacking, *n* = 174				EDF, *n* = 922			Snacking, *n* = 170		
Dietary pattern at 4 years	*n*	OR	95%CI	*n*	OR	95%CI	Dietary pattern at 4 years	*n*	OR	95%CI	*n*	OR	95%CI
Model 1[‡]													
Healthier (*n* = 754)	234	1·00		57	1·00		Healthier (*n* = 721)	247	1·00		48	1·00	
EDF (*n* = 753)	515	**4·48**	**3·51, 5·70**	55	**1·84**	**1·20, 2·83**	EDF (*n* = 780)	563	**4·68**	**3·68, 5·97**	47	**2·04**	**1·30, 3·21**
Snacking (*n* = 215)	91	**2·38**	**1·64, 3·43**	62	**6·00**	**3·88, 9·54**	Snacking (*n* = 250)	112	**2·34**	**1·64, 3·36**	63	**8·39**	**5·28, 13·32**
Model 2[§]													
Healthier (*n* = 754)	234	1·00		57	1·00		Healthier (*n* = 721)	247	1·00		48	1·00	
EDF (*n* = 753)	515	**4·48**	**3·52, 5·71**	55	**1·85**	**1·21, 2·84**	EDF (*n* = 780)	563	**4·70**	**3·68, 5·98**	47	**2·04**	**1·30, 3·20**
Snacking (*n* = 215)	91	**2·36**	**1·63, 3·41**	62	**5·94**	**3·73, 9·46**	Snacking (*n* = 250)	112	**2·35**	**1·64, 3·37**	63	**8·39**	**5·27, 13·31**

EDF, Energy-dense Foods dietary pattern; OR, odds ratio; 95% CI, 95% confidence interval.

***** Statistically significant associations are highlighted in bold.

[†] Corresponds to letter (b) in figure 1.

[‡] Model 1 is adjusted for maternal (education, BMI) and child's (non-exclusive breastfeeding, physical exercise practice, daily screen time, and exact age at the 4 years' follow-up) characteristics.

[§] Model 2 includes variables in Model 1 and is further adjusted for BMI z-score at child's 4 years of age.

Table 5. Association of children's dietary patterns at 4 years with adiposity at 7 years of age, *n* = 3,473*,†

		Girls, *n* = 1722			Boys, *n* = 1751		
Adiposity at 7 years§	Dietary pattern at 4 years‡	n	*β*	95%CI	n	*β*	95%CI
BMI z-score							
Model 1ǁ	EDF	753	**0·142**	**0·029, 0·256**	780	-0·014	-0·135, 0·107
	Snacking	215	-0·147	-0·317, 0·023	250	-0·125	-0·297, 0·047
Model 2¶	EDF	753	**0·075**	**0·009, 0·140**	780	-0·014	-0·093, 0·065
	Snacking	215	-0·010	-0·105, 0·085	250	-0·087	-0·197, 0·023
FM%							
Model 1ǁ	EDF	753	0·082	-0·019, 0·183	780	-0·033	-0·134, 0·068
	Snacking	215	-0·068	-0·220, 0·083	250	-0·016	-0·160, 0·128
Model 2¶	EDF	753	0·053	-0·171, 0·058	780	0·008	-0·077, 0·092
	Snacking	215	-0·057	-0·171, 0·058	250	-0·035	-0·153, 0·083
FMI, kg/m^2							
Model 1ǁ	EDF	753	**0·108**	**0·008, 0·209**	780	-0·024	-0·125, 0·077
	Snacking	215	-0·058	-0·210, 0·093	250	-0·026	-0·170, 0·118
Model 2¶	EDF	753	**0·071**	**0·000, 0·142**	780	0·012	-0·067, 0·090
	Snacking	215	-0·044	-0·147, 0·059	250	-0·043	-0·153, 0·066
W/Ht ratio							
Model 1ǁ	EDF	753	**0·136**	**0·036, 0·237**	780	0·015	-0·087, 0·116
	Snacking	215	-0·067	-0·218, 0·084	250	0·011	-0·133, 0·155
Model 2¶	EDF	753	**0·094**	**0·023, 0·164**	780	0·049	-0·028, 0·125
	Snacking	215	-0·007	-0·109, 0·096	250	-0·019	-0·125, 0·087

β, regression coefficient; 95%CI, 95% confidence interval; BMI z-score, body mass index standard deviation scores; EDF, energy-dense foods dietary pattern; FM%, fat mass percent; FMI, fat mass index; W/Ht, waist-to-height.

***** Statistically significant associations are highlighted in bold.

† Results correspond to letter (d) in figure 1.

‡ The reference category (healthier dietary pattern) is not shown in order to avoid redundancy.

§ Age- and sex-specific BMI z-scores defined according to the World Health Organization's criteria (7,21). To facilitate comparability, FM%, FMI and W/Ht ratio were converted into z-scores using the sex-specific mean and standard deviation observed in the study sample.

ǁ Crude model.

¶ Model adjusted for maternal (education, BMI) and child's (non-exclusive breastfeeding, physical exercise practice, daily screen time, exact age at the 4 years' follow-up and each respective adiposity indicator at 4 years) characteristics.

PAPER V

Protein intake and dietary glycemic load of 4-year-olds and association with adiposity and fasting serum insulin at 7 years of age: sex-nutrient and nutrient-nutrient interactions

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[Submitted]

Protein intake and dietary glycemic load of 4-year-olds and association with adiposity and fasting serum insulin at 7 years of age: sex-nutrient and nutrient-nutrient interactions

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ABSTRACT

Background: The role of protein intake (PI) at preschool age on later adiposity is understudied and prospective studies also examining dietary Glycemic Load (GL) are lacking.

Objective: To evaluate the association of PI and GL at 4 years with adiposity and Fasting Serum Insulin (FSI) 3 years later, and to examine the possible interaction between PI and GL on these associations, by sex.

Design: This prospective study included 1999 children enrolled in the population-based birth cohort, Generation XXI (Porto-Portugal, 2005-2006). Diet at 4 years was assessed by 3-days food diaries. Energy-adjusted PI and GL (g/d) were converted into sex-specific tertiles (T). At 7 years, World Health Organization's Body Mass Index (BMI) z-scores were defined. Using the sample's sex-specific means and standard deviations, z-scores were also computed for Fat Mass Index (FMI), Waist-to-Height ratio (W/Ht) and FSI. Associations were estimated by linear regression coefficients (β) and 95% confidence intervals (95%CI).

Results: PI was positively associated with BMI in girls (T2 *vs* T1, β=0.187, 95%CI 0.015-0.359) and boys (T3 *vs* T1, β=0.205, 95%CI 0.003-0.406), being associated with FSI only in boys (T3 *vs* T1, β=0.207, 95%CI 0.011-0.404, *P*-interaction=0.026). GL was also associated with BMI only in boys (T3 *vs* T1, β=0.362; 95%CI 0.031-0.693, *P*-interaction=0.006), in whom significant interactions between GL and PI were found on the association with FMI (*P*=0.019) and W/Ht (*P*=0.039). Boys in the 3rd T of both GL and PI at 4 years had higher FMI (β=0.505; 95%CI 0.085-0.925) and W/Ht (β=0.428; 95%CI 0.022-0.834) at 7 years.

Conclusions: In both girls and boys, PI at preschool age is positively associated with later BMI, being positively associated with FSI only in boys. GL is associated with adiposity only in boys, in whom it seems to interact with PI enhancing increased adiposity.

INTRODUCTION

Obesity is considered to be consequence of a sustained positive energy balance, influenced by several complex multifaceted factors (1), but the role of particular macronutrients in childhood obesity remains unclear. A systematic review published in 2014 (2) concluded that energy-restriction, rather than macronutrient distribution , can improve weight status in children. Yet, in a large randomized intervention trial, reduction of protein intake during infancy has been associated with lower weight at 2 years (3) as well as lower obesity prevalence at 6 years of age (4). Also, low carbohydrate diets showed a significant short-term beneficial effect on Body Mass Index (BMI) among overweight children when compared to low-fat diets (2).

Evidence on the association of higher protein intake during the first 2 years of age with accelerated growth and higher BMI is supported by several prospective studies (5), but the role of its intake at later ages during childhood is still not clear, since research in older children is scarce (6, 7) and prospective studies examining protein intake at preschool age are almost inexistent (8). Likewise, although low effectiveness of energy-restricted low-fat diets in obesity control led to the reappearance of the interest in low-carbohydrate and low-glycemic diets (9), research on Glycemic Index (GI) and Glycemic Load (GL) during early childhood is also limited (10).

The association between high protein intake and adiposity has been proposed to be mediated by changes in hormonal status such as enhanced secretion of insulin and insulin-like growth factor I (IGF-I), promoting cell growth and proliferation (11, 12). Dietary carbohydrates and blood glucose also affect insulin secretion, and amino acids produce an additional effect on insulin secretion when ingested with glucose (13). In addition, both amino acids and glucose – along with growth factors (insulin and IGF-I) – stimulate signaling pathways that regulate cell growth and proliferation (i.e., the mammalian target of rapamycin) (14). Indeed, evidence suggests that a reduction in protein content of infant formula diminishes insulin-releasing amino acids, decreasing circulating levels of insulin and insulin-like growth factor I (IGF-I) and resulting in lower weight gain (15), and that diets low in GI/GL significantly decrease insulin resistance among children and adolescents either overweight or obese (16). In addition, sexual dimorphism in response to central insulin (17), in circulating insulin and IGF-I since very early ages (18), and in the endocrine response to early protein intake (19, 20) have been reported.

We hypothesized that child's sex could modify the association of dietary factors with adiposity and that there might be an interaction between protein intake and dietary GL on the association with adiposity. As such, the present study aimed to evaluate the association of protein intake and dietary GL at 4 years of age with adiposity and fasting serum insulin (FSI) at 7 years of age, to evaluate whether child's sex modified these associations and to examine the possible interaction between protein intake and dietary GL on these associations.

SUBJECTS AND METHODS

Subjects

This prospective cohort study was based on the population-based birth cohort Generation XXI (Porto, Portugal, 2005-2006), described elsewhere (21). In brief, a total of 8647 live newborns were enrolled between April 2005 and August 2006 at all the public maternity units from the metropolitan area of Porto, Portugal.

At 4 and 7 years of age, the entire cohort was invited to be re-evaluated (86% and 81% participation proportion, respectively). Evaluations were performed in face-to-face interviews or, for those families not able to participate in-person (20% and 15% at 4 and 7 y, respectively), it was performed by telephone using a shorter version of the questionnaire.

The current analysis considered a subsample of singleton children with complete 3-days food diaries at the age of 4 years (*n*=2415). Children with diseases that might influence dietary intake [e.g., cerebral palsy or food allergy, *n*=14] were not included. After exclusion of outliers in reporting of dietary intake (i.e., daily energy intake >3 times the interquartile range, *n*=1) a total of 2121 children evaluated in-person at both 4 and 7 years of age remained for analyses. We further excluded children with missing information on birthweight and/or gestational age (*n*=10) as well as those whose mothers had incomplete information on weight or height (*n*=112), a total of 1999 children remaining for final analyses. Valid information on body composition was available for 1878 children and, for a subsample of 1404 children, information on FSI was also available.

All the phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (22). The study was approved by the University of Porto Medical School / S. João Hospital ethics committee and a signed informed consent according to Helsinki was required for all participants.

Dietary data

Dietary intake was assessed by 3-days food diaries as previously detailed (23). In brief, participants were instructed to describe all foods and beverages consumed (reporting the commercial trade names, if applicable) and the amount in grams, units or household measures. Out-of-home meals in the absence of a parent were reported by the child day-time caregiver. Meals prepared at home were recorded with details for recipes, including ingredients and methods of preparation.

Energy and nutrient intakes were calculated using the software Food Processor SQL version 2004-5 (ESHA Research, Salem, Oregon, USA) which is linked to the Food Composition Table of the United States of America Department of Agriculture (24). For Portuguese foods and recipes new codes were created with national nutritional information.

For the purpose of this study, mean daily intakes of energy (kcal) and mean absolute intakes of macronutrients (g/d) were calculated for each child considering the mean of the 3-days food diaries. For descriptive purposes, daily percentage of energy from carbohydrates and protein were computed. In addition, also for descriptive purposes, daily protein intake at 4 years of age relative to reference weight (g/kg/day) was calculated using the World Health Organization's (WHO) standards to calculate reference body weight (25).

GI values were assigned to foods reported in food diaries, according to a standardized procedure adapted from a previously published methodology (26). The concept of GI was proposed more than 30 years ago (27) as a classification system of the blood glucose-raising potential of carbohydrate-containing foods representing carbohydrate quality, and is defined as the incremental area under the blood glucose response curve elicited by 50 g available carbohydrate of a test food expressed as percentage of the response produced by 50 g of carbohydrates from a reference food (27, 28). Dietary GL was proposed later (29, 30) as a measure of the overall glycemic effect of food, incorporating both quality and quantity of carbohydrates.

In the current study, GI values were assigned according to the glucose scale, using as reference Table A1 of the International Tables of GI and GL Values (31) and updates released on the Sydney University's website (32). Foods were either assigned: i) a published GI; ii) the GI of a close match; iii) a calculated GI (e.g., for recipes, the GI of each ingredient and recipe composition were used to calculate a GI value); iv) a nominal GI (e.g., vegetables with no published GI were assigned a nominal value of 40); or no GI (i.e., foods with <1 g carbohydrate per 100 g). The content of available carbohydrate (total carbohydrate minus fiber) of each serving was multiplied by the food's GI and GL was computed as the sum of these products. The average GI was obtained by dividing daily GL by the total daily carbohydrate intake.

Anthropometric variables and insulin

Anthropometric measurement was performed by trained staff and a detailed description of procedures followed is described elsewhere (33, 34). Sex-specific z-scores of birthweight for gestational age were computed according to the Canadian reference (35) and used as continuous in analyses. At child's 7 years, age- and sex-specific BMI standard deviation scores (BMI z-scores) were computed according to the WHO's criteria (36), also used as continuous variables.

Body composition was assessed by tetrapolar bioelectric impedance (BIA 101 Anniversary®, Akern, Florence, Italy), according to standard procedures (37). Equipment outputs were converted into fat-free mass by Schaefer's equation (38) and Fat Mass Index [kg/m^2 (FMI)] was derived accordingly. Waist circumference was measured at the umbilicus level according to standard procedures (39) and Waist-to-Height (W/Ht) ratio was calculated. These variables were converted into z-score units [mean=0, standard deviation (SD)=1] using the sex-specific means and SD observed in the current sample.

After an overnight fast, a venous blood sample was collected before 11 a.m., according to standard procedures, after applying a topical analgesic cream (EMLA cream). Insulin was measured using electrochemiluminescence immunoassay, was expressed in µIU/mL and was further converted into sex-specific z-scores using means and SD observed in the current sample.

Potential confounding factors

Potential confounders selected based on previous literature were: maternal education, defined as the number of completed schooling years at child's delivery; maternal BMI at child's 4 years of age; maternal smoking status at child's 4 years of age (smoking 1-10 or >10 cigarettes/day, *vs.* non-smoking); child's sex; duration of non-excusive breastfeeding (<6 *vs.* ≥6 months); child's exact age at the 4 years follow-up; and time spent practicing structured physical exercise (hours/week) plus hours per day spent in front of a screen (television, computer, or game devices) as proxies of physical activity at 4 years of age.

Statistical analysis

The association of exposures (protein intake and dietary GL) at the age of 4 years with the outcomes (BMI z-scores, FMI, W/Ht ratio and FSI at 7 years of age) was assessed by regression coefficients (β) and respective 95% confidence intervals (95%CI) estimated by linear regression models.

In order to examine the effect of protein intake and dietary GL independently of energy content, both were adjusted using the nutrient residual method (40). Energy-adjusted protein intake and dietary GL were obtained from sex-specific regressions and predicted protein intake (or dietary GL) for mean energy intake (1581 and 1670 kcal/day in girls and boys, respectively) were added to enhance interpretability (40). These energy-adjusted dietary variables were further categorized into sex-specific tertiles.

Dietary GL represents an interaction between carbohydrates and GI (i.e., multiplying carbohydrates by GI means that higher GI has increased effect at higher intakes of carbohydrates). As such, models including dietary GL also included carbohydrates and GI, both energy-adjusted by the residuals method (40).

Total energy intake was also included in all models. Potential confounding factors were individually assessed in each model and those that did not change the associations of interest were not included in final analyses; maternal smoking status, child's exact age and daily screen time. Hence, besides total energy intake, models were adjusted for maternal education and BMI as well as for child's characteristics: birthweight adjusted for gestational age; duration of non-exclusive breastfeeding; and time practicing structured physical exercise at 4 years of age.

Protein intake and dietary GL were examined both separately and in models considering their mutual adjustment. Tests for interactions between child's sex and protein intake or dietary GL were formally assessed by product terms added into fully adjusted models. The interaction between protein intake and dietary GL on the association with BMI z-scores, FMI, W/Ht ratio and FSI was also tested by product terms added into fully adjusted models.

Statistical analysis was conducted using SPSS statistical software package version 22 (SPSS Inc., Chicago, IL, USA) and a significance level of 5% was adopted.

RESULTS

Characteristics of mothers and children are presented in **Table 1**. Mean protein intake at child's 4 years of age was similar between girls and boys, when considering both protein intake relatively to reference weight (4.4 and 4.5 g/kg/day, respectively) and percent daily energy intake from protein (18.8% and 18.6%, respectively). Dietary GL in girls (mean=99.8, SD=22.56) was slightly lower than in boys (mean=106.7, SD=23.52).

When considering protein intake separately from dietary GL and models adjusted for maternal and child's characteristics (**Table 2**, Model 2), energy-adjusted protein intake at 4 years of age was positively associated with BMI z-scores 3 years later in both girls and boys. Energy-adjusted dietary GL (Table 2, Model 2), was positively associated with BMI z-scores only in boys (T3 *vs.* T1, β=0.335, 95%CI 0.004-0.666, *P*-trend=0.045).

Models including protein and dietary GL simultaneously (Table 2, Model 3), showed that protein intake remained positively associated with BMI z-scores in both girls (T2 *vs.* T1, β=0.187, 95%CI 0.015-0.359, *P*-trend=0.266) and boys (T3 *vs.* T1, β=0.205, 95%CI 0.003-0.406, *P*-trend=0.045). Likewise, dietary GL remained significantly positively associated with BMI z-scores only in boys (*P*-interaction=0.006).

No statistically significant interaction between protein intake and dietary GL was found on the association with BMI z-scores, and therefore the product term was not included in the final model. In contrast, in boys, statistically significant interactions between protein intake and dietary GL were observed on the association with W/Ht ratio (*P*-interaction=0.039) and FMI (*P*-interaction=0.019). As such, final models included interaction terms. The association of these interactions with W/Ht ratio and FMI are depicted in **figures 1 and 2**, respectively: boys in the highest tertile of both protein intake and dietary GL, when compared to those in the first tertile for both dietary variables, had significantly higher W/Ht ratio (β=0.428, 95%CI 0.022-0.834) and FMI (β=0.505, 95%CI 0.085-0.925).

As shown in **Table 3** (Model 3); Protein intake at 4 years of age was positively associated with FSI at 7 years of age only in boys (*P*-interaction=0.026). When compared to boys in the 1^{st} tertile, boys in the highest tertile of protein intake at 4 years of age showed a statistically significant increase of 0.208 z-score units in FSI at 7 years. The interaction between protein intake and dietary GL on the association with FSI showed borderline statistical significance (*P*-value=0.051), and thus was not included in the final model.

DISCUSSION

This study shows that higher protein intake at preschool age is associated with higher BMI in both girls and boys. In addition, in boys, protein intake at 4 years of age is positively associated with subsequent serum insulin levels and dietary GL is positively associated with BMI. Moreover, among boys, this study shows an interaction between protein intake and dietary GL on the association with adiposity.

As far as could be ascertained, the only prospective study examining protein intake at preschool age and later obesity risk was conducted in Germany in a sample of 203 children (8), showing that higher total protein intake at 5-6 years was positively associated with BMI at 7 years of age and that formal examination of a possible interaction between protein and sex did not indicate a significant effect modification. Yet, other studies have reported sexual dimorphism in the association between protein intake and adiposity. In a large prospective cohort study conducted in the Netherlands (41), a borderline significant interaction between sex and protein intake on fat mass percent was found; higher protein intake at 13 months was positively associated with fat mass only in girls. In contrast, in a sample of 90 children from Iceland (42), protein intake at 9-12 months was positively associated to BMI at 6 years only in boys. Concerning dietary GL, a prospective study including 380 children from Germany (10), found no evidence of an association between dietary GL at 2 years and adiposity at 7 years of age and the analysis did not indicate an interaction between dietary GL and child's sex.

Sex-specific associations observed in the current study could be explained by sex-differences in the importance of fat *versus* protein and carbohydrates in metabolism. It has been proposed (17) that differences in sensitivity to central insulin and leptin between males and females, which are apparent since very early ages, reflect that females are physiologically more prone to use fat as substrate, while males rely more on glucose and protein metabolism. In order to assess if the effect of energy intake was independent of its source in girls and boys, sex-specific models including only energy intake were compared with models further including energy from macronutrients. Likelihood ratio tests indicated that, in girls, the effect of energy intake was independent of all macronutrients. In contrast, in boys, although the effect of energy intake was independent of energy supplied by fat, it was dependent of energy supplied either by protein or carbohydrates. This suggests that total energy intake could be more relevant in girls, while – in boys – energy intake is dependent of protein and carbohydrate intakes.

The association of both protein intake and dietary GL with specific measures of adiposity only in boys may be reflecting the higher reliance on protein and carbohydrates reported in males. The positive association between protein intake and serum insulin only found in boys gives strength to this possibility. Insulin and IGF-I have been proposed as intermediate steps in the pathway between protein intake and adiposity. In an study conducted in 83 healthy infants (20), intervention with whole milk – when compared to infant formula – resulted in higher concentration of IGF-I in boys while there was not effect in girls, suggesting differing endocrine responses to protein intake. In contrast, in a large randomized controlled trial (19), the IGF-I axis of female infants showed a stronger response to high protein intake, but no effect on BMI was observed. Insulin is more highly correlated with total fat mass in males who are also more sensitive to central insulin (17).

A major finding of the present study is that dietary GL seems to interact with protein intake, enhancing increased total and central adiposity in boys. Both amino acids and glucose, along with growth factors (insulin and IGF-I), play important roles in signaling pathways that regulate cell growth and proliferation [i.e., the mammalian target of rapamycin (mTOR)] (14). High protein supply may result in higher levels of branched-chain amino acids increasing secretion of IGF-I and insulin and resulting in adipogenic activity. Considering that both glucose and amino acids enhance insulin secretion and that amino acids have an additional effect on insulin secretion when ingested with glucose (13), as well as that boys are more sensitive to central insulin, the interaction between dietary GL and protein may have biological plausibility, as suggested by the borderline statistical significance of their interaction on the association with serum insulin. Yet, as serum insulin was considered at 7 years of age, we cannot assure if it is an intermediate step or if it is a consequence of higher adiposity.

Other underlying mechanisms might explain present findings. In some cohorts (43, 44), distinct BMI trajectories by child's sex have been reported, girls showing older age and lower BMI at infancy peak and earlier adiposity rebound. Also, among infants showing catch-up growth, boys catch-up more rapidly, which could reflect the anabolic effects of early infancy sex hormone (45). In addition, boys appear to be more sensitive to growth hormone (46) and have shown higher sensitivity to nutritional intervention during infancy in particular circumstances (i.e., preterm infants) (47). Hence, it is possible that distinct growth trajectories, distinct endocrine responses, or sex-dependent sensitivities might arise at distinct sensitive periods.

Misreporting of food consumption is a possible limitation that needs to be addressed. Yet, dietary intake was assessed by 3-day food diaries. Considering that food diaries have been described as the most suitable method for assessment of intake at the individual level, particularly in children (48), and that instructions were given to parents in order to minimize misreporting and increase quality of dietary assessment (e.g., food diary given to day-time caregiver), misreporting is expected to be diminished. Selection bias may have occurred, since there is a high burden inherent to collection of information by food diaries (49) which resulted in a high number of undelivered, incomplete or incorrectly filled records. Comparison between this sample and the remaining cohort showed no statistically significant differences relatively to maternal BMI and child's sex, although mothers included in this analysis were slightly more educated (mean=11.5, SD=4.22 *vs.* mean=10.8 complete schooling years, SD=4.30; $P<0.001$). As food diaries require literate respondents (49), this difference was expected. However, given that Cohen's effect size values are low (<0.2) (50), differences are likely due to large sample size rather than to substantial differences between participants.

Assessment of dietary GL in epidemiologic studies also has some methodological limitations. The number of foods recorded in food diaries clearly exceeds the number of items with a measured GI, hampering the assignment of GI values to databases (26) and calculation of GI for mixed dishes raises controversy. Yet, GI calculation has been reported to be valid (28) and it is the only practical approach in large population-based studies.

The prospective design, the age frame considered and the evaluation of several adiposity indicators and serum insulin are considerable strengths of this study. Furthermore, large sample size constitutes a major strength, enabling examination of interactions. Also, as participants are part of a population-based cohort regularly followed, enabled assessment of several potential confounding factors.

In conclusion, current results support that protein intake at preschool age is positively associated with later BMI in both girls and boys, and with serum insulin in boys. Also, the present study shows that higher dietary GL at 4 years of age is positively associated with later BMI only in boys, in whom it appears to interact with protein intake enhancing the effect on adiposity. Future studies should further examine possible interactions between child's sex and nutrients, as well as nutrient-nutrient interactions, and should evaluate the effect on endocrine responses to protein intake and dietary GL in order to elucidate on possible underlying mechanisms.

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Table 1. Demographic, dietary, and anthropometric characteristics by child's sex, n = 1999 [a, b]

	Girls	Boys
	n=973 (48.70%)	n=1026 (51.3%)
Maternal characteristics		
Age at child's delivery (years)	30.2 (5.12)	30.0 (5.00)
Education at child's delivery (years)	11.4 (4.30)	11.4 (4.20)
BMI at child's 4 y (kg/m^2)	26.4 (5.17)	26.2 (4.73)
Overweight at child's 4 y (≥25.0 kg/m^2)	512 (52.6)	545 (53.1)
Child's dietary characteristics		
Non-exclusive breastfeeding ≥6 months	487 (50.1)	515 (50.2)
Dietary characteristics at 4 years		
Energy (kcal/day)	1580.9 (279.68)	1669.9 (299.43)
Protein (g/day)	73.8 (14.33)	77.5 (16.00)
Protein (g/kg of reference body weight/day)	4.4 (0.91)	4.5 (0.98)
Protein (% of total daily energy)	18.8 (2.54)	18.6 (2.58)
Available carbohydrate (g/day)	180.5 (36.90)	192.6 (38.64)
Available carbohydrate (% total daily energy)	45.6 (4.13)	46.1 (4.11)
Average daily glycemic index (%)	54.7 (3.49)	54.9 (3.42)
Total dietary glycemic load (g/day)	99.8 (22.56)	106.7 (23.52)
Child's characteristics		
At birth		
Weight (g)	3163.8 (457.09)	3242.3 (476.12)
At 7 years of age		
BMI z-score [d]	0.7 (1.15)	0.7 (1.24)
Waist-to-Height ratio	0.5 (0.05)	0.5 (0.05)
Fat Mass Index (kg/m^2)	3.3 (2.41)	2.6 (2.12)
Fasting Serum Insulin (µIU/mL)	5.8 (4.00)	5.1 (3.48)

[a] Mean (SD)
[b] n (%)
[c] Sex-specific z-scores of birthweight for gestational age according to the Canadian reference (35)
[d] Age- and sex-specific BMI z-scores according to the WHO (36)

Table 2. Association of protein intake and dietary glycemic load at 4 years of age with BMI z-scores at 7 years of age, according to child's sex [a,b]

		Model 1	Model 2	Model 3
	n	*β* (95%CI)	*β* (95%CI)	*β* (95%CI)
Protein intake (g/d) [c]				
Girls				
T1 (≤69.7)	326	Ref		Ref
T2 (69.8-77.5)	323	0.165 (-0.012, 0.342)	**0.174 (0.010, 0.339)**	**0.187 (0.015, 0.359)**
T3 (>77.5)	324	0.065 (-0.112, 0.242)	0.084 (-0.081, 0.249)	0.110 (-0.075, 0.295)
P-trend		0.473	0.323	0.266
Boys				
T1 (≤72.7)	345	Ref		Ref
T2 (72.8-81.0)	335	0.074 (-0.111, 0.260)	0.077 (-0.100, 0.255)	0.085 (-0.101, 0.272)
T3 (>81.0)	346	**0.222 (0.038, 0.406)**	**0.213 (0.036, 0.391)**	**0.205 (0.003, 0.406)**
P-trend		**0.018**	**0.018**	**0.045**
Dietary Glycemic Load (g/d) [d]				
Girls				
T1 (≤94.7)	315	Ref		Ref
T2 (94.8-104.3)	329	0.047 (-0.184, 0.278)	0.067 (-0.147, 0.281)	0.060 (-0.154, 0.273)
T3 (>104.3)	329	0.017 (-0.338, 0.371)	0.071 (-0.257, 0.399)	0.072 (-0.256, 0.399)
P-trend		0.913	0.660	0.661
Boys				
T1 (≤101.7)	342	Ref	Ref	Ref
T2 (101.8-111.0)	344	0.086 (-0.151, 0.323)	0.031 (-0.196, 0.258)	0.040 (-0.187, 0.268)
T3 (>111.0)	340	**0.404 (0.058, 0.749)**	**0.335 (0.004, 0.666)**	**0.362 (0.031, 0.693)**
P-trend		**0.021**	**0.045**	**0.031**

Ref, reference category; T, tertile.

[a] Age- and sex-specific BMI z-scores were defined according to the WHO (36).

[b] Statistically significant results are highlighted in bold.

Model 1 includes: [c] protein intake (energy-adjusted by the residuals method), and total energy intake; or [d] dietary glycemic load, glycemic index and carbohydrate intake (all energy-adjusted by the residuals method), and total energy intake.

Model 2 includes variables in model 1 further adjusted for maternal (education at child's delivery and BMI at child's 4 y of age) and child's (z-scores of birthweight for gestational age (35), non-exclusive breastfeeding, and weekly time practicing structured physical exercise at 4 y) characteristics.

Model 3 includes variables in model 2, plus: [c] dietary glycemic load, glycemic index, carbohydrate intake (all energy-adjusted by the residuals method); or [d] protein intake (energy-adjusted by the residuals method).

Table 3. Association of protein intake and dietary glycemic load at 4 years of age with fasting serum insulin at 7 years of age, according to child's sex [a,b]

	n	Model 1	Model 2	Model 3
		β (95%CI)	*β* (95%CI)	*β* (95%CI)
Protein intake (g/d) [c]				
Girls				
T1 (≤69.7)	222	Ref		Ref
T2 (69.8-77.5)	230	0.014 (-0.166, 0.194)	0.006 (-0.173, 0.186)	0.011 (-0.173, 0.195)
T3 (>77.5)	210	0.013 (-0.169, 0.194)	-0.002 (-0.184, 0.180)	0.031 (-0.168, 0.231)
p-trend		0.890	0.983	0.759
Boys				
T1 (≤72.7)	237	Ref		Ref
T2 (72.8-81.0)	233	-0.060 (-0.237, 0.118)	-0.057 (-0.232, 0.118)	0.002 (-0.183, 0.186)
T3 (>81.0)	242	0.136 (-0.040, 0.312)	0.138 (-0.037, 0.313)	**0.207 (0.011, 0.404)**
p-trend		0.134	0.126	**0.035**
Dietary Glycemic Load (g/d) [d]				
Girls				
T1 (≤94.7)	229	Ref		Ref
T2 (94.8-104.3)	237	0.159 (-0.087, 0.405)	0.155 (-0.090, 0.400)	0.160 (-0.087, 0.406)
T3 (>104.3)	226	0.000 (-0.362, 0.361)	0.016 (-0.344, 0.376)	0.022 (-0.340, 0.383)
p-trend		0.991	0.937	0.920
Boys				
T1 (≤101.7)	242	Ref	Ref	Ref
T2 (101.8-111.0)	235	-0.234 (-0.565, 0.098)	-0.229 (-0.556, 0.098)	-0.193 (-0.520, 0.135)
T3 (>111.0)	235	-0.219 (-0.457, 0.019)	-0.211 (-0.446, 0.023)	-0.181 (-0.416, 0.054)
p-trend		0.179	0.180	0.261

Ref, reference category; T, tertile.

[a] Fasting serum insulin was converted into z-scores using sex-specific means and SD of the current sample.

[b] Statistically significant results are highlighted in bold.

Model 1 includes: [b] protein intake (energy-adjusted by the residuals method), and total energy intake; or [c] dietary glycemic load, glycemic index and carbohydrate intake (all energy-adjusted by the residuals method), and total energy intake.

Model 2 includes variables in model 1 further adjusted for maternal (education at child's delivery and BMI at child's 4 y of age) and child's (z-scores of birthweight for gestational age (35), non-exclusive breastfeeding, and weekly time practicing structured physical exercise at 4 y) characteristics.

Model 3 includes variables in model 2, plus: [c] dietary glycemic load, glycemic index, carbohydrate intake (all energy-adjusted by the residuals method); or [d] protein intake (energy-adjusted by the residuals method).

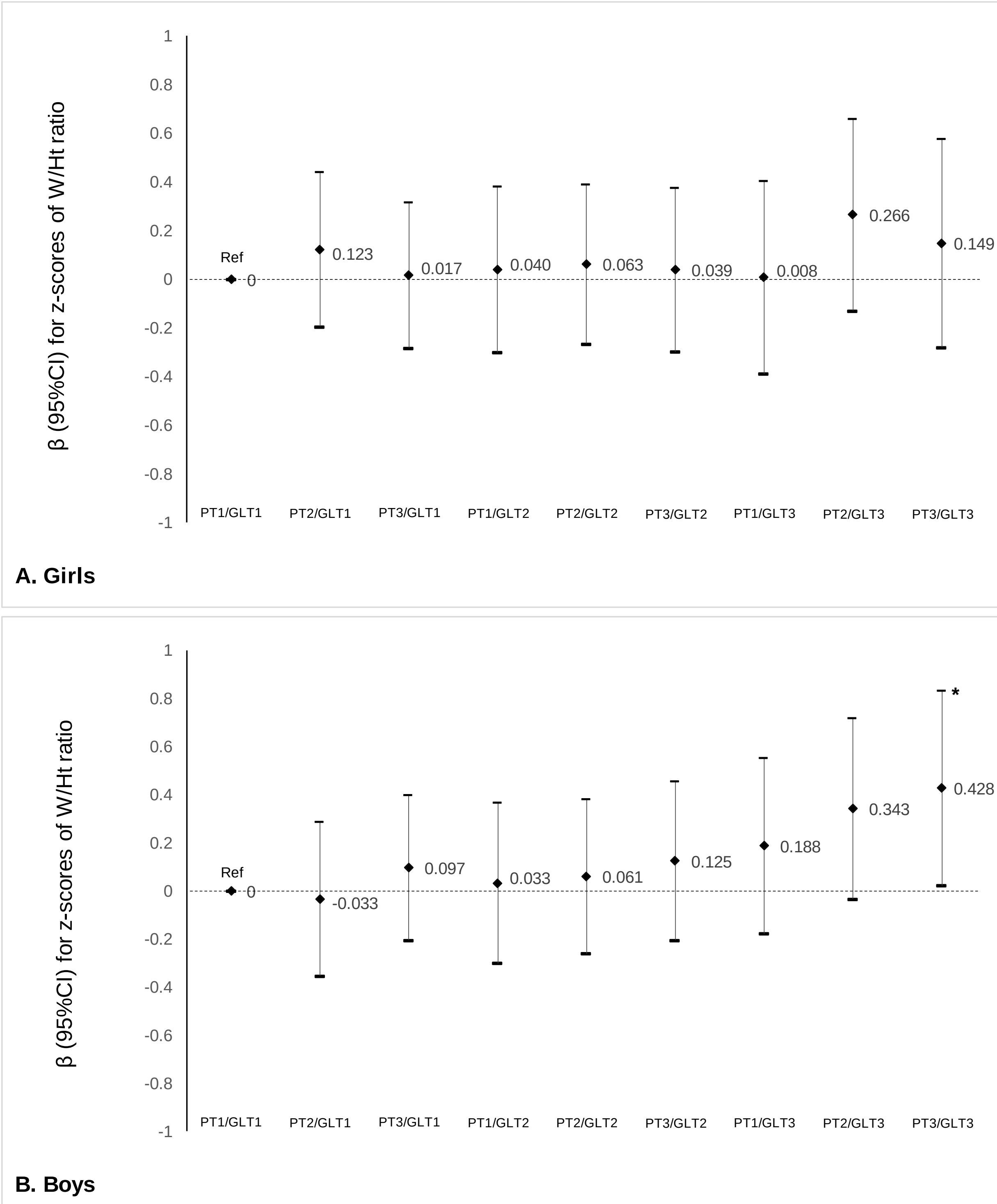

Figure 1. Interaction of protein intake and dietary GL on the association with W/Ht ratio

Interaction between dietary glycemic load and protein intake on the association with waist-to-height (W/Ht) ratio in girls (A) and boys (B). The interaction was assessed as a product term with multiplication of Protein intake Tertiles (PT) by dietary Glycemic Load Tertiles (GLT) and children in the first tertile of both variables (PT1/GLT1) were considered as the reference category (Ref). *$p<0.05$ *vs.* reference category. Models are adjusted for total energy intake plus maternal (education, BMI) and child's (birthweight-for-gestational age, non-exclusive breastfeeding, time practicing structured physical exercise) characteristics.

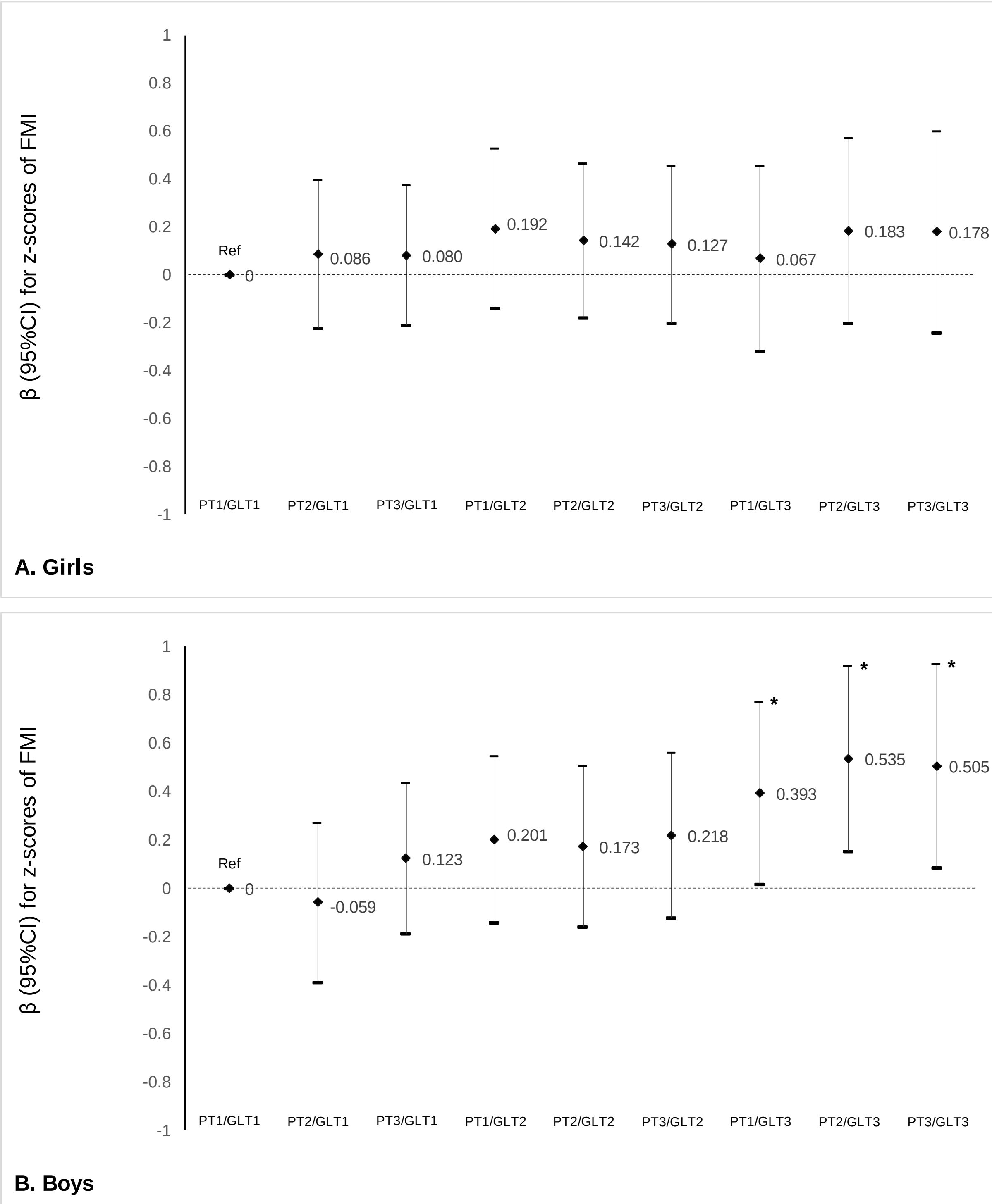

Figure 2. Interaction of protein intake and dietary GL on the association with FMI

Interaction between dietary glycemic load and protein intake on the association with fat mass index (FMI) in girls (A) and boys (B). The interaction was assessed as a product term with multiplication of Protein intake Tertiles (PT) by dietary Glycemic Load Tertiles (GLT) and children in the first tertile of both variables (PT1/GLT1) were considered as the reference category (Ref). *p<0.05 vs. reference category. Models are adjusted for total energy intake plus maternal (education, BMI) and child's (birthweight-for-gestational age, non-exclusive breastfeeding, time practicing structured physical exercise) characteristics.

GENERAL DISCUSSION AND CONCLUSIONS

This dissertation examined potential determinants of 4-year-old children's diet (e.g., family characteristics, plus maternal characteristics and behaviors) and, in turn, evaluated the association between the child's diet and later adiposity, using different measures of adiposity as well as distinct approaches to evaluate dietary habits (single foods, nutrients and dietary patterns).

In the current children's sample three distinct dietary patterns were identified and seem to be comparable to patterns identified among preschool children from other countries. Two studies conducted in the UK identified three dietary patterns practiced at the ages of 3 (253) and 4 (134) years: a 'traditional' pattern characterized by meat and vegetables; a 'junk' pattern characterized by high-fat processed foods and snacks, comparable to the present EDF pattern; and a 'health conscious' pattern rich in vegetarian style foods, rice, pasta, fruit, cheese and fish, comparable to the current Healthier pattern. In addition, in the study examining 3-year-old children from the UK (253), a 'snack' dietary pattern – partly comparable to the current Snacking pattern – was identified. Two other studies identified dietary patterns comparable to the present EDF pattern, namely a 'Processed and fast foods' pattern identified in multi-time points in French children (133) and a 'Snacky' pattern identified in Greek 4-year-olds (254).

In the present study, SEP of the mother at 12 years of age was considered as a possible upstream determinant of children's diet. Lower socioeconomic conditions at mothers' 12 years of age indirectly increased the possibility of her child following less healthy dietary patterns later on, while lower maternal education at delivery directly increased this possibility. Studies on determinants of preschool children's dietary patterns did not examine early patterns of maternal SEP, hampering comparisons of indirect effects and direct effects.

The indirect association between early maternal SEP and child's diet might be explained by several pathways such as later maternal education attainment, subsequent family characteristics and/or later maternal behaviors. Although we did not aim to evaluate such pathways, the conceptual framework enabled to observe that early SEP of the mother is associated with her child's diet many years later. Parents of low SEP have been reported as less likely to model healthy eating habits (229) and, keeping in mind that childhood SEP is correlated with later SEP (255), this could partly explain the overall effect observed for early maternal SEP.

Findings relative to maternal education are partly comparable to those found in other populations of preschool children (133, 134, 253, 254, 256), children being less likely to follow unhealthier dietary patterns when the mother was more educated. This may be explained by an increased nutrition knowledge in more educated mothers that would facilitate practice of healthier dietary habits.

Earlier socioeconomic conditions may also influence other subsequent characteristics within the family context. Distinct family characteristics were found to be associated with different dietary patterns. Children with only older siblings were more likely to follow a dietary pattern high in EDF, a finding comparable to results from other studies (132-134, 256). Older children have been reported to be more exposed to EDF (257) and older siblings may influence younger children through modelling of dietary habits, as evidence on siblings suggests (56). In contrast, children with parents as main day-time caregiver and having only younger siblings were more likely to follow the Snacking dietary pattern. Concerning parents as main day-time caregivers, keeping in mind that children model behaviors from their peers (258, 259), lower exposure to other children at the kindergarten may explain this finding. Relatively to siblings, it is possible that mothers attending to younger children are overburdened and have less time to prepare healthy meals.

Conceptually, parental diet is a proximal determinant of children's diet that is influenced by upstream factors, notably socioeconomic influences. In this study maternal diet was the key factor associated with children's diet, above and beyond socioeconomic and demographic characteristics. Comparison of these findings with those of other studies is difficult because, as far as could be ascertained, only one study (256) examined overall measures of diet for both mother and child at preschool age. Like in the present study, maternal diet was the key influence on the quality of 3-year-old children's diet, even after adjustment for maternal (education attainment, BMI and smoking) and child's (birth order, and time spent watching television) characteristics and behaviors.

Although not using overall measures of diet, studies considering specific food groups also show consistent positive associations between parental – particularly maternal – and child's consumption of fruit and vegetables(106-111, 125), fruit (112-116), milk (105, 117, 118, 260), sugar-sweetened beverages (107, 111, 119-122) and EDF or snacks (107, 112, 114, 115, 123).

It was expected that maternal diet was strongly associated with children's diet as they share the same household food environment. It is likely that parents' food selection is closely related with foods consumed by them. Hence, both availability and modelling may explain associations between parental and child's intake. These two influences are difficult to separate because they naturally co-occur (53). As such, maternal intake observed in the present study is likely representing both availability and modelling, especially considering that these children consume most meals at home (40).

As emphasized by Savage *et al* (95), parents play a critical role in determining which foods will become familiar to their children, act as gatekeepers and are the first social model of the child; as such, what is served in family meals will dictate what children eat and learn to like. Considering the strong association between parental and children's diet and that the most effective interventions aiming to prevent childhood obesity are multiple approaches including home and community components, it is surprising that most intervention studies have been conducted in the school setting (14-16).

Maternal child-feeding practices were also associated with children's diet; both the perceived monitoring and the restriction patterns were inversely associated with children's unhealthier dietary patterns. As far as could be ascertained, no studies examined maternal child-feeding patterns in other populations. Yet, relatively to children's diet, studies evaluating broader ranges of foods or dietary patterns have reported comparable findings; monitoring was negatively associated with unhealthier eating (82), and restriction was positively associated with a wholesome pattern (261).

In respect to specific maternal child-feeding practices and the evaluation of their association with particular food groups, some maternal child-feeding practices such as overt control were positively associated with the odds of children consuming specific food groups above the recommendations. Additionally, with the exception of pressure-to-eat, maternal practices were inversely associated with consumption of EDF. Comparison with other studies is hampered as no studies were found using a perspective of non-compliance with dietary guidelines as below or above intervals of adequacy. Nevertheless, some studies are partially comparable to the current research. Restrictive child-feeding practices have been associated with lower consumption of EDF and higher consumption of fruit and vegetables by young children in other studies (261, 262).

However, prospective studies (263, 264) show restriction to be associated with worse dietary habits later on. Considering that in a prospective study conducted in this cohort (265) higher BMI at 4 years was significantly positively associated with later maternal restriction and not the opposite, we cannot exclude the possibility of reverse causation and it is possible that children's dietary habits – such as consumption of EDF – are prompting maternal restrictive practices. Also, we cannot exclude that restriction at child's 4 years is positively associated with later unhealthier eating habits in these children.

Present findings suggest that overt and covert control may be slightly different with respect to children's consumption of fruit and vegetables. Overt control was associated with consumption of this food group above recommended levels, which might indicate that parental firmness is a distinct theoretical concept from covert control (87). Pressure-to-eat was also associated with consumption above recommendations for dairy. These findings may be related with the overt nature of these practices, both being therefore perceived by the child. Other studies support this hypothesis. Pressure to eat has been positively associated with overt control (87, 266) and, in a study previously conducted in the present sample (93), a similar correlation was observed. The association between pressure to eat and consumption of dairy may have additional explanations. It has been suggested that children play an active role in response to parenting practices (267, 268), and it is possible that, although parents pressure for both food groups, dairy may be more easily accepted by children than fruit and vegetables, depending on children's enjoyment of food. In addition, when compared to other practices, pressure to eat has a distinct nature and is related to parents' tendency to push the child to eat more food (66), especially when they perceive the child to be thin or a picky eater (53).

In summary, several family and maternal characteristics and behaviors were associated with children's dietary habits, supporting that the family context is a very important influence to children's dietary habits. In turn, children's diet tracked during early childhood and specific aspects of dietary intake at the age of 4 years were associated with later adiposity.

Although both consumption of EDF and BMI showed tracking between 2 and 4 years of age, no association between consumption of EDF at 2 years of age and BMI at 4 years was observed. Not finding an association between consumption of EDF and BMI from 2 to 4 years of age may be explained by several aspects such as the early age frame considered, the ability of young children to compensate for extra energy (269, 270), a relatively short time of follow-up or a low frequency of exposure.

In other prospective studies with young children an association between snacks (271, 272), fast food (156, 157), sugar-sweetened beverages (273-275), and EDF (157, 276, 277) with later adiposity was also not found. In addition, the association between snacking and adiposity in children is inconclusive (144, 158). Yet, it should be emphasized, that EDF were associated with lower dietary quality in another study in this sample frame (39).

A dietary pattern characterized by high consumption of EDF followed at 4 years of age was positively associated with adiposity 3 years later in girls. Comparison with other studies is difficult as those considering dietary patterns at preschool age and later adiposity (164) did not report sex-specific associations. In the analysis from 2 to 4 years of age, we formally examined the possible interaction between consumption of EDF and sex on the association with BMI, but found no evidence of an effect-modification by sex. Considering that from 4 to 7 years an overall measure of diet was used, the EDF pattern may better account for the cumulative effects of foods and nutrients (152) enabling to observe an association in girls.

Theoretically, distinct temporal associations of dietary patterns and adiposity between girls and boys could be possible. Girls appear to develop complex social interactions earlier, being more influenced by peers and earlier than boys (278-280). Hence, a conceptual model was used to examine possible sex-differences. However, at the ages considered, such differences were not found and the sex heterogeneity found in the association of the EDF pattern with adiposity could have other underlying explanations.

Sexual dimorphism in body composition could also explain different associations in girls and boys. Sex-differences in total and regional adiposity are apparent since early ages, namely at preschool age (281). Girls have higher relative body fat and lower visceral adipose tissue and these differences emerge before puberty (281, 282). If higher body fat in girls was the main explanation for the interaction found in this study, it would be expected that – when measures of total adiposity were considered (e.g., BMI or FMI) – significant associations were observed in girls, and it could be expected that – when a measure of central adiposity was considered – significant associations were also observed in boys in whom visceral adipose tissue has been reported to be higher. Yet, the EDF dietary pattern was positively associated with W/Ht ratio only in girls. It is possible that, in this population, the early age considered is too soon to observe differences in W/Ht ratio. As such, considering that girls have higher abdominal subcutaneous adiposity (283), the association of the EDF pattern with W/Ht ratio may be reflecting an increase in abdominal subcutaneous adipose tissue.

Women are considered at higher risk of obesity (284) as they have higher propensity to store body fat than men. It has been suggested (285) that women's metabolic predisposition to store more fat may be an evolved adaptive advantage for reproductive success and that the association between adiposity and reproductive success may start at birth. The energy costs of reproduction (e.g., gestation and lactation) could be a potent adaptive force driving adipose metabolism in women. Also, the ability to deliver "fatter" female infants and children may – in past environments – have had reproductive benefits in terms of earlier age at menarche and more resilient ovarian function during adulthood, increasing total reproductive life span (285). Yet, in current obesity-promoting environments, this evolved adaptive pattern may contribute to higher adiposity in females (285). Considering that the association of the EDF dietary pattern with body fatness was consistent in several measures of adiposity among girls, it is possible that the interaction observed – if true – is reflecting a higher propensity of girls to gain fat. Despite these considerations, males and females are both susceptible to obesity and it is possible that the EDF pattern is associated with adiposity at later ages in boys.

This research also found that sex modified the effect of protein intake/dietary GL on adiposity. A prospective study (207) examined the association of protein intake at preschool age with later adiposity in 203 children enrolled in the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study. Higher total protein intake at 5-6 years was positively associated with BMI at 7 years of age, but formal examination of an interaction between protein and sex did not indicate a significant effect modification. Prospective studies focusing on protein intake in the first 1-2 years of age have reported sexual dimorphism in its association and adiposity. A large prospective cohort study conducted in the Netherlands (224, 225) reported a borderline significant interaction between sex and protein intake on FM% and a significant interaction on FMI, protein intake at the age of 13 months showing a stronger association with body fatness at 6 years in girls. In contrast, in a sample of 90 children from Iceland (192), protein intake at 9-12 months was positively associated to BMI at 6 years only in boys. In addition, in the ambit of the DONALD cohort (215), a prospective study including 308 children did not find evidence of an association of dietary GL at 2 years of age with adiposity at 7 years of age. Again, formal examination of a possible interaction between sex and dietary GL did not suggest significant effect modification. However, considering the small sample sizes in these studies, low statistical power to detect an interaction may be a limitation.

Findings of the present research indicate a significant sex-effect modification on the association of protein intake and dietary GL with adiposity. A possible explanation for these results are differences in the metabolism between males and females. As previously highlighted, women appear to be metabolically inclined to store more fat (284). Also, they seem to use more fat than men as an energy substrate during periods of sustained exertion, showing higher rates of fat oxidation, while men are more likely to up regulate glucose and amino acid metabolism during sustained exercise bouts (285). These differences appear to be associated with estrogen that, when administered to men, increases fat oxidation and decreases amino acids and glucose metabolism (285). Hence, it has been proposed (285) that women are physiologically more prone to store fat as well as to use it as fuel under sustained increased demand, while men rely more on glucose and protein metabolism.

In order to assess if the effect of energy intake was independent of its source in girls and boys, sex-specific models including only energy intake were compared with models further including energy from macronutrients. Likelihood ratio tests indicated that, in girls, the effect of energy intake was independent of all macronutrients. In contrast, in boys, although the effect of energy intake was independent of energy supplied by fat, it was dependent of energy supplied either by protein or carbohydrates. This suggests that total energy intake could be more relevant in girls, while – in boys – energy intake is dependent of protein and carbohydrate intakes.

In fully adjusted models energy intake was positively associated with FMI in girls, but not in boys in whom positive associations with adiposity were only found for macronutrients. These differences may explain why the EDF pattern – significantly higher in total energy than the other patterns – was associated with all measures of adiposity only in girls. In addition, the association of both protein intake and dietary GL with specific measures of adiposity only in boys may be reflecting higher reliance on protein and carbohydrates reported in males (285).

The positive association between protein intake and serum insulin only found in boys gives strength to this possibility. Insulin and IGF-I have been proposed (286) as intermediate steps in the pathway between protein intake and adiposity. In an study conducted in 83 healthy infants (287), intervention with whole milk – when compared to infant formula – resulted in higher concentration of IGF-I in boys while there was not effect in girls, suggesting differing endocrine responses to protein intake.

In contrast, in a large randomized controlled trial (204), the IGF-I axis of female infants showed a stronger response to high protein intake, but no effect on BMI was observed. Insulin is more highly correlated with total fat mass in males that are also more sensitive to central insulin, while females are more sensitive to central leptin. These differences emerge early in life, as reflected in higher circulating leptin in pregnancies where the fetus is a female, higher leptin levels in females at birth, and intrinsically higher insulin resistance in girls (288, 289). These differences do not reflect only sexual dimorphism in total adipose tissue, and have been suggested (285) to reflect sex-differences in the importance of fat *versus* carbohydrate and protein in metabolism.

A major finding of the present thesis is that dietary GL seems to interact with protein intake, enhancing increased total and central adiposity (i.e., FMI and W/Ht ratio) in boys. Both amino acids and glucose, along with growth factors (insulin and IGF-I), play important roles in signaling pathways that regulate cell growth and proliferation [i.e., the mammalian target of rapamycin (mTOR)] (218). High protein supply may result in higher levels of branched-chain amino acids increasing secretion of IGF-I and insulin which can promote adipogenic activity (201). Considering that both glucose and amino acids enhance insulin secretion and that amino acids have an additional effect on insulin secretion when ingested with glucose (216), as well as that boys are more sensitive to central insulin, the interaction between dietary GL and protein intake on adiposity may have biological plausibility.

Other underlying mechanisms might explain present findings. In some cohorts (290, 291), distinct BMI trajectories by child’s sex have been reported, girls showing older age and lower BMI at infancy peak and earlier adiposity rebound. Also, among infants in whom catch-up growth was observed, boys showed more rapid catch-up than girls, which could be reflecting the anabolic effects of early infancy sex hormone (292). In addition, boys appear to be more sensitive to growth hormone (293) and have been reported to show higher sensitivity to nutritional intervention during infancy in particular circumstances (i.e., preterm infants) (294). Hence, it is possible that distinct growth trajectories, distinct endocrine responses, or sex-dependent sensitivities might arise at distinct sensitive periods.

Some methodological approaches also deserve to be discussed. Using a perspective of dietary inadequacy as below or above a range of adequacy hampered comparison with other studies. However, consideration of food-based dietary guidelines may enable an interpretation more closely linked to the diet-health relationship as well as closer to nutrition recommendations. In addition, this approach reflects current trends in recommendations of some macronutrients which are moving towards ranges (295), and avoids choice of single cut-points that could increase the possibility of misclassification. Furthermore, the relationship between dietary exposures and a given health-related outcome usually is not linear (296), assuming a J or U shape.

Published evidence suggests that high consumption of vegetables and whole grain foods may, in particular circumstances, be detrimental to children (297-299). A safe range of fiber has been previously suggested (300), but current recommendations for ages above 1 year are extrapolated from adults (301). Hence, it is possible that exaggerated consumption of fruit and vegetables in early childhood may be unnecessary and/or imprudent, particularly if high food volumes (e.g., bulky foods) are not balanced with other foods resulting in excessively low energy density that may affect growth (35).

Identification of dietary patterns by latent class analysis was an advantage, as this methodology is especially well suited for categorical variables asymmetrically distributed, common in FFQ's data. It avoids subjective choice of cut-points on underlying dimensions (latent classes or dietary patterns) as the classification is provided by the model (i.e., the model finds the smallest number of latent classes by adding classes stepwise until the it fits the data well). Moreover, it can be compared to factor analysis enabling comparison with studies that use the more common methodology of principal component analysis (162).

The conceptual framework used to examine determinants of children's dietary patterns is also an advantage enabling to better understand upstream and downstream factors, overall and direct effects, as well as factors most relevant to the child's diet. Additionally, the use of composite measures to assess early maternal SEP, maternal child-feeding practices, maternal diet and children's diet was advantageous as overall measures can better capture their inherent complexity. Another advantage of the present research is that it addressed not only determinants of adiposity, but also determinants of preschool children's diet for which research is still very scarce (130), notably maternal overall diet plus overt and covert control.

Evaluation of distinct adiposity indicators was essential to understand sex-differences in the associations between diet and adiposity, since BMI is a proxy of adiposity that does not distinguish between fat and lean mass and relative fatness (i.e., FM%) cannot be assessed independently of free-fat mass, being important to use an index adjusted for height (302) – FMI – that has been reported to perform better in monitoring changes in adiposity among Portuguese obese children aged 3-9 years (303).

Furthermore, assessment of W/Ht ratio as surrogate of central adiposity was also important for interpreting the sex-differences observed. Likewise, assessment of serum insulin was crucial to understand sex-differences. Moreover, sample size and inclusion of children enrolled in a large population-based birth cohort regularly followed were also advantages, allowing assessment of several potential confounding and interaction factors.

Inclusion of subsamples in analyses and exclusion of subjects due to missing data could have introduced some bias. We conducted sensitivity analyses fitting models with the highest possible number of subjects for each variable and compared them with models considering complete-subject analysis and results were very similar. In addition, although comparison of each subsample with the remaining cohort showed statistically significant differences in maternal education (slightly higher in included subjects), given that Cohen's effect size values were not high, differences relatively to the remaining cohort are likely due to large sample size, rather than to large differences between participants (304).

Restricting the analysis of dietary patterns at 7 years to the consumption categories observed at 4 years deserves to be discussed. This approach was defined *a priori* in order to assess if children switched between dietary patterns between the two moments, rather than to identify new patterns at 7 years. Since median probabilities of class membership support an acceptable classification of individuals into each latent class at both ages and that similarity of dietary patterns at 4 and 7 years of age has been shown by other authors (134), this approach was deemed appropriate.

The strong tracking of adiposity and dietary patterns across the two ages could have hampered assessment of temporal associations between diet and adiposity. Considering that the association between the EDF pattern at 4 years with later adiposity observed in girls was statistically significant in crude models as well as in those adjusted for adiposity at 4 years, it is unlikely that tracking of adiposity in time overcame the association between diet at 4 years and later adiposity. Also, given that cross-sectional Spearman's correlations between diet and adiposity at 4 years in girls were very low and non-significant, it is unlikely that adiposity measures at 4 years were intermediate steps in the pathway between diet and adiposity. As such, adjustment for adiposity at 4 years was considered appropriate. In contrast, in the study examining protein intake and dietary GL, in cross-sectional analysis at 4 years of age, protein intake was significantly positively associated with adiposity in boys (p=0.003). As such, adiposity at 4 years could be an intermediate step and adjustment for birthweight for gestational age was considered more adequate.

The use of a FFQ to assess children's diet may also be considered a limitation as dietary intake was estimated by frequency rather than by quantities. Inclusion of questions on portion size in FFQ is controversial as it was shown to provide little additional information and may reduce validity (305). In the subsample of children with complete 3-days food diaries at 4 years, the validity and reliability of the FFQ were assessed by Pearson's correlation coefficients and intraclass correlation coefficients for key food groups as measured by both methods. Significant positive moderate Pearson's correlations were found for vegetable soup (r=0.54, p<0.001), fruit (r=0.42, p<0.001), milk (r=0.46, p<0.001), and yogurts (r=0.48, p<0.001), and not so strong for cheese (r=0.35, p<0.001), fish (r=0.27, p<0.001), vegetables eaten on a plate (r=0.26, p<0.001), eggs (r=0.21, p<0.001), and non-carbonated sugar-sweetened beverages (iced tea r=0.29, p<0.001; juices r=0.22, p<0.001). Intraclass correlation coefficients varied from 0.54 (95%CI 0.51-0.56) for vegetable soup to 0.17 (95%CI 0.11-0.32) for juices, showing fair-to-moderate agreement for food items most frequently consumed. Taking into account that at 4 years of age a 3-days food diary may not be the ideal method to represent consumption of foods eaten less frequently, the week-to-moderate correlations and fair-to-moderate agreement found for foods most frequently consumed support an acceptable validity and reliability of the FFQ. Moreover, comparison of the dietary patterns with 3-days food diaries showed that the EDF pattern was significantly higher in energy when compared to the other two patterns.

In order to assess social desirability bias, we stratified the analysis by maternal weight status. As overweight mothers reported higher consumption of crisps and soft drinks and lower consumption of fruit and vegetables, this bias seems unlikely. Still, mothers may have incorrectly reported foods eaten out-of-home. Considering that at preschool age these children took most meals at home (40), we do not expect that out-of-home consumption would result in different findings. Additionally, relatively to food diaries, this limitation is expected to be reduced as parents were advised to resort to the help of children's day-time caregivers.

Nevertheless, associations between maternal and child's diet may have been overestimated, since assessment was performed at the same time by the same individual. Considering that information was collected by trained interviewers, overestimation may have been diminished. In addition, keeping in mind cognitive limitations in young children for self-reporting diet (306), this approach was considered adequate.

Not including fathers must also be discussed. Fathers from different socioeconomic conditions appear to be increasingly involved in feeding their child (307) and two-parent families have shown to be associated with some dietary behaviors (49, 308) in children. Maternal marital status was considered in order to clarify possible differences between two- and one-parent families, but no differences were found. As such, taking into account that mothers spend more time feeding young children (54, 309), we do not expect that inclusion of the father would change our conclusions.

Main Conclusions

This study supports a transgenerational influence of less favorable socioeconomic conditions on children's diet. Early maternal lower SEP indirectly increases the odds of exposing children to unhealthier dietary patterns, while lower maternal education at child's delivery directly increases these odds.

Distinct family characteristics are associated with different dietary patterns; children with only older siblings are more likely to follow a dietary pattern high in EDF, while those having only younger siblings and being cared by parents during day-time are more likely to follow a dietary pattern lower in foods eaten at main meals and higher in those usually consumed as snacks.

Specific maternal behaviors are associated with children's dietary patterns, above and beyond demographic and socioeconomic factors and the conceptual framework enabled to observe that maternal diet is the most important factor to children's diet. Children's whose mother have lower dietary quality are significantly more likely to follow unhealthier dietary patterns high in EDF. Some maternal child-feeding practices may be beneficial to children's diet since they are associated with lower prevalence of inadequate consumption of EDF and fruit and vegetables.

Some of these findings could be translated into public health messages. The family context is an important factor for the development of dietary habits of young children, being particularly important to emphasize that early dietary behaviors already show persistence in time. Findings also suggest that guidance of mothers regarding their diet and their role as models could be very relevant as it is a modifiable factor strongly associated with children's diet.

Moreover, interventions may need to give particular attention to lower socioeconomic groups, and – keeping in mind that socioeconomic conditions tend to persist across generations – public health policies should also target upstream factors (such as education level) in this generation of children in order to improve the probability of better dietary habits in their offspring.

Dietary habits and adiposity show tracking during early childhood and inadequate dietary habits at preschool age are associated with subsequent adiposity. Furthermore, child's sex seems to interact with dietary factors on the association between diet and adiposity.

A dietary pattern high in EDF at 4 years is consistently positively associated with later adiposity only in girls, while higher protein intake at preschool age is positively associated with all adiposity measures and serum insulin at 7 years of age in boys, being associated only with BMI in girls.

Furthermore, dietary GL is positively associated with adiposity only in boys, in whom it seems to interact with protein intake enhancing increased adiposity. This finding may be reflecting the role that both amino acids and glucose, along with growth factors such as insulin, play in signaling pathways that regulate cell growth and may be related to higher sensitivity to central insulin in boys.

Consumption of EDF should be avoided since very early ages, as it tends to persist in time. Although an association with higher adiposity was only observed in girls at 7 years of age, this association may become apparent in boys at later ages, as is suggested by the association of higher dietary GL and BMI in boys. Indeed, in a public health perspective, consumption of EDF should be avoided in both girls and boys as they have little nutritional interest, have been associated with lower dietary quality, are consumed in large portions that contribute to higher energy intake and may promote obesity in the long-term. Finally, it should be emphasized that EDF tend to be aggregated with other foods such as meat, eggs, and processed meat resulting in a dietary pattern high in protein intake and dietary GL that may contribute to higher insulin resistance and adiposity.

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