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Obesity-related dietary patterns in 7 years-old children from Generation XXI and their effects in cardiometabolic health later in life

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Esta dissertação foi realizada no âmbito da coorte Geração XXI, desenvolvida no Departamento de Ciências da Saúde Pública e Forenses e Educação Médica da Faculdade de Medicina da Universidade do Porto e no Instituto de Saúde Pública da Universidade do Porto.

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

I. **Identification of dietary patterns at 7 years-old that explain body mass index at 10 years-old: comparison of three methodological approaches in the Generation XXI birth cohort**

II. **Data-driven dietary patterns at 7 years-old and their association with cardiometabolic health at 10 years-old**

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LIST OF ABBREVIATIONS

AUC – Area Under the Curve

BMI – Body mass index

CI – Confidence interval

DASH – Dietary Approach to Stop Hypertension

DBP – Diastolic blood pressure

DQI – Diet Quality Index

DQI-CH – Diet Quality Index for Young Children

EDF – Energy-dense food

FFQ – Food frequency questionnaire

HDI – Healthy Diet Indicator

HDL-c – Cholesterol of high-density protein

HEI – Healthy Eating Index

HOMA-IR – Homeostatic model assessment - insulin resistance

LCA – Latent Class Analysis

LDL – Low-density protein

LDL-c – Cholesterol of low-density protein

MDS – Mediterranean Diet Score

PCA – Principal Component Analysis

PLS – Partial Least Squares

ROC – Receiver Operating Characteristics curves

RRR – Reduced Rank Regression

SBP – Systolic blood pressure

SD – standard deviation

SPSS – Statistical Package for Social Sciences

TG – Triglycerides

RESUMO

Introdução

Uma alimentação pouco saudável tem um importante papel no surgimento da doença, nomeadamente da obesidade e das suas co-morbilidades associadas. No entanto, do ponto de vista metodológico, é complexo analisar o consumo alimentar. Atualmente são reconhecidas às abordagens tradicionais várias limitações metodológicas. Por sua vez, a definição de padrões alimentares tem ganho interesse em epidemiologia nutricional, especialmente aqueles que tentam explicar um resultado de saúde específico. Desta forma, torna-se relevante entender quais os padrões alimentares que podem explicar a obesidade e se estes podem desencadear o aparecimento de fatores de risco cardiometabólico mesmo em idades pediátricas.

Objetivos

Este trabalho teve dois objetivos principais:

i) identificar padrões alimentares aos 7 anos de idade relacionados com a obesidade aos 10 anos, através de três diferentes abordagens metodológicas (artigo I)

ii) avaliar se os diferentes padrões alimentares identificados aos 7 anos de idade podem contribuir para o aumento dos valores dos parâmetros cardiometabólicos aos 10 anos de idade (artigo II).

Métodos

Foram utilizados dados da coorte de nascimento de base populacional Geração XXI. Na avaliação inicial (2005/2006), 8647 recém-nascidos e 8495 mães foram recrutados em todas as maternidades públicas da região metropolitana do Porto (Norte de Portugal).

Avaliações presenciais aos 4, 7 e 10 anos de idade foram realizadas por entrevistadores treinados, através de questionários estruturados. Neste estudo, foram utilizados dados prospetivos das avaliações realizadas no recrutamento, aos 7 e aos 10 anos de idade. Informação relativa ao sexo da criança, peso ao nascimento, idade gestacional, escolaridade e idade materna foi recolhida aquando do recrutamento ou obtida através de processos clínicos. Aos 7 anos de idade foram também recolhidos dados comportamentais, nomeadamente sobre o consumo alimentar através de um questionário validado de frequência alimentar referente aos 6 meses anteriores, aplicado por entrevistadores treinados ao principal cuidador da criança. Parâmetros antropométricos, nomeadamente peso e estatura, foram medidos por observadores treinados aos 7 e 10 anos de idade, de acordo com procedimentos padronizados e o z-score do índice de massa corporal (IMC) foi calculado de acordo com os critérios da Organização Mundial de Saúde. A pressão arterial sistólica e diastólica (PAS e

PAD) e amostras biológicas foram obtidas e diferentes parâmetros [(glicose, triglicerídeos, colesterol da lipoproteína de alta densidade (HDL-c), da lipoproteína de baixa densidade (LDL-c) e HOMA- resistência à insulina] foram analisados aos 10 anos de idades e padronizados com base na idade e sexo (os z-scores de pressão arterial foram também ajustados para a estatura).

Para identificar padrões alimentares aos 7 anos de idade, três métodos diferentes foram utilizados: regressão linear, análise de componentes principais (PCA) e *partial least squares* (PLS). PCA é um método que agrega o consumo de alimentos sem explicar diretamente o resultado, explicando a máxima variância das variáveis preditoras (grupos de alimentos), enquanto o PLS deriva padrões alimentares baseados na máxima variância das variáveis preditoras (grupos de alimentos aos 7 anos de idade) e das variáveis resposta (z-score do IMC aos 10 anos de idade). Os itens alimentares relacionados com cada padrão alimentar derivado por PCA ou PLS foram aqueles com cargas fatoriais padronizadas |>0,20| e por regressão linear foram aqueles com valores de beta padronizados de maior magnitude. Padrões alimentares que foram identificados num estudo anterior por análise de classes latente aos 7 anos de idade também foram estudados.

No artigo (i), Curvas *Receiver Operating Characteristics* (ROC) foram construídas para comparar a capacidade de cada método para discriminar entre obesidade e não-obesidade aos 10 anos de idade. Realizaram-se modelos de regressão linear para estimar a associação entre caraterísticas sociodemográficas e de início de vida com o padrão alimentar mais explicativo das variáveis preditoras e de resposta (n=4698). Coeficientes de regressão e intervalos de confiança a 95% (IC 95%) foram calculados (covariáveis: sexo da criança, idade gestacional, peso ao nascimento, vivência com irmãos, prática de exercício físico aos 7 anos de idade, escolaridade e idade materna).

Para dar resposta ao objetivo ii) coeficientes de regressão linear e intervalos de confiança a 99% (IC 99%) foram calculados para avaliar a associação entre os padrões alimentares identificados aos 7 anos de idade e os parâmetros cardiometabólicos aos 10 anos de idade (covariáveis: peso ao nascimento, idade gestacional, idade e escolaridade materna e prática regular de exercício físico pela criança aos 7 anos de idade) (n=3350).

A análise estatística foi realizada usando o software R® (*The R Project for Statistical Computing*), versão 3.4.0 para Windows e o IBM SPSS® (*Statistical Package for Social Sciences*), versão 24.0™.

Resultados

Artigo I

O padrão alimentar identificado por regressão linear (método padrão) explicou 4,30% da variância do z-score do IMC e apenas 0,11% dos grupos alimentares. O método PLS identificou dois padrões alimentares, sendo o primeiro padrão (PLS-1) aquele que explicou uma maior variância dos grupos alimentares (4,14%) aos 7 anos de idade e do z-score do IMC (3,74%) aos 10 anos e foi caraterizado por ser um padrão rico em alimentos de elevada densidade energética e produtos de carne processados e pobre em sopa de legumes. A variância cumulativa explicada pelos grupos de alimentos destes dois padrões alimentares foi de 10,06% e do z-score de IMC foi de 4,23%. O método PCA também derivou dois padrões alimentares; a variância cumulativa explicada por este método relativamente aos grupos de alimentos foi de 13% e apenas 0,23% do z-score de IMC.

Considerando a capacidade de discriminar a obesidade três anos mais tarde (aos 10 anos de idade), o método PLS apresentou um maior poder discriminatório [área sob a curva (AUC) = 0,63, IC 95%: 0,61; 0,65) do que o PCA.

Seguir um padrão alimentar aos 7 anos de idade relacionado com obesidade aos 10 anos (PLS-1) é mais provável em crianças com maior peso ao nascimento ($\hat{\beta}$=0,116; IC 95%: 0,033; 0,198), não praticantes de exercício físico regular aos 7 anos ($\hat{\beta}$=0,155; IC 95%: (0,064; 0,246) e com mães mais novas ($\hat{\beta}$=-0,022; IC 95%: -0,028; -0,015) e menos escolarizadas ($\hat{\beta}$=-0,050; IC 95%: -0,057; -0,042).

Artigo II

Um padrão alimentar relacionado com obesidade seguido aos 7 anos de idade (PLS-1) associou-se a um aumento dos níveis de quase todos os parâmetros de risco cardiometabólico aos 10 anos de idade: PAS ($\hat{\beta}$=0,052, IC 99%: 0,022; 0,082), PAD ($\hat{\beta}$=0,043, IC 99%: 0,022; 0,065), TG ($\hat{\beta}$=0,065, IC 99%: 0,026; 0,104), HDL-c ($\hat{\beta}$=-0,059, IC 99%: -0,099; -0,019), LDL-c ($\hat{\beta}$= 0,040, IC 99%: 0,001; 0,080) e HOMA-IR ($\hat{\beta}$= 0,110, IC 99%: 0,071; 0,149). O IMC aos 10 anos é um mediador das associações estudadas, uma vez que, no geral, a magnitude das associações foi fortemente atenuada após ajuste.

Os padrões alimentares identificados pelos outros métodos (PCA e análise de classes latentes) não mostraram uma associação consistente com o perfil cardiometabólico das crianças. Contudo, PCA-1 (rico em alimentos densamente energéticos e pobre em fruta, sopa e peixe) associou-se igualmente a um aumento da pressão arterial e resistência à insulina aos 10 anos.

Conclusão

Dos três métodos utilizados para identificar padrões alimentares, o PLS parece ser o melhor método para explicar a obesidade em crianças em idade escolar, uma vez que explicou uma grande variância dos grupos de alimentos aos 7 anos de idade e do z-score do IMC aos 10 anos e apresentou ainda um maior poder discriminatório para classificar a obesidade aos 10 anos de idade.

Um padrão alimentar rico em alimentos de elevada densidade energética, refrigerantes, carne processada e com menor consumo de sopa aos 7 anos de idade explicou uma maior variância do z-score do IMC aos 10 anos e foi mais frequentemente adotado por crianças com maior peso ao nascimento, menos ativas fisicamente aos 7 anos e com mais mães mais novas e menos escolarizadas. Este padrão associou-se prospectivamente com um aumento dos parâmetros cardiometabólicos aos 10 anos de idade, nomeadamente pressão arterial sistólica e diastólica, triglicerídeos, colesterol LDL-c e resistência à insulina e diminuição do colesterol HDL. O z-score do IMC aos 10 anos de idade explicou parte destes efeitos.

Estes resultados enfatizam a importância da aquisição de padrões alimentares saudáveis desde idades precoces, de forma a prevenir o desenvolvimento de fatores de risco cardiometabólicos no futuro. Também traz à discussão a utilidade da definição de padrões alimentares explicativos de um resultado em saúde específico, de forma a ultrapassar a frequente dificuldade em relacionar o consumo alimentar com a doença.

Palavras-chave: Crianças; Estudos de coorte; Hábitos alimentares; Padrões alimentares; Obesidade; *Partial Least Squares*; Saúde cardiometabólica

ABSTRACT

Introduction

An unhealthy diet has an important role in the onset of disease, namely obesity and their associated co-morbidities. However, methodologically, diet is not easy to analyse. Currently, traditional approaches have several methodological limitations recognized and in turn, dietary patterns have gained interest in nutritional epidemiology, especially those that attempt to explain a specific health outcome. In this way, it becomes relevant to understand which dietary patterns can be explanatory of obesity and whether these can trigger the development of cardiometabolic risk factors even at paediatric ages.

Aims

This work had two main objectives:

i) to identify dietary patterns at 7 years-old that are related with obesity at 10 years, by three different methodological approaches (paper I);

ii) to evaluate whether the different dietary patterns derived at 7 years of age may contribute to an increase of levels of cardiometabolic parameters at 10 years of age (paper II).

Methods

Data are from the population-based birth cohort Generation XXI. At baseline (2005/2006), 8647 newborns and 8495 mothers were recruited from all public maternity hospitals from Porto metropolitan area (Northern Portugal).

Face-to-face follow-up assessments at 4, 7 and 10 years of age were performed, and information was collected by trained interviewers through structured questionnaires. In this study, prospective data from baseline, 7 and 10 years of age were used. Information regarding child's sex, birth weight, gestational age and mother's education and age was questioned at baseline or obtained through medical records. At 7 years-old, data on behaviours were also collected, namely a validated food frequency questionnaire covering the previous 6 months, applied by trained interviewers to the main caregiver of the child. Anthropometrics at 7 and 10 years-old, namely weight and height were measured by trained personnel according to standardized procedures and the body mass index (BMI) z-score was calculated according to the World Health Organization criteria. Systolic and diastolic blood pressure (SBP and DPB) and blood samples were obtained and different parameters [(glucose, triglycerides, high-density lipoprotein (HDL-c) and low-density lipoprotein (LDL-c) cholesterol, and HOMA-insulin resistance] were obtained at 10 years-old, and then standardized based on age and sex (for blood pressure z-scores were also adjusted for height).

To derive dietary patterns at 7 years of age three different methods were used: linear regression, principal component analysis (PCA) and partial least squares (PLS). PCA is a method that aggregates food consumption without directly explaining the outcome and explains the maximum variance of the predictor variables (food groups), while the PLS method derives dietary patterns based on the maximum variance of the predictor variables (food groups at 7 years-old) and response variables (BMI z-score at 10 years). The food items related to each dietary pattern derived by PCA or PLS were those with standardized factor loadings |>0.20| and by linear regression were those with higher standardized beta values. Dietary patterns derived in a previous study by latent class analysis (LCA) at 7 years-old were also studied.

In paper (i), Receiver Operating Characteristics (ROC) curves were performed to compare the ability of each method to define dietary patterns to discriminate between obesity and non-obesity at 10 years of age. Linear regressions were performed to estimate the associations between sociodemographic and early life characteristics with the dietary pattern with a greater explanation of predictor and response variables (n=4698). Regression coefficients and 95% confidence intervals (95% CI) were calculated (covariates: child's sex, gestational age, birth weight, living with siblings, physical activity practice at 7 years of age, maternal education and age).

To answer the objective ii), linear regression models were calculated to assess the association between dietary patterns derived at 7 years of age and cardiometabolic parameters at 10 years-old and 99% confidence intervals (99% CI) were calculated (covariates: child's birth weight, gestational age, maternal age, maternal education and child's regular practice of physical activity at 7 years-old) (n=3350).

Statistical analysis was performed using R® software (The R Project for Statistical Computing), version 3.4.0 for Windows and the IBM SPSS® (Statistical Package for Social Sciences), version 24.0 ™.

Results

Paper I

The dietary pattern identified by linear regression (standard method) explained 4.30% of the variance of the BMI z-score and only 0.11% of the food groups. By PLS, two dietary patterns were derived; the first (PLS-1) was the one that explained a greater variance of both the food groups (4.14%) at 7 years of age and the z-score BMI (3.74%) at 10 years of age and was rich in energy-dense foods, soft drinks and processed meat and low in vegetable soup. The cumulative variance explained by the food groups of these two dietary patterns was 10.06% and the BMI z-score was 4.23%. The PCA method also derived two dietary patterns;

the cumulative variance explained by these two dietary patterns in relation to food groups was 13% and only 0.23% of the BMI z-score.

Considering the ability to discriminate obesity three years later (at 10 years-old), the PLS method presented a higher discriminatory power [area under the curve (AUC)=0.63, 95% CI: 0.61; 0.65) than the PCA method.

Following at 7 years-old an obesity-driven dietary pattern (PLS-1) was more likely among children who were born heavier (β=0.116; 95% CI: 0.033; 0.198) and who did not practice physical exercise at 7 years-old (β=0.155; 95% IC: (0.064; 0.246), and from younger (β=-0.022; 95%CI: -0.028; -0.015) and less educated (β=-0.050; 95 IC%: -0.057; -0.042) mothers .

Paper II

An obesity-driven dietary pattern followed at 7 years of age (PLS-1) was associated with an increase in levels of most cardiometabolic risk parameters at 10 years-old: SBP (β=0.052, 99%CI: 0.022; 0.082), DBP (β=0.043, 99%CI: 0.022; 0.065), TG (β=0.065, 99%CI: 0.026; 0.104), HDL-c (β=-0.059, 99%CI: -0.099; -0.019), LDL-c (β=0.040, 99%CI: 0.001; 0.080) and HOMA-IR (β=0.110, 99%CI: 0.071; 0.149). BMI at 10 years is a mediator of the studied associations once the overall magnitude of the effect was strongly attenuated after adjustment for.

Dietary patterns derived by the other methods (PCA and LCA) did not showed so consistent associations with the children's cardiometabolic profile. However, PCA-1 (rich in energy-dense foods and low in fruit, soup and fish) was also associated with increased blood pressure and insulin resistance at 10 years of age.

Conclusion

Of the three used methods to derive dietary patterns, PLS seems to be the best method to explain obesity in school-aged children, since it explained a greater variance of the food groups at 7 years-old and the BMI z-score at 10 years-old and had higher discriminatory power for classifying obesity at 10 years of age.

A dietary pattern rich in energy-dense foods, soft drinks and processed meat and low in vegetable soup at 7 years of age had higher explained variance of BMI z-score at 10 years-old and was more frequently followed by children with higher birth weight, less physically active at 7 years and from younger and less educated mothers. This dietary pattern was prospectively associated with an increase of cardiometabolic parameters at 10 years of age, such as SBP, DBP, triglycerides, LDL-c and insulin resistance and a reduction in HDL-c. The BMI z-score at 10 years of age explained part of these effects.

This work emphasizes the importance of acquiring healthy dietary patterns from an early age in order to prevent the onset of obesity and the development of cardiometabolic risk factors in the future. It also adds important insights in discussing the usefulness of defining dietary patterns that explain a specific outcome in overcoming the usual difficulty of relating diet with the disease.

Key-words: Children; Cohort studies; Food habits; Dietary patterns; Obesity; Partial Least Squares; Cardiometabolic health

INTRODUCTION

1. DIETARY PATTERNS ANALYSIS

Diet plays a major role in the well-being and health of populations. In general, a diet that includes foods rich in fats, particularly trans-fatty acids, added sugars, salt and sugary drinks is not considered as a healthy diet (1, 2). In contrast, fruits and vegetables are rich in bioactive compounds, such as vitamins, fiber and phenolic compounds and there are mostly considered protective foods against various diseases (3).

Diet quality tend to decline from early childhood through adolescence (4, 5), since this transition covers some social changes that translate into food deviations, namely a greater consumption of snacks, fast-foods and foods away from home, which determines an increased consumption of energy-dense foods and a lower consumption of nutrient-dense foods (4, 6). Generally, diet quality and variety can be achieved when dietary recommendations are followed (6). It is believed that children who consume a greater variety of fruits and vegetables are more likely to have a better overall diet quality (7).

Having a healthy diet is essential throughout lifespan (8). Several diseases are known to be potentiated by a poor diet (9). For that reason, there has been an increasing interest in assessing what people eat and showing the relationship with the onset of disease.

However, diet is an exposure quite complex (8) to measure. Previously, in nutritional epidemiology to assess whether a food was related to an outcome (disease), the analysis was performed with a single nutrient or food (10). However, this approach had some limitations because we do not eat isolated foods or nutrients (11, 12) and no food groups contain all the nutrients needed for a varied and healthy diet (13). Diet is composed of several foods that interact with each other (12, 14-16), and in some cases, foods have a high intercorrelation, which makes difficult the interpretation of results (14, 17). Furthermore, the effect of only one nutrient or food may be too small that it is hard to detect (18, 19), but when detected in a dietary pattern, their cumulative effects can be sufficient large to be identified (18). Besides that, the consumption of individual foods is less stable over time than dietary preferences at all (8).

Thus, in nutritional epidemiology, the interest in detecting and evaluating dietary patterns has increased and this approach emerged to evaluate the overall diet quality (18, 20) and the association between foods/nutrients and health outcomes (8, 21). Dietary patterns represent the consumption of a set of foods and nutrients that are commonly consumed together (11). So, dietary patterns can be more predictive of disease risk comparing to the isolated analysis of a single nutrient or food (18), since they describe better the eating complexity (11, 22) and becomes more reliable. They accurately describe the real consumption and it is easier to the public understand the results (17, 18).

To identify and evaluate dietary patterns there are different methodological approaches: *a priori* methods (theoretically defined), *a posteriori* methods (empirically derived) (23) and hybrid methods (15). Figure 1 presents some examples of the most used approaches to define dietary patterns.

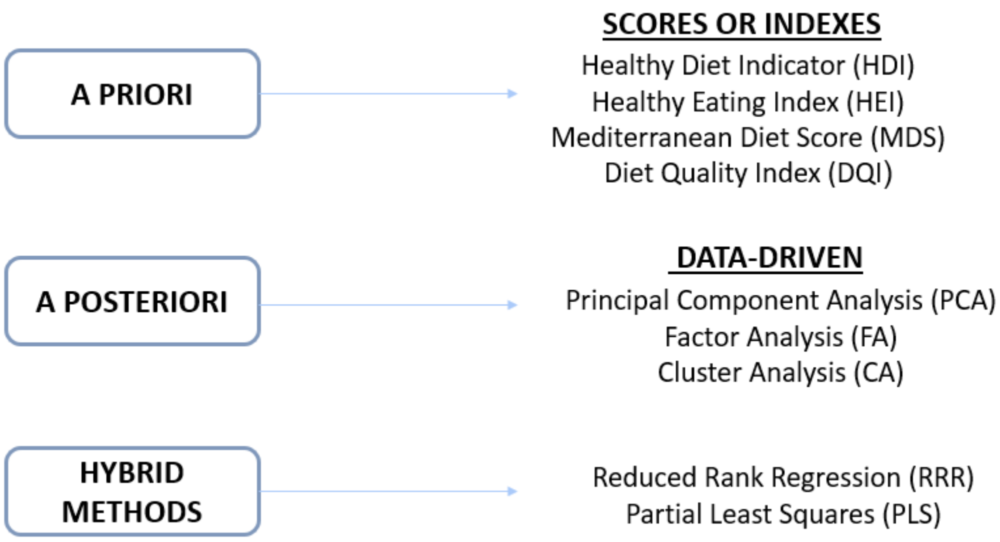

Figure1: Distinction between *a priori*, *a posteriori* and hybrid methods to define dietary patterns.

A priori methods

The *a priori* methods, or commonly called hypothesis-oriented approaches, are created based on dietary recommendations or known healthy diets (12, 18). They can be used as a quick and simple method of assessment of diet quality and many indices or scores have been developed and validated all over the years (24). According to this method, foods are grouped taking into account prior knowledge of the association of foods and health outcomes (12) and indices or scores are created to evaluate food/nutrient intake (5), in order to assess the degree of adherence to a certain dietary pattern (e.g. Mediterranean Diet Pattern) or dietary recommendations (for instance food guides, adherence to the World Health Organization guidelines or country-specific recommendations) (18, 20, 24, 25), based on empirical evidence of health benefits (26).

To create an index or score it is essential to define the items to be included, the cut-off values and also the scoring of the variables (12). Thus, these choices are dependent on the researcher and include a large degree of subjectivity (12, 18). They can also be defined based on different sources of dietary consumption. In a systematic review, the most common dietary methods applied to assess food intake were food frequency questionnaires and 24-hours recalls, but mostly indexes or scores used multiple dietary methods (27). In general, the included variables are foods, food groups or nutrients that are established in the literature as to be healthy and unhealthy (12, 27).

There are certain dietary patterns that have been associated with beneficial health outcomes. In Europe, The Mediterranean Diet (28) is undoubtedly the one with more accumulated evidence of its effects on health. It is characterized by a high consumption of vegetables, fruit, nuts, olive oil, unrefined cereals, fish, low to moderate milk intake, low consumption of meat and meals are accompanied with moderate amounts of red wine (9, 29, 30). The adherence to Mediterranean pattern has been broadly studied (12) and several studies revealed many benefits (31-33). A meta-analysis showed that a 2-point increase in adherence to the Mediterranean diet showed an 8% reduction in all-cause of death, a 10% reduction in disease from death and/or incidence of cardio- and cerebrovascular diseases and reduction in the appearance of other diseases, such neoplastic or neurodegenerative diseases (34).

Because there are a lot of different dietary recommendations, especially between countries, there are also a huge variety of dietary indices. The most commonly used indexes or scores are the following: Healthy Eating Index (HEI); Diet Quality Index (DQI); Dietary diversity score; Mediterranean Diet Score (MDS) and Healthy Diet Indicator (HDI) (28). Some of these indices were later adapted from the original ones, namely to incorporate the different dietary recommendations of each country (12). At the same time, due to the high number of adaptations and their country-specific profile (28), this became a disadvantage by the difficulty of comparing results between studies. *A priori* dietary patterns have also other disadvantages, as dietary indices or scores do not describe the overall diet pattern (15) and most dietary recommendations are not disease specific. In addition, the rationale and the weightings for the constructed indexes or scores are not very well described in the literature (26). Some indices have the same weight for all variables, assuming that all variables have the same importance, while other authors attributed different weights to different items, based on a subjective criterion (12). Furthermore, many of the indices are not appropriate for studies with children, since dietary recommendations are usually created to be followed by adults (5). However, there are some indexes or scores that were developed to be used in children, such as the Revised Children's Diet Quality Index and Diet Quality Index for young children (DQI-CH). The first one was based on dietary recommendations from the United States and the second one was developed to measure the compliance with the Flemish dietary guidelines, both applied in children between 2 and 5 years-old (24). However, the HEI was also adapted to Youth Healthy Eating Index in children between 9 and 14 years of age and this index focuses in healthy and unhealthy foods and eating behaviour (35). Consequently, the usefulness of *a priori methods* in younger populations is limited (27), particularly when we want to apply to Portuguese data.

A posteriori method

A posteriori methods are also known as data-driven approaches and determine dietary patterns based on the available data (8). To use this technique, it is necessary to use statistical methods (25). These methods are also called data-reduced techniques (22), since they reduce the original variables in a set of variables that explain the maximum possible variance, generally called predictor variables. They derive dietary patterns only based on available data and they are independent of definitions about what a healthy dietary pattern means. However, because of that reason, they are always population- and data-specific (15).

In general, it is possible to use *a posteriori* methods based on information collected by different dietary methods, namely 24-hour recalls, dietary records and food frequency questionnaires (18, 36).

There are different methods described in the literature to derive *a posteriori* dietary pattern (18). The most commonly used methods are factor analysis, principal component analysis (PCA) and cluster analysis (8, 11, 17).

The Factor analysis is a data-reduced technique that aggregates the food items based on the degree they are correlated with each other in the dataset (18, 37).

Principal component analysis is similar to factor analysis (10). This approach combines foods that explain a high proportion of food intake data and identifies foods that are regularly consumed together, so considering the degree to which food items or food groups correlate with each other, this method aggregates them (8). PCA derives uncorrelated dietary patterns by the construction of linear combinations of food intake data through explained variation of all predictor variables that are the original dietary variables (10, 22, 38). Individual indexes or scores are created for each pattern (factor) and individuals are ranked according to their food consumption that are highly weighted in the factor (10). However, PCA does not take into account the outcome measure (19, 22) and the interpretation of the results are more challenging (39).

Cluster analysis separates individuals according to their similar food intake. It aggregates individuals in mutually exclusive clusters if they have a similar dietary pattern, so if individuals are located into two different clusters, it means that they do not share the same food intake (8, 15). The investigator has to decide on the number of clusters, but there are statistical tests to support this decision (8).

Hybrid methods

Hybrid methods combine a *priori* information with exploratory statistical methods (with available data to the population under study) and allow to obtain dietary patterns based on a specific outcome, thus they are likely to be more relevant to the population (15). This approach is limited to the health outcomes that have accumulated sufficient knowledge, in order to choose the intermediate or response variables under study (8).

Reduced rank regression (RRR) was introduced in nutritional epidemiology by Hoffmann et al. in 2004 (19). RRR tries to identify dietary patterns that are associated with a specific disease, combining exploratory methods and *a priori* knowledge from epidemiologic nutritional studies (8, 40). In this method, there are two sets of variables: the predictors (food intake data) and response variables (disease-related nutrient or disease-specific biochemical markers) (19). Applying this approach, linear functions of predictors are derived, explaining as much variation as possible in a set of intermediate response variables that are important to the outcome under study (8, 10, 38) – response variables. The maximum number of factors derived by this technique has to be equal to the number of response variables (14). To choose the response variables, it is necessary that there is enough evidence of the influence of these variables in the outcome under study (8). However, the dietary patterns created are limited to the included response variables and dietary patterns that do not specifically include other response variables cannot be derived (22). Several studies are already derived dietary patterns using this technique associated with many health outcomes, including obesity, and some of them in children (14, 38, 41-45).

Partial Least Squares (PLS) is a recent technique for deriving dietary patterns (36) and it is a mix between PCA and RRR. In this method, there are two set of variables – predictors and response variables (22). Instead of attempting to explain mostly the response variables and predictor variables, such as RRR and the PCA do, respectively, the PLS tries to maximize the explained variance between these two types of variables (36). So, the extracted dietary patterns explained a maximum variance in dietary intake (predictor variables) and in the response variables related to health or disease (22). The first pattern derived is the one that explains a higher percentage of the variance (19). PLS is a method that was more commonly used in bioinformatics and chemometrics research (22), however there are already some studies that use this method to derive patterns. PLS was used for instance, to evaluate the association of the dietary patterns and bone health (22, 46) and myocardial infarction (41), but almost none were used in relation to obesity (36), since it is not yet widely used in nutritional epidemiology.

Although *a posteriori* approaches and hybrid methods introduce advantages, disadvantages cannot be discarded. The effect of a food or a nutrient may be not always visible because the effect can be diluted in the dietary pattern (18). Also, arbitrary choices are made, such as the selection of food groups, the number of factors (patterns) to be retained, the decision of which factors are relevant and in the cut-off decision of the loadings factors. Labelling dietary patterns can be also a challenge, since that is a choice of the researcher and, for instance a "traditional" dietary pattern of a country can be considerably different from another country (8), which also become more difficult the comparison between studies.

As dietary patterns seem to track over time (47, 48) it is also important to understand how food preferences and choices are developed throughout life.

2. EARLY DEVELOPMENT OF FOOD CHOICES

Food behaviours develop from infancy; this is a critical period to develop healthy eating habits (21). It is possible to recognize the smell and taste of foods still in the intrauterine environment, due to the swallow of amniotic fluid which is believed to contain the organoleptic characteristics of the food that the mother eats during pregnancy (49, 50). Breastfeeding has also a positive impact on food acceptance and enjoyment, once it promotes the acceptance of at least the flavours that were present in the breast milk (51). It is exclusively recommended by the World Health Organization until the age of six months (52) because of the many benefits it brings to infants and mothers.

Children develop their eating behaviours as well as the acceptance for certain types of food, considering their eating experiences and through the direct observation of others around them (4), like family members and particularly parents. Up to 5 years of age, children go through a transition period to adult diet and during this period they learn how to eat, what foods are common to their culture, how much should be eaten (50) and they have sensitivity to develop food preferences (53).

Although, children's eating behaviour develops early in life, during this process some problematic eating behaviours can be established (54). This can range from binge eating or disinhibited eating until picky eating (55). The latter is characterized by children who are peculiar in their diet (56). In general, picky eaters and neophobic children are described in the literature as children who exhibit limited food intake and variety as well; their food is very different from the rest of the family members and they have strong food likes and dislikes (57-59). The major difference between the two definitions is when children are reluctant essentially to try new foods they are called neophobic, whereas picky eaters do not like eating unfamiliar foods as well as familiar ones (54, 60). According to a review, the prevalence of picky eater among the studies ranged from 5.6% to 50%, depending on the children's age studied, the definition used and the assessment method (61). The consumption of vegetables, fruits and meat tends to be lower in children who are considered neophobic when compared to children who are not (49, 54, 59), as well as less food variety (58, 59). This inadequate food variety may lead to lower nutrient intake and might result in the appearance of later complications (58). In some studies, overeating appears to be associated with the development of obesity, and children who are picky eaters are more prone to have low weight (56). These problematic eating behaviours once manifested in childhood tend to be a precursor to maladaptive eating later in life (62).

Children as well as adults tend to reject food according to their sensory characteristics (54). Sweet and salty tastes are the flavours that the infants are more prone to prefer when they are born (4, 50). However, it should be noted that it is usual for foods such as vegetables to be more rejected by children since their taste are generally bitter. But, as more often their intake is experienced (some food exposures are required), it becomes more familiar and their acceptance tends to increase (49).

3. CHILDHOOD OBESITY EPIDEMIOLOGY

Obesity is a multifactorial disease (63) and there are several mechanisms involved in its development, such as genetic, environmental and behavioral factors (64, 65).

Obesity represents a major public health challenge (53, 65-67). It has been increasing worldwide (5, 12) and is also a well-recognized problem in Europe (68). In 2016, 39% of the worldwide adult population aged 18 years-old and more were overweight and 13% were obese (69). Furthermore, with the increasing prevalence of obesity, the direct costs with this pathology also increases (70, 71). The global economic impact of obesity, in 2014, was estimated to be 2.8% of the gross domestic product (72). Besides that, obesity can also lead to a lower productivity at work, absenteeism and higher disability (67).

Still in 2016, 41 million children under 5 years of age and 340 million children and adolescents (5-19 years old) were overweight or obese worldwide (69). According to the latest data of the National Food, Nutrition and Physical Activity Survey, conducted in a representative sample of the Portuguese general population during 2015-2016, 22.3% of Portuguese are obese and 34.8% are overweight. Among children the burden is also high: 7.7% of children under 10 years-old are obese, and the prevalence of overweight children reached 17.3% (73). When we compare these data with European numbers, according to the European Childhood Obesity Surveillance Initiative, , the countries from Southern Europe (Greece, Italy, Spain and Portugal) have the highest overweight (more than 30%) and obesity prevalence (higher than 13%) in children aged 6-9 years-old (74). Another study that evaluated the prevalence of overweight and obesity in European children below 10 years old showed that the prevalence of these two conditions combined can be 10% in Northern countries whereas in the Southern countries can reach 40% (66).

Obesity triggers several short and long-term complications (75) and a higher risk for developing chronic diseases (76). Nowadays, obese children are facing with comorbidities that previously were only observed in adults (64), such as hypertension, dyslipidaemia, heart disease, type 2 diabetes, osteoarthritis, gallbladder disease and respiratory problems (64, 76). Besides that, depression, sleep and eating disorders and social isolation are the main physical and psychological complications that obesity can leads to (64) and these problems affect a lot of children, due to a loss of self-esteem (65). In addition, in 2014, 5% of worldwide deaths were attributed to obesity (67).

Thus, there are many reasons to prevent and reduce the prevalence of obesity, particularly in the paediatric age, as obese children and adolescents are much more likely to become obese adults than children with normal weight (64). Another reason is that adults find much more difficult to reduce their weight from the time they become obese (64). In addition, changing habits in adults, including rooted eating habits for several years, become much more difficult to modify.

Childhood and the first years of life are the ideal periods to prevent poor eating habits, which is also the period when children contact with more types of foods, as there is a progressive transition from infantile food to an adult diet with a huge range of foods (50).

3.1 FACTORS FOR CHILDHOOD OBESITY DEVELOPMENT

There are several factors that can contribute for the onset of obesity and many risk factors may change with age (17). That is why it is of utmost relevance to understand what the determinants of obesity are, since childhood obesity tends to track into adulthood (77).

All over the years, we are observing a paradigm change: food industry growth, unhealthy food marketing by the media, high fibre diets replaced by a diet rich in simple carbohydrates and higher in fat and energy-dense foods (78). Besides that, nowadays, parents have less time to prepare the meals (21) and consequently, take away and fast-food are gaining increasing relevance and importance in the food market because they become convenient foods (79).

Obesity can be the result of the intercorrelation of several factors, in a multi-level framework. Regarding an ecological approach for the aetiology of childhood overweight and also for their prevention, there are three major domains that can influence the weight status: i) community and demographic factors; ii) parenting, feeding and parent characteristics and iii) child’s behaviour; they are the community, household and individual levels, respectively (80). The most distal domain is characterized by the socioeconomic status, accessibility of foods and restaurants, neighbourhood safety and ethnicity. Parent characteristics are related to the type of food available at home, the parent’s eating and physical activity and, consequently, their weight status, the nutritional knowledge and the promotion of healthy habits. The more proximal domain is related to the child's behaviour, namely food habits, practice of physical exercise and time spending in sedentary behaviours (80). Some of these determinants will be described in more detail in this chapter.

Socioeconomics factors are a strong determinant of food consumption that influences the quality of infant's feeding (21). Children belonging to a low socioeconomic status seems to eat fewer fruits and vegetables and have higher fat intake compared with those with a higher socioeconomic status (53). A study that analysed the prevalence of overweight and obesity in European children below 10 years of age (n=18 745) reported a higher prevalence of obesity in population groups with lower education and income levels (66).

Concerning the access to food, a study that evaluated the proximity of fast-food restaurants to US schools, showed that more than an half of schools have a fast-food restaurant near their facilities, which facilitates access to unhealthy foods to children (81). This study demonstrated also that these children were more likely to be overweight and consumed more soda and less fruit and vegetables servings (81). Portion size of foods has increased since 1970 (82, 83) and this increase has paralleled the increasing prevalence of obesity (84). Fast-food restaurants have introduced menus, some particularly developed to children but with adult-sized portions and many food items were two to five times larger than the originally marketed (53).

Parental-related factors can also influence the child's eating behaviour. Parents are the ones that purchases food for all the family, so they determine what type of food is available at home (healthy or unhealthy) (21), and consequently, what their children eat and how their food preferences are established (50, 53). In a Brazilian study, mothers who considered their work as a priority, paid less attention to the preparation of meals (21), which affects the teachings communicated to the children, particularly related to healthy eating. Furthermore, children whose parents are overweight are more prone to have higher weight, especially because they tend to show similar dietary patterns, so children eat what their family eats and if their parents do not have a healthy diet, their food consumption will not be healthy as well (53). Additionally, there are also the parents who prepare the size of the portions to serve to their children (50). Thus, when parents prepare a large portion meal (above child' needs), children tend to eat all the meal, just because the food is placed in front of them (50, 53). The mealtime structure also affects the development of eating behaviour (53). Children who eat meals with other family members consume more healthy foods (53) and less soft drinks. Another factor that seems to be relevant is the parent's knowledge/perception about nutrition and children's nutritional status. For instance, authors of a Canadian study compare the perceptions of the parents about their children's weight and the real weight. They identify that a large proportion of parents did not recognise their children as obese or overweight (85). In addition, another study demonstrated that parents underestimated their children's overweight and overestimated the ones that have lower weight (86), highlighting the importance of the knowledge and an easy recognition by parents in order to an early intervention. Parents' teachings about food can influence children's eating habits. In a study that assessed the nutritional knowledge of mothers, it was found that mothers that have a higher nutritional knowledge, feed their children with more fruit and vegetables and less fast-food and sugared drinks compared with mothers that have a lower nutritional knowledge (87).

Nonetheless, parental feeding practices may also be influenced by the characteristics of the children, such as age, behaviour and weight (88). For instance, if the child is overweight or otherwise underweight, the parents also behave differently (4, 88). In the first case, they tend to restrict children's food intake and in the second situation, parents pressure the children to eat (88). However, when excessively, neither of the two parental controls in relation to infant feeding seems to have a long-term effect (4). These children seem to have less self-regulation in their caloric intake and less preference for the foods that they are obliged to eat by their parents (4). Parents of children who eat small quantities of food, tend to pressure their children to eat more, but nevertheless often the opposite effect occurs, since children regard the meal time as something they do not enjoy (89).

The development of obesity will also depend deeply on child's individual behaviours (50), such as child's physical exercise, as well as whether has sedentary behaviours. Higher levels of television watching and low time spending in sports or physical exercise increase the risk for weight gain (90).

Thus, as stated above, there are several factors influencing the child's weight. Perhaps, it is for this reason that many of the school-based interventions had little success (50). Strategies for interventions in the prevention of obesity must have to include the maximum contexts that can influence children' eating habits and weight (multifactorial approach) (50), in order to prevent overweight in the future and eventually other associated complications, such as cardiometabolic risk factors.

4. DIET AND CARDIOMETABOLIC HEALTH

Cardiovascular diseases are the leading cause of death globally (91). In Portugal, in 2005, as well as in 2016, ischemic heart disease was the first cause of death (92), showing that cardiovascular diseases are still a major public health problem and must be diminished.

One of the main causes for the appearance of several chronic diseases is due to the poor diet that we face nowadays (9). Most of foods available in the market contains a large amount of salt (93), particularly those that are tastier, which can explain the high consumption of this mineral. More than a half of children who have higher blood pressure when they are children are more likely to have hypertension in adulthood (94), suggesting that hypertension tend to persist from childhood to adulthood. Besides that, children who eat more fruits and vegetables are more prone to have lower blood pressure during childhood (95) and the reduction in salt intake, accompanies a reduction of hypertension.

Foods that are rich in added fats were positively associated with increased triglycerides (96). By their turn, fibre intake has a protective effect for the onset of obesity, and consequently for the development of cardiovascular risk factors, for example, lower levels of insulin resistance. Furthermore, it is more likely to have a higher risk of cardiovascular disease in adulthood once these cardiometabolic risk factors persists in childhood (97).

Obesity has a strong impact in the health and it is associated with several co-morbidities, in particular cardiometabolic risk factors such as dyslipidaemia, abnormal glucose tolerance and hypertension. For instance, atherosclerosis can start in childhood and is more prevalent with the presence of others cardiometabolic risk factors (93).

Some dietary patterns were evaluated and associated with cardiometabolic risk factors. The Dietary Approach to Stop Hypertension (DASH) is a dietary pattern that was developed in order to low or control the blood pressure. This diet promotes the intake of fruit and vegetables, including whole grains, low-fat dairy products, fish and nuts and emphasizes the lower consumption of fat, beverages with sugar, sweets and red meat (98). In that way, this is associated with a decreased systolic and diastolic blood pressure (99). Besides that, as DASH diet highlights the consumption of healthy foods, it promotes the consumption of several protective nutrients, contributing to a decrease of fat and refined carbohydrate consumption and consequently, may contribute to low the low-density protein cholesterol (LDL-c) concentrations and cholesterol levels (99). Similarly, the Mediterranean diet is characterized by the consumption of mostly healthy foods, as was described above. For that reason, it has also some advantages in cardiometabolic health (33).

Some *a posteriori* dietary pattern derived by different techniques were also associated with cardiometabolic health or cardiometabolic risk factors (44, 100-102). However, most studies are conducted in adolescence, have a cross-sectional design and dietary patterns are not related with a specific outcome.

To the best of our knowledge, dietary patterns derived by different sound methodologies were not very well explored in 7 years-old children, namely its prospective association with cardiometabolic health in later life.

AIMS

This study aims to identify data-driven dietary patterns at 7 years-old and whether following these patterns has some influence in the cardiometabolic health three years later. The specific objectives of this work are described below and are presented by papers.

Paper I

To identify dietary patterns at 7 years-old explaining body mass index (BMI) at 10 years-old, to evaluate their predictive value for classifying obesity, and to assess their early life influences.

Paper II

To evaluate if dietary patterns followed at 7 years of age, in particular an obesity-related dietary pattern, has an effect on cardiometabolic health indicators at 10 years-old.

PAPERS

PAPER I

Identification of dietary patterns at 7 years-old that explain body mass index at 10 years-old: comparison of three methodological approaches in the Generation XXI birth cohort

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ABSTRACT

Introduction: Most approaches to identify dietary patterns do not consider the explanation of a specific outcome, such as obesity. At some extent, this could explain the lack of consistent associations between diet and obesity-related outcomes.

Objective: To compare three different methodological approaches [linear regression, principal component analysis (PCA) and partial least squares (PLS)] to derive dietary patterns at 7 years-old; to evaluate the predictive value of those to explain body mass index (BMI) later in life (at 10 years-old); to assess associated early life factors.

Methods: Children from the birth cohort Generation XXI (Northern Portugal, 2005-2006) with information of interest at 7 and 10 years-old were included (final sample n=4698, excluding twins). Diet was assessed by a validated food-frequency questionnaire (38 items/food groups). Measured BMI z-scores were calculated according to the World Health Organization criteria. PCA aggregated food consumption without directly explaining the outcome and PLS derived dietary patterns that explained the maximum variance in predictor (38 items/food groups at 7 years-old) and response variables (BMI z-score at 10 years-old). The food items/groups with factor loadings >|0.2| were considered to be relevant to the dietary patterns. Receiver Operating Characteristics (ROC) curves were performed to compare the ability to discriminate between obesity and non-obesity by the different methods.

Results: By linear regression (standard method), a significant positive association of cakes and soft drinks and a negative association of vegetable soup with BMI at 10 years of age were identified. This dietary pattern explained 0.11% of food groups and 4.30% of the response variable. By PCA, two dietary patterns were derived. The cumulative variance explained was 13.0% of food groups and only 0.23% of BMI z-score. PLS derived two dietary patterns that explained 10.1% of food groups and 4.23% of BMI z-score. The first dietary pattern derived by PLS was more strongly associated with BMI z-score at 10 years-old ($\tilde{\beta}$=0.193; 95%CI: 0.166; 0.221) and showed an area under the curve (AUC)=0.63, 95%CI: 0.61; 0.65. The food items with relevant contribution to this dietary pattern were processed meats, energy-dense foods and soft-drinks and a low consumption of vegetable soup. This dietary pattern was more likely followed by children from younger and less educated mothers, children who were born heavier and who did not practice regular physical activity at 7 years-old.

Conclusions: PLS seems to be the best method to derive dietary patterns, as it tries to explain the variability of both food intake and the weight status. A dietary pattern higher in processed meats and energy-dense foods and drinks and with lower vegetable soup intake at 7 years-old explained better the BMI z-score at 10 years-old.

Key-words: Body mass index; Children; Cohort studies; Feeding behaviour; Food habits; Partial Least Squares

Introduction

Diet plays an important role in preventing the onset of disease (1, 2). Consequently, an inadequate diet is a modifiable leading risk factor for many chronic diseases, such as obesity (3, 4), whose prevalence has been increasing worldwide reaching public health proportions (5-9). However, due to its complexity, it is not easy to study the overall diet effects (10, 11). Analysis of one unique nutrient or food and its association with health or disease, nowadays has less value for multifactorial diet-related diseases, because its methodological limitations (12). We do not eat isolated foods and nutrients, and these interact with each other (13-15), which makes difficult to examine; the effect of a single nutrient can also be too small to be detected (12) and can be confounded by the overall diet effect.

Hence, analyses of dietary patterns, studying diet as a whole, have been gaining interest in nutritional research (16, 17). Dietary patterns represent the set of foods and nutrients that are consumed together, being a usual method to study the role of diet and their association with health outcomes (13). Nowadays, three main different approaches to derive dietary patterns have been described - *a priori, a posteriori* and hybrid methods*.* The *a priori* approach evaluates diet quality through scores or indexes according to the adherence of dietary recommendations or known dietary patterns based on prior scientific evidence (10, 18-20). Factor analysis and principal component analysis (PCA) are widely applied methods in nutritional epidemiology and there are considered *a posteriori* methods (10); they try to explain the maximum variance of predictor variables, that is food intake (21).

Reduced rank regression (RRR) and partial least squares (PLS) are considered hybrid methods since they combine *a priori* information with exploratory statistics of dietary data (14, 22). Both methods work with two set of variables: the predictors (food groups) and response variables (usually nutrients or biomarkers that are related to the study outcome) (23). RRR creates uncorrelated patterns that are related to the outcome of interest (24) and tries to explain the maximized variance of *a priori* selected set of response variables. PLS is a mix method between PCA and RRR since it tries to explain the maximum variance in the predictor variables as well as in the response variables (24).

Despite there are several studies deriving dietary patterns, most of them do not consider the explanation of the study outcome, which could in part explain the lack of consistent associations between diet and the disease, and there are fewer prospective analyses, especially with data from school-aged children. To the best of our knowledge this is the first study deriving and comparing dietary patterns using linear regression, PCA and PLS methods with prospective data from school-age children. The aims of this study are: i) to identify dietary patterns at 7 years-old explaining body mass index (BMI) at 10 years-old; ii) to evaluate the predictive value of those dietary patterns for classifying obesity; iii) to assess early life factors associated with the dietary patterns identified.

Methods

Study design and participants

Generation XXI is the first prospective population-based birth cohort of Portuguese new-borns, aiming to monitor the growth, development and health of children up to adulthood. In 2005 and 2006, 8647 new-borns and 8495 mothers were recruited in all public maternity hospitals in the Porto metropolitan area (Northern Portugal) and enrolled at baseline. Of the invited mothers, 91% agreed to participate, after signed a consent form. When the children completed 4 years-old, the entire cohort were invited to be re-evaluated (participation rate: 86%) and the same occurred when children were 7 (participation rate: 80%) and 10 years-old (participation rate: 76%).

This study includes a prospective analysis with data from the 7 and 10 years-old evaluation. Twins (n=246) and participants who had no dietary data (n=1848) at 7 years-old and information in variables of interest (n=90) were excluded. Thus, this analysis included 4698 children who had complete information on the variables of interest.

To compare our sample (n=4698) with the remaining non-participating cohort, differences in baseline characteristics were checked. No differences were found regarding children’s sex, but in the study sample, mothers were slightly more educated (mean=11.2; standard deviation (SD)=4.3 vs. mean=9.5; SD=4.0; $p<0.001$) and older (mean=29.9; SD=5.2, vs. mean=27.9; SD=5.9; $p<0.001$) comparing to the remaining cohort non-included in this analysis. However, according to Cohen effect size the magnitude of the differences was not high and these differences are possibly due to the large sample size and not because of the different characteristics of the participants (25).

At every evaluation, a signed informed consent according to the Declaration of Helsinki was required. The study was approved by the University of Porto Medical School/S. João Hospital Centre ethics committee and Ethical Principles for Medical Research Involving Human Subjects.

Data collection

Face-to-face interviews using structured questionnaires were performed by trained researchers to collect information on maternal and children’s characteristics or retrieved from medical records. Maternal age, education, child’s sex, birth weight and gestational age were obtained at baseline. At 7 years old, physical activity and living with siblings were obtained, among several other variables, such as children’s diet. A validated food frequency questionnaire (FFQ) covering the previous 6 months was applied by a trained interviewer to the main caregiver(s) to assess habitual dietary intake at 7 years-old. The FFQ was composed of 38 food items/groups and 9 response options which varied from “never” to “more than 4 times a day”. It was previously validated in a similar sample from Generation XXI, by comparing data with 3-day food records (n=3441) and biomarkers (n=80) (26). Compared with food records, the highest intraclass correlation coefficients (ICC) obtained was for ‘vegetable soup’ (ICC=0.430). Significant correlation coefficients were observed for all nutrients; the average of correlation coefficients was 0.42. When compared with serum biomarkers in a sub-sample of 80 children at 7 years of age, the de-attenuated correlation coefficients were: r=0.282 (p=0.120) for plasma concentration of vitamin A, r=0.425 (p=0.027) for folate.

Anthropometrics were collected at 7 and 10 years-old evaluation by trained staff according to standard procedures (27). Weight was measured in light clothing and without shoes, by using a scale (TANITA®, Arlington Heights, IL, USA) (to the nearest 0.1kg). Height was measured using a stadiometer (SECA®, Hamburg, Germany) (to the nearest 0.1cm). Children were then classified according to the age- and sex-specific BMI reference z-scores developed by the World Health Organization (28) into obese (BMI > 2 SD) and non-obese (29).

Dietary patterns definition

Three methodological approaches to derive dietary patterns were followed: linear regression, PCA and PLS.

Linear regression was used as the standard method, as this technique linearly measures the relationship between a dependent variable (BMI z-score) and one or several independent variables (the 38 FFQ food groups). All foods were standardized (z-score) when entered in the models, to allow comparisons between them. The final score was estimated by multiplying the ß-regression coefficients by the standardized food groups and summed up.

PCA works with one set of variables, that are the predictor variables (food groups) and tries to explain the maximum variance as possible of these variables (13).

In the literature, PLS uses two sets of variables: predictor and response variables and tries to explain the maximum variance of these two (14). Predictor variables are usually the food groups and the response variables are generally nutrients or biomarkers that act as an intermediate step to explain the outcome. As the nutrients or biomarkers are often closely related to food groups, thus explaining a large percentage of variance, we tried to present a different approach that is using BMI z-score as a response variable, which is less related to food groups and directly related to the outcome of interest in this study, that is obesity. So, this response variable was chosen because we wanted to identify dietary patterns that best predict obesity at 10 years-old. Thus, the 38 food groups were used as the predictor variables and the BMI z-score as the response variable.

Dietary pattern score was extracted as a continuous variable and it was calculated as a sum of the product of the food group intake of each participant and its corresponding factor loading. Food groups or food items having factor loadings >|0.2| were considered to be relevant to the dietary pattern and as higher the positive value of the factor loading, greater the contribution of that food or food group to the factor (dietary pattern) (30).

In linear regression, the number of factors (dietary patterns) retained equals the number of response variables (in our case, one). The number of factors to retain in PCA was decided based on the acceleration factor; two factors were retained using a scree plot as a guide. For PLS, we retain the same number of factors to be easily comparable between methods.

Statistical Analysis

Characteristics of participants were described as means and standard deviation for numerical variables with symmetric distribution, and as the percentage for categorical variables.

Linear regression models were performed to estimate the association of sociodemographic and early life characteristics with the dietary pattern with a greater explanation of the response and predictor variables. Three models were run: model 0 - crude model; model 1 – adjusted for child's sex, gestational age, birth weight and maternal age and education: and model 2 – model 1 plus adjustment for living with siblings and physical activity practice at 7 years of age.

No adjustment has been made for energy intake since it is likely to be an intermediate step between diet and health outcomes, such as obesity. However, even though, we tested if the consumed calories at 7 years-old influenced the results, but it did not change the magnitude of estimates. We also checked whether breastfeeding was a relevant confounder, but it was not, and thus was not included in the final model.

The explained variance of food groups and BMI was similar by sex and the effects of the dietary patterns on BMI at 10 years-old were the same, thus analyses were conducted with both sexes together.

Receiver Operating Characteristics (ROC) curves were performed to compare the ability to discriminate between obesity and non-obesity by the different methods. The area under the curve (AUC) was determined and describes the discriminatory power of a test (measure that combines sensitivity and specificity) with values ranging from 0 to 1. The ideal value of the AUC is 1; as much closer to this value, the greater the discriminatory power; the AUC equal to 0.5 means a random value (no discriminatory ability) (31).

The significance level was set at 5%. Analyses to derive dietary patterns were performed using the R software (The R Project for Statistical Computing), version 3.4.0 for Windows, and linear regression models were obtained by IBM SPSS (Statistical Package for Social Sciences), version 24.0™.

Results

Table 1 presents the participants' characteristics. Boys represent 51.3% of the study sample. The mean birth weight was 3.196 kilograms, the mean gestational age was 38.7 weeks, and 16.6% of children were obese at 10 years-old. Mothers had a mean age of 29.9 years and 11.2 years of education.

Standardized betas for the factor (dietary pattern) extracted by linear regression and standardized factor loadings for factors extracted by PCA and PLS are shown in table 2.

Food items such as yoghurt, cheese, bread, cakes and carbonated soft drinks were positively and significantly associated with the dietary pattern extracted by linear regression (strongest standardized beta values). On the other hand, food items such as whole milk, vegetable soup, breakfast cereals and cookies were negatively associated with the dietary pattern. This single factor explained 4.30% of BMI z-score, although it only explained 0.11% of the variance of the food groups (table 2).

Foods most related to factors derived by PCA and PLS were identified as those with standardized factor loadings |>0.20|. Ice cream, sausage, meat salty snacks, fish snacks, pizza, hamburger, French fries, cookies, cakes, chocolate, sugar, candies, butter or margarine, coffee, coke, ice tea, nectar juices and carbonated and non-carbonated soft drinks were food items positively associated with PCA-factor 1, and in contrast, fish, vegetable soup and fruit were inversely associated. This factor explained a low percentage of the children's BMI z-score at 10 years-old (0.23%) and a higher percentage of the variance of food groups (7.0%). In PCA-factor 2, a dietary pattern presenting factor loadings with almost reversed associations compared to PCA-factor 1 was found; the explanation of the outcome (BMI z-score) was null (0.00%), but it explained 6.0% of the variance of food groups.

Lastly, two dietary patterns were derived by the PLS method. In the first factor, ice cream, cheese, sausage, ham, bread, cakes, coffee, coke, carbonated and non-carbonated soft drinks had higher standardized factor loadings, thus these were the food items that were positively associated with this pattern. The items negatively and significantly associated were whole milk and vegetable soup. However, in PLS-2, energy-dense foods and sugary drinks were items that had standardized negative factor loadings; fish was the only food item positively associated. PLS was the only method that was able to explain the greater variance in both the predictor variables (food groups) as in the response variable (BMI z-score), particularly PLS-factor 1. It explained 4.14% of variance of food groups at 7 years-old and 3.74% of variance of BMI z-score at 10 years-old. PLS-factor 2 was able to explain a higher variance of the food groups (5.92%), but it only managed to explain 0.49% of the outcome.

In figure 1, the ability to discriminate obese children from non-obese for each method is presented by ROC curves. Considering the area under the curve, PCA-factor 1 was the method with the lowest value (AUC=0.55, 95%CI: 0.53-0.57), followed by linear regression (AUC=0.63, 95%CI: 0.61-0.65) and PLS-factor 1 (AUC=0.63, 95%CI: 0.61-0.65). As these estimates are higher than 0.5 it means that these dietary patterns identified at 7 years of age have discriminatory power to classify obese children at 10 years-old.

As factor 1 derived from PLS had higher explanatory value of both food groups and BMI z-score, it was tested which sociodemographic and early life characteristics were its main determinants (table 3). In multivariate analysis (model 2), maternal age ($\hat{\beta}$=-0.022; 95%CI: -0.028; -0.015) and education ($\hat{\beta}$=-0.050; 95%CI: -0.057; -0.042) were inversely associated with this dietary pattern. Children's with higher birth weight ($\hat{\beta}$=0.116; 95%CI: 0.033; 0.198) and those who did not practice physical activity at 7 years-old ($\hat{\beta}$=0.155; 95%CI: (0.064; 0.246) were more likely to follow this dietary pattern. Child's sex, gestational age, and living with siblings were not significantly associated with the dietary pattern identified.

Discussion

In scientific literature, dietary patterns have been identified by different methodological approaches (11, 12). In 2004, Hoffman et al. (23) described a new method to extract dietary patterns. In that study, the authors highlighted the main advantages of reduced rank regression over PCA; the latter does not consider the variance of the response variables, so it means that the identified dietary patterns may not be related to a specific outcome (32), whereas RRR does. RRR is a hybrid method (33) that derives dietary patterns that explain as much response variance as possible, but also consider *a priori* knowledge to choose which response variables should be included (usually nutrients or biomarkers) (23). Currently, this method is widely used in nutritional epidemiology (34-38), and comparisons between this method and previously widely used methods (21, 39), particularly PCA have been made.

PLS and RRR are both hybrid methods for extracting dietary patterns, however PLS tries to explain the maximum variance of both the predictor variables and the response variables. In RRR, the derived patterns are directly related to the chosen nutrients or biomarkers, in the same number, and these are intermediate steps for the health outcome. In this study, we explored a new approach and instead of using response variables, such as nutrients or biomarkers, as usually happen, we used directly BMI z-scores as the outcome (our response variable). Thus, the results are not easily comparable with other authors who have used biomarkers or nutrients as response variables. However, a study from 2016 has identified dietary patterns through PCA and RRR at 1 year of age and their relationship with body composition at 6 years of age. This study used as predictor variables the food groups of the FFQ and similarly to our study, did not used biomarkers or nutrients as response variables, but a fat mass index and a fat-free mass index instead. The two patterns derived by PCA and RRR, explained 0.5% and 2.6% of the response variables, respectively (40), lower than the variance of BMI z-scores explained in our study by using PLS (~5%).

Another authors (39) compared three different methods (PCA, PLS and RRR) to derive dietary patterns related to the risk of myocardial infarction in adults and showed that PCA and PLS produced patterns more related to the outcome than RRR. Besides that, in case of partial knowledge about the disease, PLS seems to be preferably used instead RRR (14). Nevertheless, in the literature, there are some studies using PLS as a method to derive dietary patterns and its association with cancer or bone health, but to the best of our knowledge, a single one was associated with obesity (22).

In our representative study of Portuguese children, PCA was not useful to detect the explained variance of the response variable (0.23% in the two factors) when compared to the PLS technique (4.23% in both factors). Of the three methods, PLS was the one that optimized the explained variance between the predictors (food groups) and the response variable (BMI z-score at 10 years-old). Besides that, PLS showed a greater discriminatory power for classifying obesity at 10 years-old by observing the area under the ROC curves, that from our knowledge so far, it was not described before.

The food items more consumed at 7 years of age that were more strongly associated with an obesity-driven dietary pattern at 10 years of age were energy-dense foods and soft drinks and a low consumption of whole milk and vegetable soup. A high intake of soft drinks (41) and a low intake of vegetables (42) have been previously associated with childhood obesity, but the overall effect of this dietary choices was unknown. In a study, children who followed a healthy dietary pattern was negatively associated with the prevalence of total and abdominal obesity, while the ones who followed the Western dietary pattern, that was characterized by high intake of red meat and refined grains, were more likely associated (30). In general, a systematic review found that empirical dietary patterns higher in energy-dense, high-fat and low fiber favors young people to later appearance of overweight and obesity (43).

From a public health perspective, it is also relevant to study the characteristics of children who tend to follow an obesity-driven dietary pattern. In our analysis, we identified that factor 1 from PLS was more likely among children from younger and less educated mothers and children who were born heavier and who did not practice regular physical activity at 7 years-old. These results reinforce the idea that maternal characteristics and intrauterine development are crucial factors to the development of healthy dietary behaviors (44, 45). Nonetheless, more sedentary behaviors later in life have also their influence.

This study has several strengths, such as the inclusion of a large sample size of children (almost 5000 children) from a population-based birth cohort. It allowed several assessments at different ages and the collection of a wide range of variables across time, thus adjustment of models was performed for different confounding variables at different stages of life. Anthropometric assessments were performed by trained professionals and were not self-reported, thus the outcome under study was objectively measured and not prone to underestimation, as usually happens with self-reported measures. The FFQ used in the current study, although small and limited to 38 food/food groups was previously validated in this cohort (26), which contributed to the increased internal validity of the study. Under-reporting usually is a cause of concern when comparing obese vs. non-obese subjects. Although it is documented that obese subjects usually report less food and caloric intake than they actually do, in our study, obese children do not have lower caloric intake, suggesting that at this age underreporting does not seem to be a relevant threat.

Nevertheless, we cannot discard measurement errors associated with the collection of dietary intake that can, at least in part, be responsible for the weak association of dietary patterns with obesity (they only explained a maximum of 5% of the BMI z-scores variance), regardless of the method used to derive them. Another possible explanation is related with the change of dietary patterns along time that was not captured in the current study, as dietary patterns were assessed in a single moment, at 7 years-old, but previous studies have showed a tracking of diet across childhood (46). Obesity is a multifactorial disease for which multiple components interact; diet represents only one piece of the very complex puzzle.

Conclusion

Results from this study suggest that PLS seems to be the best method to derive obesity-related dietary patterns of school-aged children. It explains the variance of both predictors (food groups) and response variables (BMI z-scores) and had a better discriminatory power to classify obesity later in life (at 10 years-old).

A single dietary pattern followed at 7 years of age, rich in high-energy-dense foods, soft drinks and low in vegetable soup explained ~5% of BMI z-score at 10 years-old. Children from younger and less educated mothers and children who were born heavier and who did not practice regular physical activity at 7 years-old were more likely to follow this dietary pattern.

This study reinforces the importance of establishing healthy eating habits from an early age for preventing obesity at older ages. It also gives methodological support to the definition of dietary patterns related to obesity during childhood.

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Table 1 – Characteristics of mothers and children from the Generation XXI cohort, included in this study

Mother and children's characteristics (n=4698)	
Maternal age (years), mean (SD)	29.9 (5.18)
Maternal education (years), mean (SD)	11.2 (4.31)
Child's sex, n (%)	
Male (%)	2411 (51.3)
Female (%)	2287 (48.7)
Gestational age (weeks), mean (SD)	38.7 (1.68)
Child's birth weight (kg), mean (SD)	3.196 (0.487)
Regular physical activity at 7 years-old, n (%)	
Yes	4021 (85.6)
No	677 (14.4)
Living with siblings, n (%)	
Yes	2867 (61.0)
No	1831 (39.0)
Child's BMI z-score at 7y, mean (SD)	0.72 (1.17)
Child's BMI z-score at 10y, mean (SD)	0.72 (1.23)
Child's BMI z-score at 10 y, n (%)	
Non – obese	3920 (83.4)
Obese	778 (16.6)

Abbreviations: SD: standard deviation; BMI: body mass index

Table 2 - Standardized beta and standardized factor loadings and explained variance (%) for dietary patterns derived from linear regression, principal component analysis (PCA) and partial least squares (PLS)

Food items	Standardized Beta	Standardized factor loadings			
	Linear Regression	PCA -1	PCA - 2	PLS - 1	PLS - 2
Chocolate milk	-0.018	0.187	-0.053	0.055	**-0.207**
Whole milk	**-0.066**	-0.014	-0.031	**-0.298**	-0.179
Semi-skimmed or skimmed milk	-0.014	0.002	**0.253**	-0.023	-0.046
Yoghurt (all type)	**0.039**	0.170	**0.268**	0.167	-0.062
Ice cream	0.024	**0.312**	0.041	**0.252**	**-0.197**
Cheese	**0.029**	0.088	**0.319**	**0.278**	-0.014
Eggs	0.014	0.129	**0.222**	0.124	-0.093
Meat (chicken, turkey, rabbit, pig, cow)	-0.015	0.133	0.081	-0.033	-0.171
Sausage	0.024	**0.443**	-0.038	**0.268**	**-0.334**
Ham, chorizo	0.026	0.191	**0.286**	**0.303**	-0.113
Meat salty snacks	-0.011	**0.376**	0.066	0.160	**-0.357**
Fish (including molluscs and seafood)	0.028	**-0.289**	**0.356**	-0.092	**0.309**
Fish snacks	0.005	**0.380**	0.023	0.144	**-0.333**
Pizza, hamburger	0.006	**0.312**	-0.016	0.180	**-0.255**
Vegetable soup	**-0.123**	**-0.274**	**0.328**	**-0.566**	-0.110
Boiled vegetables (plate)	0.016	-0.189	**0.534**	0.027	0.171
Raw vegetables (plate)	0.004	-0.150	**0.538**	0.064	0.124
Fruit	-0.007	**-0.239**	**0.530**	-0.158	0.164
Bread	**0.050**	0.066	**0.414**	**0.213**	0.034
Breakfast cereals	**-0.056**	0.181	0.170	-0.191	**-0.324**
Rice, potatoes, pasta	-0.024	-0.085	**0.212**	-0.189	0.010
French fries, chips	-0.011	**0.468**	-0.044	0.167	**-0.435**
Crackers	-0.024	0.156	**0.320**	-0.058	**-0.227**
Other cookies and biscuits	**-0.055**	**0.304**	-0.030	-0.170	**-0.404**
Cakes	**0.040**	**0.313**	0.044	**0.248**	-0.163
Chocolate and chocolate snacks	-0.028	**0.211**	-0.005	-0.084	**-0.247**
Sugar	-0.022	**0.351**	0.109	0.168	**-0.381**
Candies	-0.012	**0.464**	0.068	0.123	**-0.450**
Butter or margarine	0.005	**0.206**	**0.265**	0.174	-0.177
Coffee (included in the milk)	0.023	**0.268**	0.043	**0.269**	-0.195
Black or green tea	0.012	0.114	0.052	0.122	-0.075
Infusions	0.000	0.069	**0.200**	0.053	-0.086
Coke	0.021	**0.426**	0.043	**0.332**	**-0.303**
Carbonated soft drinks	**0.031**	**0.400**	0.043	**0.296**	**-0.263**
Ice Tea	-0.014	**0.417**	-0.058	0.138	**-0.399**
Nectar juices	0.013	**0.289**	0.161	0.175	**-0.225**
Non-carbonated soft drinks	0.010	**0.354**	0.041	0.192	**-0.291**
Natural fruit juice	-0.008	0,002	**0.305**	0.020	-0.044
β for **BMI z-score**	**0.207**	**0.046**	**0.011**	**0.193**	**0.070**
(95% CI)	(0.179; 0.235)	(0.018; 0.075)	(-0.018; 0.039)	(0.166; 0.221)	(0.042; 0.098)
Explained variance % of food groups	**0.11**	**7.0**	**6.0**	**4.14**	**5.92**
Explained variance % of BMI z-score	**4.30**	**0.23**	**0.00**	**3.74**	**0.49**

Standardized betas (p<0.05) and standardized factor loadings >|0.2| are presented in bold-type.
Abbreviations: PCA: principal component analysis; PLS: partial least squares; BMI: body mass index; CI: confidence intervals

Table 3 – Association of sociodemographic and early life characteristics with PLS – factor 1 dietary pattern.

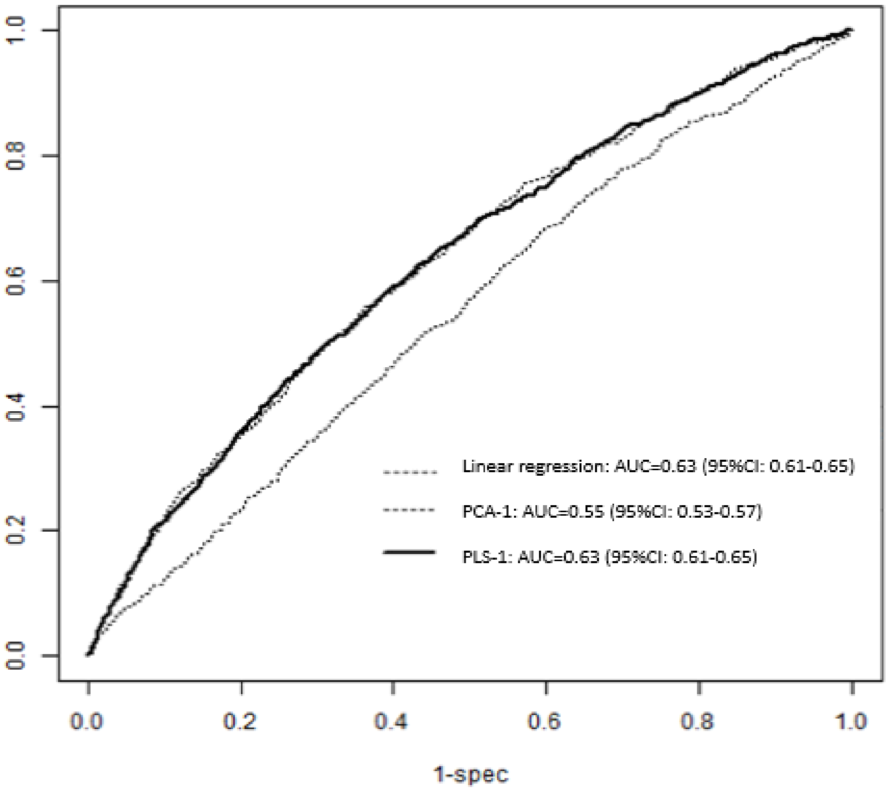
	Crude **β̂ (95% CI)**	**Model 1** **β̂ (95% CI) ***	**Model 2** **β̂ (95% CI) ****
Child's sex*			
Male	1 (ref.)	1 (ref.)	1 (ref.)
Female	-0.006 (-0.070; 0.059)	0.003 (-0.060; 0.067)	0.006 (-0.058; 0.069)
Gestational age (weeks)	0.015 (-0.004; 0.034)	-0.010 (-0.033; 0.014)	-0.009 (-0.033; 0.015)
Child's birth weight (per kg)	**0.089 (0.023; 0.155)**	**0.116 (0.034; 0.199)**	**0.116 (0.033; 0.198)**
Maternal age (years)	**-0.027 (-0.033; -0.021)**	**-0.022 (-0.028; -0.016)**	**-0.022 (-0.028; -0.015)**
Maternal education (years)	**-0.055 (-0.063; -0.048)**	**-0.052 (-0.059; -0.045)**	**-0.050 (-0.057; -0.042)**
Living with siblings at 7y			
Yes	1 (ref.)	1 (ref.)	1 (ref.)
No	0.032 (-0.034; 0.098)	-0.008 (-0.074; 0.058)	-0.006 (-0.072; 0.060)
Regular physical activity practice at 7y			
Yes	1 (ref.)	1 (ref.)	1 (ref.)
No	**0.277 (0.185; 0.368)**	**0.155 (0.064; 0.246)**	**0.155 (0.064; 0.246)**

Abbreviations: β̂: beta-regression coefficients; 95%CI: 95% confidence intervals; y: years; ref: reference category
Statistically significant results are shown in bold-type ($p<0.05$)

*Beta-regression coefficients adjusted for child's sex, birth weight, gestational age, maternal age and education.

** Beta-regression coefficients adjusted for child's sex, birth weight, gestational age, maternal age and education, living with siblings and regular physical activity practice at 7 years.

Figure 1 – Discriminatory power of methods to classify obesity by using the Area Under the Curve of ROC curves.



Abbreviations: AUC: Area Under the Curve; ROC: Receiver Operating Characteristics curve; PCA-1: Principal Component Analysis – factor 1; PLS-1: Partial least squares – factor 1, sens: sensitivity; spec: specificity; CI: Confidence interval.

PAPER II

Data-driven dietary patterns at 7 years-old and their association with cardiometabolic health at 10 years-old

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ABSTRACT

Introduction: A poor diet can be a potential contributor for the appearance of a worst cardiometabolic profile. Diet is usually represented as single foods or dietary patterns not related with a specific outcome, and its cardiometabolic effects at early ages is not clearly understood.

Objective: To assess whether dietary patterns, namely an obesity-related dietary pattern, derived at 7 years of age, have an effect on cardiometabolic health at 10 years-old.

Methods: This study uses data from the Generation XXI cohort (n=8647 children, Northern Portugal, 2005-2006). Dietary data were collected by a validated food frequency questionnaire at 7 years-old and dietary patterns were previously derived through partial least squares (PLS) (explains variance of food groups, but mostly BMI z-score), principal component analysis and latent class analysis (both explaining variance of food groups). At 10 years-old, systolic (SBP) and diastolic (DBP) blood pressure were measured, and blood samples were drawn to analyse cardiometabolic parameters [(glucose, triglycerides (TG), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c) and HOMA-insulin resistance], standardized based on age and sex. After excluding twins, follow-up losses and participants without information of interest, 3350 children were studied. Linear regression coefficients and 99% confidence intervals [$\hat{\beta}$ (99%CI)] were computed (covariates: child's sex and birth weight, gestational age, physical activity, maternal age and education).

Results: A dietary pattern that takes into account the explanation of BMI z-score at 10 years-old (PLS-1 - characterized by the intake of processed meat, energy-dense foods and a lower intake of vegetable soup) was the only significantly associated with most cardiometabolic parameters: SBP ($\hat{\beta}$=0.052, 99%CI: 0.022; 0.082), DBP ($\hat{\beta}$=0.043, 99%CI: 0.022; 0.065), TG ($\hat{\beta}$=0.065, 99%CI: 0.026; 0.104), HDL-c ($\hat{\beta}$=-0.059, 99%CI: -0.099; -0.019), LDL-c ($\hat{\beta}$=0.040, 99%CI: 0.001; 0.080) and HOMA-IR ($\hat{\beta}$=0.110, 99%CI: 0.071; 0.149). After further adjustment for BMI at 10 years-old, the magnitude of the associations was weakened. The other methods to derive dietary patterns did not show a consistent significant association with cardiometabolic health at 10 years of age.

Conclusions: The adherence at 7 years of age to a dietary pattern that is related to obesity, and rich in energy-dense foods, processed meat and low in vegetable soup, may increase SBP, DBP, triglycerides, LDL-c and HOMA-IR and reduce HDL-c at 10 years-old. BMI at 10 years-old explained part of these effects.

Key-words: Children; Cohort studies; Dietary patterns; Obesity; Cardiometabolic health

Introduction

Obesity has a strong impact in health and has a particular harmful effect in children and adolescents (1). Childhood obesity tend to persist into adulthood (2) and triggers the appearance of cardiometabolic risk factors: dyslipidaemia, abnormal glucose tolerance and hypertension (3). Furthermore, it is more likely to have a higher risk of cardiovascular disease in adulthood once these cardiometabolic factors persist during childhood (1). A systematic review highlighted that overweight and obese children have a worse cardiometabolic risk profile than children with normal weight (4). Nevertheless, from what age this happens and if it occurs at early ages, remains unclear (4).

From the literature it is known that atherosclerosis develops from childhood (4, 5) and levels track over time, meaning that children with higher levels of LDL-cholesterol are more likely to have higher levels in adulthood (6), but the manifestation of the disease seldom starts in childhood or adolescence (5).

One of the main causes for the appearance of several chronic diseases, such as obesity and cardiovascular diseases is a poor diet (7, 8). Children who eat less fruits and vegetables are more prone to have higher blood pressure levels during childhood (9) and more than a half of children who have higher blood pressure when they are young are more likely to have hypertension in adulthood (1), showing that hypertension also persists over the years.

However, it is important to assess the overall diet effects that are not easy to evaluate due to their complexity (10). Dietary patterns represent a broader approach, closer to a real-world scenario (11), examining diet-disease relationships. There are three major methods to derive dietary patterns*: a priori*, *a posteriori* and hybrid methods. The latter can create uncorrelated dietary patterns that are related to a specific outcome of interest (12, 13). In previous studies, we used *a posteriori* methods (14, 15) and a hybrid method to derive dietary patterns at 7 years-old that were associated with body mass index (BMI) z-score at 10 years-old (14).

Most studies that have previously derived *a posteriori* dietary patterns attempt to establish a relationship between these and adiposity rather than cardiometabolic parameters (1, 16). In addition, they often have a cross sectional design, are conducted in adolescents and do not try to explain an outcome. So, this study aims to assess if dietary patterns followed at 7 years of age, in particular an obesity-related dietary pattern, influence cardiometabolic parameters three years later (at 10 years of age).

Methods

Study design and participants

This study included children from the Generation XXI, an ongoing prospective population-based birth cohort from the Northern Portugal, previously described elsewhere (17). Mothers and their children were recruited between April 2005 and August 2006 at all public maternities of the metropolitan area of Porto. These maternity units were responsible, at enrolment, for 91.6% of the deliveries in the whole catchment population. Mothers were invited to participate 24 to 72 hours after delivery. Of all eligible mothers, 91% agreed to participate (8495 mothers and 8647 children). All cohort participants were invited to participate at 4, 7 and 10 years-old follow-up, whose participation rates were 86%, 80% and 76%, respectively.

The present study sample includes a prospective analysis with data from baseline, and from 7 and 10 years-old follow-up. Data from 3350 children were included in the analysis, after excluded twins (n=246), participants without dietary data (n=1848), without cardiometabolic data (n=1325) and without information on variables of interest (n=113).

We compared our sample characteristics (n=3350) with the remaining non-participating cohort at baseline. In our study, the mothers were slightly more educated (mean=11.1; standard deviation (SD)=4.2 vs. mean=10.1; SD=4.3; $p<0.001$) and older (mean=29.9; SD=5.3, vs. mean=28.4; SD=5.7; $p<0.001$) comparing to the remaining cohort. However, these differences are likely due to the large sample size and not because of the different characteristics of the participants. According to the Cohen effect size (18) that was <0.30 the magnitude of the differences was not high.

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

Data collection

Dietary patterns analyses

Three different methodological approaches to derive dietary patterns have been previously used by our research group: principal component analysis (PCA), partial least squares (PLS) (14) and latent class analysis (LCA) (15).

PCA is an *a posteriori method* that works with one set of variables (predictor variables), and it derives uncorrelated factors, that are linear combinations of the original data (19). However, it does not explain a specific health outcome. Previously, in 7 years-old children form the Generation XXI, two dietary patterns were identified by PCA, that explained 13% of the variance of food groups (PCA-1: 7.0%; PCA-2: 6.0%) and 0.23% of the variance of BMI z-scores (table 1). PCA–1 was positively associated with the consumption of energy-dense foods like sugary drinks, sweets and salty snacks and negatively associated with the consumption of fish, vegetable soup and fruits, whereas PCA–2 was characterized by the intake of yoghurt, cheese, eggs, bread, fish, natural fruit juice, fruit and vegetables, including soup (table 1).

PLS is called a hybrid method because it balances between the variance explanation of predictor variables and the response variables as well (12, 19). The last could be intermediate steps to a specific outcome or the outcome itself. Previously, we identified dietary patterns at 7 years-old that explained BMI z-scores at 10 years-old. Consequently, we used as predictor variables the 38 food groups from a previously validated food frequency questionnaire (FFQ) (20) filled out by the main caregivers, and as response variable the BMI z-score at 10 years-old. Of all derived dietary patterns, the first pattern derived by PLS was the one with a greater explanation of the predictor variables (food groups), as well as the response variable (BMI z-score) ($\hat{\beta}$=0.193, 3.74%) (table 1). In addition, this dietary pattern derived by PLS showed a greater discriminatory power for classifying obesity at 10 years-old by observing the area under the Receiver Operating Characteristics (ROC) curve (AUC=0.63). This dietary pattern was characterized by a higher intake of processed meats and energy-dense foods, namely cakes and soft drinks and a lower intake of vegetable soup. The second pattern derived through PLS had a lower intake of energy-dense foods, sugary drinks and a high intake of fish (table 1).

In this paper, another three dietary patterns derived previously in the Generation XXI cohort by latent class analysis were studied (15). LCA is an exploratory method that identifies mutually exclusively subgroups of individuals (in categories) with similar dietary patterns, being a person-centred approach (21, 22). It only explains the variance of predictor variables. The three dietary patterns derived were called "Energy-dense foods" (EDF), "Snacking" and "Healthier". The "EDF" pattern was characterized by the intake of sweets, soft drinks, salty pastry and processed meat whereas the "Snacking" dietary pattern was composed by foods that typically are not consumed at main meals, like snacks and had lower intake in fish, meat, eggs, rice, pasta, potatoes and vegetables on a plate. The "Healthier" pattern was higher in healthy foods and lower in unhealthier ones.

Cardiometabolic health

Venous blood samples were obtained by trained nurses in our research centre, after an overnight fasting at 10 years-old follow-up. Afterwards, the samples were placed in a centrifuge, and then they were centrifuged at 3500 rpm for 10 minutes and stored at -80ºC in appropriate freezers.

In the laboratory, several methods were used to analyse the various biochemical parameters under study: glucose, triglycerides (TG), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c) and homeostatic model assessment-insulin resistance (HOMA-IR). UV enzymatic assay (hexokinase method) was used to measure the glucose and electrochemiluminescence immunoassay was used to measure the insulin. TG and HDL-c were measured using an enzymatic colorimetric assay and LDL-c was calculated by the Friedewald equation (23). HOMA-IR was calculated as “glucose (mg/dL) x insulin (µU/mL) / 405” formula.

Systolic (SBP) and diastolic blood pressures (DBP) were measured twice using an automatic sphygmomanometer (Medel® ELITE, S.Polo de Torrile, Italy) at the right brachial artery, with at least 5-min intervals. At the end, if there was a difference greater than 5 mmHg between the two measurements, then a third measurement was performed. If this did not happen, the mean value of SBP and DBP measurements were used.

Age- and sex- specific z-scores for all biochemical parameters were calculated, based on the sample’s distribution. For systolic and diastolic blood pressures, in order to avoid misclassification of children who are very tall or short, z-scores were adjusted for age, sex and height, according to the American Academy of Paediatrics (24).

Covariates

Maternal age and education, child’s sex, gestational age and birth weight were variables collected at baseline through face-to-face interviews or retrieved from medical records.

At 7 years-old follow-up, behaviours such as the practice of regular physical activity and dietary intake were asked about by trained researchers. A qualitative FFQ was administrated to the main caregiver(s) of the child, covering the dietary intake of the preceding 6 months. This FFQ collected information on 38 food groups and answers were on a six-point scale, ranging from ‘never’ to ‘more than 4 times a day’. It was previously validated in a similar sample from Generation XXI, by comparing data with 3-day food records (n=3441) and nutrient biomarkers (n=80) (20).

Anthropometrics (weight and height) were measured by trained professionals according to standard procedures (25). Weight was measured in light clothing and without shoes, by using a scale (TANITA®, Arlington Heights, IL, USA) (to the nearest 0.1kg). Height was measured using a stadiometer (SECA®, Hamburg, Germany) (to the nearest 0.1cm). Children were then classified according to the age- and sex-specific BMI reference z-scores developed by the World Health Organization (26).

Statistical analysis

Characteristics of participants are described as means and standard deviation (SD) for continuous variables with normal distribution. Categorical variables are shown as the total number and their corresponding percentage.

Each dietary pattern score (PLS – factors 1 and 2; PCA – factors 1 and 2) was extracted as a continuous variable. The patterns extracted by LCA were a categorical variable: “EDF”, “Snacking” and “Healthier”, the last used as reference category.

To estimate the association of all dietary patterns derived at 7 years-old with each cardiometabolic variable at 10 years-old, linear regression models were run. Crude and adjusted beta-regression coefficients (β) and their 99% confidence intervals (99%CI) are presented and a total of three models were created: 1) a crude model; 2) model 1, adjusted for child’s birth weight, gestational age, maternal age, maternal education and child’s regular practice of physical activity at 7 years-old; 3) model 2, further adjusted for body mass index at 10 years-old.

A sex interaction in the associations was tested, however, as no significant differences were found, all analyses were performed with the two sexes together.

Several independent variables (dietary patterns) and cardiometabolic risk factors (dependent variables) were compared. To correct for multiple comparisons testing, the significance level was set at 1%. Statistical analyses were performed using IBM SPSS® (Statistical Package for Social Sciences), version 24.0™.

Results

Children and their mother’s characteristics are presented in table 2. The study sample includes 52% of boys, children who born with a mean gestational age of 38.7 weeks (SD=1.6), and a mean birth weight of 3.205 kg (SD=0.480). Their mothers had a mean age of 29.7 years (SD=5.3) and 11.1 mean years of education (SD=4.3) at baseline. At 7 years-old, 85.4% of children had a regular practice of physical activity outside school and at 10 years-old, 17.0% were obese. The cardiometabolic profile of these children is also shown in table 2. Values were not standardized as z-scores, to allow observing the absolute values (with more clinical relevance), showing overall a normal range for this age (27).

Table 3 shows the associations of the dietary patterns derived at 7 years-old, through the different methodological approaches, with each cardiometabolic parameter assessed at 10 years-old. Children who followed an obesity-driven dietary pattern at 7 years (PLS-1 - characterized mainly by the intake of processed meat, energy-dense foods and a lower intake of vegetable soup) were more likely to have increased blood pressure, in multivariate analysis (model 1): SBP ($\hat{\beta}$=0.052, 99%CI: 0.022; 0.082), DBP ($\hat{\beta}$=0.043, 99%CI: 0.022; 0.065). They had as well higher lipids levels: TG ($\hat{\beta}$=0.065, 99%CI: 0.026; 0.104), LDL-c ($\hat{\beta}$=0.040, 99%CI: 0.000; 0.080), and HOMA-IR ($\hat{\beta}$=0.110, 99%CI: 0.071; 0.149), and lower HDL-c ($\hat{\beta}$=-0.059, 99%CI: -0.099; -0.019). With further adjustment for BMI z-scores (model 2), the associations were lost or were 50% weaker, i.e. the BMI explained half or more of the effect shown in model 1. The second factor obtained by PLS (PLS-2 - a dietary pattern that explained food groups and not z-scores of BMI, with higher intake of fish), had no significant association with cardiometabolic parameters.

Adherence to PCA-1 (explaining only food groups, particularly energy-dense foods) was positively related to SBP, DBP, TG and HOMA-IR in crude analysis. Nevertheless, after adjustment for sociodemographic and early life characteristics, only SBP ($\hat{\beta}$=0.036, 99%CI: 0.001; 0.072) and HOMA-IR z-scores ($\hat{\beta}$=0.061, 99%CI: 0.014; 0.108) remained significant (model 1). Following the second factor defined by PCA (PCA 2 - explaining food groups only, particularly dairy, fish, fruit and vegetables) does not seem to have an effect in cardiometabolic parameters at 10 years of age.

The association of dietary patterns defined by LCA (creating groups of individuals sharing similar dietary intake, but again not explaining response variables) was also studied. In univariate analyses, comparing to a “Healthier” dietary pattern, a greater adherence to the “Energy-dense food” was positively associated with higher SBP, DBP and HOMA-IR z-scores, and following a “Snaking” dietary pattern at 7 years-old was associated with higher DBP ($\hat{\beta}$=0.086, 99%CI: 0.003; 0.170) at 10 years-old. In multivariate analysis, no significant association was found.

Discussion

A higher adherence to PLS-1 at 7 years-old (rich in processed meat and energy-dense foods), previously described as obesity-related dietary pattern, was significantly associated with all cardiometabolic parameters except for glucose. Part of these effects was explained by BMI z-scores at 10 years-old. Dietary patterns derived by the other methods (PCA and LCA) did not showed so consistent associations with the children’s cardiometabolic profile. However, PCA-1 (rich in energy-dense foods and low in fruit, soup and fish) was also associated with blood pressure and insulin resistance at 10 years of age.

Different methodologies have been used to define dietary patterns. Most of them do not take into account the explanation of variance of the outcome of interest; they only explain the aggregation of food/nutrient choices. In the current study, PLS-1 – the hybrid method able to explain the food items and an outcome variable (BMI z-scores) simultaneously - was the one more consistently associated with cardiometabolic parameters. PCA and LCA methods only aggregate the food choices and cannot explain a specific outcome, such as BMI, so it may be a reason why they did not show so consistent associations with cardiometabolic parameters that have a strong link with obesity. Previous studies have identified BMI as a clinically relevant marker of different cardiometabolic risk factors (28). Thus, identifying dietary patterns that explain BMI variance could be a useful methodological approach to overcome the frequent nil associations found between diet and several diseases.

Both dietary patterns associated with the children's cardiometabolic profile (PLS-1 and PCA-1) were rich in energy-dense foods, such as fast-food (ice cream, sausages, chips, pizza, hamburgers) and soft drinks. These foods are usually richer in salt, fat and refined carbohydrates, and may contribute to the appearance of a worse profile (higher levels of almost all cardiometabolic parameters and reduced HDL-c concentrations). Previously, a review has shown that these types of energy-dense and micronutrient-poor foods are likely to raise, for instance, insulin, glucose, and lipid levels (29).

According to a recent systematic review on the association between *a posteriori* dietary patterns and cardiometabolic risk in children and adolescents (16), most of the studies conducted until now have a cross-sectional design (cannot discard reverse causality bias) and only two of them were cohort studies in adolescents and young adults. Most of the studies were not able to show significant associations with cardiometabolic risk and the authors emphasizes the need of more robust evidence. A previous review has already suggested the lack of prospective evidence in children; most of the studies related dietary patterns with adiposity and not cardiometabolic health (1).

In a cross-sectional study that evaluated the association of dietary patterns derived by factor analysis (similar to LCA, used in the current study) (labelled as healthy, transitive and Western) with cardiometabolic risk factors and obesity found that children (aged 6-13 years) who followed a Western dietary pattern (characterized by the intake of red meat, sugar and refined carbohydrates) had increased levels of blood pressure (SBP and DBP), glucose and LDL-c and lower levels of HDL-c comparing with those who have a healthier pattern (30). Obesity was also more prevalent among children following Western dietary pattern (30), suggesting that body weight can have an important role on these associations.

Similarly to our study, they found an association between a worse diet and a worse cardiometabolic profile. However, we should keep in mind that higher levels do not mean installed disease, as most children in our sample are apparently healthy. Nonetheless, our study suggests that at early ages, as 7 years, following a less healthy dietary pattern could have an effect on metabolic parameters that at long-term could lead to disease.

Another cross-sectional study also described a positive association between the Western pattern, characterized by the consumption of red meat, sweets, fast-food, fried foods, snacks and sugar sweetened beverages) with cardiometabolic risk factors, such as increased triglycerides (31).

In the present study, we tested if part of the effects of dietary patterns on cardiometabolic risk could be mediated by BMI z-scores. BMI showed to be an important mediator of the studied associations, once the overall magnitude of the effect was strongly attenuated. This means that part of the effect of diet is through its effect on body weight. Weight status seems to be important for the cardiometabolic risk profile even in paediatric ages and also reinforces the need to maintain a healthy weight. A systematic review and meta-analysis regarding healthy children aged 5 to 15 years, showed that an abnormal BMI worsens risk parameters for cardiovascular disease; overweight children are in higher risk and obese even more (4).

In the present study, PLS-2 as well as PCA-2 are dietary patterns that were characterized by the intake of vegetables, fruit and fish and a low consumption of fast- food, sweets and sugar-containing beverages. Similarly, the DASH diet promotes the intake of fruits and vegetables, whole grains, low-fat dairy products, fish and the reduction in the consumption of red meat, sweets and sugary beverages (32), in order to low blood pressure. However, as it promotes a healthy diet it also accompanied other cardiometabolic advantages, like a reduction in lipid profile (33). Nevertheless, in our study no significant associations were found, despite a slight decrease.

In addition, no significant associations were found between the dietary patterns defined by LCA and the cardiometabolic risk. However, previously we were able to show that an “Energy-dense food” dietary pattern at 4 years of age (similar to the one defined at 7 years in the present study) increased adiposity at 7 years of age only in girls (15). However, in the current study no association between these dietary patterns and cardiometabolic parameters were found, and no differences were found by sex. At older ages, it has been also suggested different associations by sex. An Australian cohort study derived an energy-dense, high-fat and low-fibre dietary pattern by reduced rank regression (a hybrid method, like PLS). This study showed a moderate tracking of this dietary pattern between 14 and 17 years-old and found that following this DP was associated with an increased insulin and HOMA values and higher fasting glucose only in boys (34).

The current study has some strengths. It has a prospective design, including a large number of participants from a population-based birth cohort. Anthropometrics and blood pressure were measured, which is more reliable than self-reporting, and blood samples were collected by trained professionals under ideal conditions. The cardiometabolic parameters were standardized according to age and sex. For SBP and DBP, the z-scores were based on gender, sex and height, classifying in this way the blood pressure according to the body size, and according to a reference population (24). Potential confounding variables were assessed at different ages. Although there are some studies that have already studied the association between dietary intake and cardiometabolic parameters, the dietary patterns in our study were derived based on the explanation of an outcome, namely the BMI z-score, which is scarce in other studies. In addition, our research group has extensive experience in dietary assessment. Previous analysis defining different dietary patterns allowed us to compare their effects in cardiometabolic health, using validated tools (20) and sounding statistical approaches.

However, this study has also some limitations. A family history of cardiometabolic risk was not considered in analyses, but some children may have some genetic potential. Nevertheless, we expect that at the population level, in a “healthy-apparent” sample, this effect should be minimal. Although we have tested different confounding variables, we cannot discard some potential residual confounding.

Conclusion

This study suggests that in an apparently-healthy population of school-aged children, food choices prospectively influence higher levels of cardiometabolic risk factors. The adherence at 7 years of age to an obesity-driven dietary pattern, rich in energy-dense foods and low in vegetable soup may increase SBP, DBP, triglycerides, LDL-c and insulin-resistance and reduce HDL-c at 10 years-old. These results emphasize how important is to promote healthy eating habits from childhood, in order to improve cardiovascular health later in life.

This study also suggests that dietary patterns that did not take into account the explanation of BMI in their definition were not consistently associated with cardiometabolic risk. Thus, hybrid methods, such as PLS used in this study, that tries to explain both predictor (food groups) as well as response variables (BMI) seem to be a better approach to show the association with cardiometabolic health from a prospective analysis. BMI at 10 years-old showed to explain part of these associations.

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Table 1 - Standardized beta and loadings and explained variance (%) for dietary patterns derived from linear regression, principal component analysis (PCA) and partial least squares (PLS)

Food items	Standardized loadings			
	PCA -1	PCA - 2	PLS - 1	PLS - 2
Chocolate milk				-0.207
Whole milk			-0.298	
Semi-skimmed or skimmed milk		0.253		
Yoghurt (all type)		0.268		
Ice cream	0.312		0.252	-0.197
Cheese		0.319	0.278	
Eggs		0.222		
Meat (chicken, turkey, rabbit, pig, cow)				
Sausage	0.443		0.268	-0.334
Ham, chorizo		0.286	0.303	
Meat salty snacks	0.376			-0.357
Fish (including molluscs and seafood)	-0.289	0.356		0.309
Fish snacks	0.380			-0.333
Pizza, hamburger	0.312			-0.255
Vegetable soup	-0.274	0.328	-0.566	
Boiled vegetables (plate)		0.534		
Raw vegetables (plate)		0.538		
Fruit	-0.239	0.530		
Bread		0.414	0.213	
Breakfast cereals				-0.324
Rice, potatoes, pasta		0.212		
French fries, chips	0.468			-0.435
Crackers		0.320		-0.227
Other cookies and biscuits	0.304			-0.404
Cakes	0.313		0.248	
Chocolate and chocolate snacks	0.211			-0.247
Sugar	0.351			-0.381
Candies	0.464			-0.450
Butter or margarine	0.206	0.265		
Coffee (included in the milk)	0.268		0.269	
Black or green tea				
Infusions		0.200		
Coke	0.426		0.332	-0.303
Carbonated soft drinks	0.400		0.296	-0.263
Ice Tea	0.417			-0.399
Nectar juices	0.289			-0.225
Non-carbonated soft drinks	0.354		0.192	-0.291
Natural fruit juice		0.305		
BMI z-score	0.046	0.011	**0.193**	**0.070**
BMI z-score (CI 95%)	(0.018;0.075)	(-0.018; 0.039)	(0.166; 0.221)	(0.042; 0.098)
% explained variance of food groups	**7.0**	**6.0**	**4.14**	**5.92**
% explained variance of BMI	**0.23**	**0.00**	**3.74**	**0.49**

Abbreviations: PCA: principal component analysis; PLS: partial least squares; BMI: body mass index; CI: confidence interval. Standardized betas (p<0.05) and standardized factor loadings >|0.2| are presented.

Table 2 – Descriptive statistics of the study sample (children and mothers) from the Generation XXI birth cohort.

Children and mother's characteristics (n=3350)	
Child's sex, n (%)	
Boys	1754 (52.4)
Girls	1596 (47.6)
Gestational age, weeks [mean (SD)]	38.7 (1.6)
Child's birth weight, kg [mean (SD)]	3.205 (0.480)
Maternal age at baseline, years [mean (SD)]	29.7 (5.3)
Maternal education at baseline, years [mean (SD)]	11.1 (4.3)
Regular physical activity at 7y, n (%)	
Yes	2862 (85.4)
No	488 (14.6)
Child's z-score BMI at 10y [mean (SD)]	0.72 (1.22)
Child's z-score BMI at 10y, n (%)	
Non - obese	2782 (83.0)
Obese	568 (17.0)
Cardiometabolic parameters at 10y	
SBP (mmHg) [mean (SD)] *	109.4 (9.5)
DBP (mmHg) [mean (SD)] *	69.0 (7.1)
Glucose (mg/dL) [mean (SD)]	87.1 (7.3)
Triglycerides (mg/dL) [mean (SD)]	67.1 (33.0)
HDL-c (mg/dL) [mean (SD)]	55.3 (10.6)
LDL-c (mg/dL) [mean (SD)]	94.0 (23.2)
HOMA-IR [mean (SD)]	2.0 (1.4)

Abbreviations: SD: standard deviation; IQR: interquartile range; kg: kilogram; y: years;
SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglycerides; HDL: high-density lipoprotein-cholesterol; LDL: low density lipoprotein-cholesterol; HOMA-IR: homeostatic model assessment-insulin resistance.

*4558 participants had blood pressure measurements.

Table 3 – Associations of dietary patterns derived at 7 years-old with cardiometabolic profile at 10 years-old in the Generation XXI birth cohort.

	z-score SBP (n=4558)	z-score DBP (n=4558)	z-score glucose (n=3350)	z-score TG (n=3350)	z-score HDL-c (n=3350)	z-score LDL-c (n=3350)	z-score HOMA-IR (n=3350)
PLS-1				ß (99%CI)			
Crude	**0.060 (0.031; 0.090)**	**0.051 (0.030; 0072)**	0.031 (-0.005; 0.059)	**0.069 (0.031; 0.107)**	**-0.073 (-0.112; -0.035)**	0.025 (-0.013; 0.064)	**0.117 (0.079; 0.155)**
Model 1[a]	**0.052 (0.022; 0.082)**	**0.043 (0.022; 0.065)**	0.034 (-0.004; 0.071)	**0.065 (0.026; 0.104)**	**-0.059 (-0.099; -0.019)**	**0.040 (0.001; 0.080)**	**0.110; (0.071; 0.149)**
Model 2[b]	0.011 (-0.018; 0.040)	**0.022 (0.001; 0.043)**	0.022 (-0.016; 0.060)	0.031 (-0.008; 0.069)	-0.028 (-0.067; 0.011)	0.025 (-0.015; 0.065)	**0.047 (0.012; 0.083)**
PLS-2							
Crude	-0.015 (-0.039; 0.008)	-0.005 (-0.022; 0.007)	-0.014 (-0.042; 0.015)	-0.003 (-0.033; 0.027)	-0.009 (-0.039; 0.022)	**0.035 (0.005; 0.065)**	-0.003 (-0.033; 0.027)
Model 1[a]	0.001 (-0.023; 0.025)	0.008 (-0.009; 0.025)	-0.011 (-0.041; 0.019)	0.005 (-0.026; 0.036)	-0.023 (-0.055; 0.008)	0.029 (-0.003; 0.060)	0.013 (-0.018; 0.045)
Model 2[b]	-0.019 (-0.042; 0.004)	-0.002 (-0.019; 0.014)	-0.016 (-0.046; 0.014)	-0.011 (-0.041; 0.020)	-0.009 (-0.040; 0.022)	0.022 (-0.009; 0.054)	-0.015 (-0.043; 0.013)
PCA-1							
Crude	**0.058 (0.024; 0.092)**	**0.038 (0.014; 0.063)**	0.040 (-0.002; 0.082)	**0.046 (0.003; 0.090)**	-0.035 (-0.079; 0.010)	-0.028 (-0.072; 0.017)	**0.081 (0.037; 0.125)**
Model 1[a]	**0.036 (0.001; 0.072)**	0.019 (-0.007; 0.045)	0.041 -(0.004; 0.086)	0.037 (-0.010; 0.083)	-0.008 (-0.055; 0.039)	-0.011 (-0.059; 0.036)	**0.061 (0.014; 0.108)**
Model 2[b]	**0.035 (0.001; 0.069)**	0.018 (-0.007; 0.043)	0.040 (-0.005; 0.085)	0.033 (-0.012; 0.078)	-0.005 (-0.051; 0.041)	-0.013 (-0.060; 0.034)	**0.054 (0.013; 0.096)**
PCA-2							
Crude	-0.018 (-0.052; 0.015)	-0.016 (-0.040; 0.008)	-0.008 (-0.050; 0.034)	0.005 (-0.038; 0.049)	-0.021 (-0.065; 0.023)	0.024 (-0.020; 0.069)	-0.015 (-0.059; 0.029)
Model 1[a]	-0.012 (-0.045; 0.021)	-0.011 (-0.035; 0.013)	-0.008 (-0.050; 0.034)	0.006 (-0.038; 0.050)	-0.026 (-0.071; 0.018)	0.020 (-0.024; 0.065)	-0.009 (-0.053; 0.035)
Model 2[b]	-0.018 (-0.049; 0.014)	-0.014 (-0.037; 0.009)	-0.009 (-0.052; 0.033)	0.002 (-0.041; 0.045)	-0.022 (-0.066; 0.021)	0.019 (-0.026; 0.063)	-0.017 (-0.056; 0.022)
LCA: EDF vs Healthy							
Crude	**0.101 (0.030; 0.172)**	**0.073 (0.022; 0.124)**	0.034 (-0.057; 0.124)	0.068 (-0.025; 0.162)	-0.025 (-0.120; 0.070)	-0.026 (-0.121; 0.069)	**0.108 (0.014; 0.202)**
Model 1[a]	0.065 (-0.009; 0138)	0.042 (-0.011; 0.094)	0.031 (-0.063; 0.125)	0.050 (-0.047; 0.148)	0.023 (-0.076; 0.122)	0.004 (-0.095; 0.102)	0.068 (-0.030; 0.166)
Model 2[b]	0.053 (-0.016; 0.122)	0.036 (-0.015; 0.087)	0.028 (-0.066; 0.122)	0.043 (-0.051; 0.138)	0.029 (-0.067; 0.125)	0.000 (-0.098; 0.099)	0.055 (-0.032; 0.142)
LCA: Snacking vs Healthy							
Crude	0.088 (-0.028; 0.205)	**0.086 (0.003; 0.170)**	-0.012 (-0.160; 0.136)	-0.000 (-0.153; 0.153)	0.008 (-0.147; 0.163)	-0.138 (-0.293; 0.017)	0.095 (-0.059; 0.249)
Model 1[a]	0.046 (-0.070; 0.163)	0.054 (-0.029; 0.138)	-0.021 (-0.171; 0.129)	-0.015 (-0.170; 0.141)	0.047 (-0.110; 0.204)	-0.117 (-0.275; 0.040)	0.051 (-0.105; 0.208)
Model 2[b]	0.074 (-0.037; 0.185)	0.069 (-0.012; 0.151)	-0.009 (-0.159; 0.140)	0.017 (-0.134; 0.168)	0.018 (-0.135; 0.171)	-0.103 (-0.260; 0.054)	0.110 (-0.029; 0.249)

[a] Model 1 is adjusted for child's birth weight, gestational age, maternal age, maternal education and practice of regular physical activity at 7y.

[b] Model 2 is adjusted for child's birth weight, gestational age, maternal age, maternal education, practice of regular physical activity at 7y and body mass index z-score at 10y.

Abbreviations: SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglycerides; HDL: high-density lipoprotein-cholesterol; LDL: low density lipoprotein-cholesterol; HOMA-IR: homeostatic model assessment-insulin resistance; PLS 1 – partial least squares – factor 1; PLS 2 – partial least squares – factor 2; PCA 1– principal component analysis – factor 1; PCA 2 – principal component analysis – factor 2; LCA: latent component analysis; EDF: energy dense foods; CI: confidence interval.

GENERAL DISCUSSION AND CONCLUSIONS

This thesis includes new evidence on deriving and comparing dietary patterns using different methodological approaches in school-age children. In particular, an obesity-related dietary pattern at 7 years-old, with greater discriminatory power for classifying obesity, was prospectively associated with cardiometabolic indicators later in life.

Three different methods to derive dietary patterns were used in this thesis, despite the other possible approaches identified in the literature (8, 10, 15). As our objective was to identify dietary patterns that are obesity-related, PCA was not considered as the best method, since the two dietary patterns derived by this method only explained 0.23% of the BMI z-score. However, for instance, if the goal is related to the clustering of food choices, PCA could be a suitable method, since it explained a greater variance of food groups (13.0%). PLS was the only method that explained concomitantly the BMI z-score at 10 years-old and also explained 10.1% of the food groups. Therefore, all the methods to assess the food intake and to derive dietary patterns have advantages and obviously, associated limitations (8, 15). As a result, it is essential to identify the objectives under study (23), in order to understand which method(s) is the best option to accomplish that.

To derive obesity data-driven dietary patterns, we used as predictor variables the 38 food groups from the FFQ at 7 years-old and as response variable the BMI z-score at 10 years of age. The majority of studies that evaluate dietary patterns associated with obesity used response variables that may be related to the outcome under study (usually nutrients or biomarkers) (14, 37, 42, 45) and for which there is prior knowledge of this association. As a result, the dietary patterns identified by this procedure are strongly related to the nutrients that were used as response variables and because of that, the explained variance is higher. However, in our study, as our response variable was the BMI z-score, and this is directly related to obesity, the dietary patterns were created based on BMI. So, this explain why our obesity-data driven dietary pattern (derived by PLS) only explained about 4% of the BMI z-score, which is a lower percentage compared to other studies that were used nutrients or biomarkers as response variables. Another explanation is because as obesity is a multifactorial disease, it has multiple causes (63). Although diet could contribute to obesity, it is not the only factor.

Our obesity data-driven dietary pattern was characterized by the intake of energy- dense foods, processed meat and a lower intake of vegetable soup. This dietary pattern was prospectively associated with higher levels of systolic and diastolic blood pressure, triglycerides, low-density protein cholesterol (LDL-c) and insulin resistance and lower high-density protein-cholesterol (HDL-c) concentrations. However, most children in our sample are apparently healthy. Nonetheless, our study suggests that at early ages, as 7 years-old, following a less healthy dietary pattern could have an effect on metabolic parameters that at long-term could lead to disease.

We also tested if part of the effects of dietary patterns on cardiometabolic risk could be mediated by BMI z-scores. BMI showed to be an important mediator of the studied associations, once the overall magnitude of the effect was strongly attenuated. This means that part of the effect of diet is through its effect on body weight. Weight status seems to be important for the cardiometabolic risk profile even in paediatric ages and also reinforces the need to maintain a healthy weight.

The other dietary patterns, especially those characterized by a healthier food intake (fruits, vegetables, whole grains, low-fat dairy products, fish and the reduction of red meat, sweets and sugary beverages), namely the PCA-2 and PLS-2, were not considered explanatory of obesity and furthermore did not appear to influence cardiometabolic health at 10 years of age. Likewise, dietary patterns identified by latent class analysis were not associated with cardiometabolic risk factors. This approach takes more into account the aggregation of food choices and does not explain a specific outcome. In this way, it reinforces the idea that deriving dietary patterns based on a specific outcome brings more advantages.

Our results bring some findings with importance in the public health framework. Evaluating dietary patterns is of great importance in nutritional epidemiology, since it evaluates the overall combinations of foods (18), which facilitates a more practical transmission of messages to the public (22). Prevention may be central to avoid the onset of obesity and their associated comorbidities. Nevertheless, a multifactorial approach is needed to change behaviors (63). Many approaches have been designed and performed but many of them are school-based interventions and did not seemed to be very successful (50). It is necessary to act at a younger age and according to broader range of strategies (50). In this study, it was found that early life and sociodemographic characteristics were determinant factors for following an obesity-related dietary pattern (especially the ones related to the mother), highlighting once more that the strategies should be not only centered in the children. These results also reinforce the idea that maternal characteristics and intrauterine development are crucial factors to the development of healthy dietary behaviors.

This work besides providing relevant information about public health messages, brings also methodological support regarding methods to derive dietary patterns related to obesity in children. As the partial least squares method is not widely used in nutritional epidemiology, more precisely in the definition of dietary patterns related to obesity, it may be more used in the future, especially in another populations, since these dietary patterns depend on the population under study.

This work presents some limitations that should be discussed. Regarding the collection of dietary intake, some errors may have occurred, which may be in part related to the weak association between dietary patterns and obesity (they only explained a maximum of 5% of the BMI z-scores variance). Nonetheless, FFQ-data was validated against food records and biochemical indicators (103), increasing the internal validity. Besides that, some children may have a family history of cardiometabolic risk, even though this was not considered in this study. As the dietary patterns that we created were data-driven, the results should be interpreted with caution, because in another samples, the findings may be not exactly the same.

On the other hand, this study was strengthened by the inclusion of a large number of children from a prospective population-based birth cohort. The design of the cohort study allowed us to collect several variables at different ages, which enable the adjustment for potential confounding variables whenever needed. It also introduces an advantage as most studies that derived dietary patterns carried out so far had a cross-sectional design and were conducted during older ages (adolescence). The anthropometric measures, blood samples and blood pressure were obtained by trained professionals, which contributed to inter-interviewer agreement. Moreover, the outcome under study was objectively measured and not self-reported, being more reliable. In our study we derived dietary patterns related to a specific outcome, which is not so common. As the derived dietary patterns are uncorrelated, the food items associated with each one is different, so, allow us to compare their effects in the cardiometabolic health. In order to check if we did not obtain random associations between the dietary pattern derived and the explained BMI z-score, we constructed Receiver Operating Characteristics (ROC) curves. As we obtained values that were higher than 0.5, we can admit that our dietary patterns had discriminatory power to classify obesity at 10 years-old. Cardiometabolic parameters were standardized based on age and sex, and blood pressure was additionally standardized based on height.

In conclusion, from all the methods used to derive dietary patterns, PLS showed better discriminatory power of obesity at 10 years of age. This research suggests that following a dietary pattern that was characterized by the intake of energy-dense foods, processed meat and a low consumption of vegetable soup was considered as explanatory of BMI z-scores distribution three years later. The adherence at 7 years of age to this dietary pattern may increase SBP, DBP, triglycerides, LDL-c and insulin-resistance and reduce HDL-c at 10 years-old. BMI is an important mediator of this effect. Children who were born heavier and from younger and less educated mothers were more prone to follow this obesity-driven dietary pattern.

From a methodological perspective, to derive dietary patterns taking into account the explanation of a specific outcome seems to be a promising approach to overcome the usual difficulty of relating diet with the disease. From a public health prospective, it is important to highlight that, following a dietary pattern that is related with obesity at school-ages can increase levels of cardiometabolic parameters at early ages as 10 years, mainly through the effect of adiposity. The family socio-economic background and the intra-uterine environment are crucial to tackle the establishment of these unhealthy eating behaviours since early life.

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