TITLE: Unhealthy dietary patterns stablished in infancy track to mid-childhood: the EU Childhood Obesity Project.

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Running title: Tracking of dietary patterns from early childhood

- (i) OSM submitted: Supplemental Table 1, 2, 3 and 4, and supplemental Figure 1 are available from the "Online Supporting Material"
- (ii) abbreviations: CORE: Core-foods Dietary Pattern, F&S: Poor Quality Fats and Added Sugars, PROT: High Protein Sources Dietary Pattern; KMO: Kaiser-Meyer-Olkin (test for sample adequacy)
- Funding support: The Childhood Obesity Project was funded by the 5th Framework Program from the European Union [grants number QLRT–2001–00389 and QLK1-CT-2002-30582]. The follow up of the participants was funded by the 6th Framework Program (with contract number FOOD-CT-2005-007036) and also by the 7th Framework Program (FP7-KBBE-2007-1, ref. nº 212652; and FP7-289346-EarlyNutrition) and its Brain Mobility Programme. This manuscript does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript;
- (iv) Conflict of Interest Disclosure: The authors declare no conflict of interest.

ABSTRACT

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- 2 Background: Dietary habits established in infancy could determine long-term health. The aims of this
- 3 work are to describe dietary patterns, the predictors of adherence to specific dietary patterns and their
- 4 tracking from 1 to 8y in a cohort of European children.
- 5 Methods: 3-day food diaries were prospectively collected at ages 1, 2, 3, 4, 5, 6 and 8y. Foods were
- 6 allocated to one of 29 food groups, which were included in exploratory factor analyses at each
- 7 children's age. Tracking of patterns through childhood was assessed by an estimated general equation
- 8 model. Results: At 1y (n=633), two patterns were identified: one labeled as "Core Foods" (CORE), since
- 9 it positively loaded for vegetables, fish, olive oil, white and red meat, and negatively loaded for ready-
- 10 to-eat infant products, sugar and confectionary. The second was positively loaded for saturated
- spreads (e.g. butter), sugar, fruit juices and confectionary, and negatively loaded for olive oil, fish and
- 12 cow's milk; this was named "Poor Quality Fats and Added Sugar" pattern (F&S). From 2 to 8y, three
- patterns were repeatedly identified: CORE, F&S, plus a "High Protein Sources" (PROT) pattern that was
- 14 positively loaded for milk, flavored milks, fish, eggs, white and processed meat, chips and olive oil, and
- 15 negatively loaded fresh fruits at almost all timepoints.
- Of those children in the highest quartiles of the CORE, F&S and PROT patterns at 2y, 45%, 72% and
- 17 36% remained in the highest quartile at 8y, respectively ((OR=2.01 (1.08, 3.8), OR= 3.6 (1.5, 8.4) and
- 18 OR= 0.80 (0.4,1.6) p=0.510)
- 19 Conclusions: Dietary patterns are established between one and two years, and track into mid-
- 20 childhood. A dietary pattern characterized by added sugar, unhealthy fats and poor consumption of
- 21 fish and olive oil was the most stable throughout childhood. Further analyses will reveal whether those
- dietary patterns are associated with metabolic disease risk.
- 24 Key words: tracking dietary patterns, exploratory factor analyses, Childhood Obesity Project, infants,
- 25 toddlers, children, development of dietary habits

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INTRODUCTION

Non-communicable diseases such as cardiovascular disease, diabetes and cancer are main drivers of morbidity and mortality in many countries and the risk of being impacted by these illnesses can in part, be prevented by having a more healthy diet (1–3).

Dietary patterns are likely established in the family environment during infancy and childhood (4). A critical window for dietary pattern development is the period just after complementary feeding commences, when the toddler begins to eat family foods. It is of interest to detect when dietary patterns are established as well as whether those dietary patterns track into to older ages, as they may influence later health. This would open a window of opportunity for educational programs at critical ages that could impact health later in life.

Commonly, single nutrients and foods are analyzed in relation to health and disease. However, foods and nutrients are consumed in combination, and the empirical analyses of dietary patterns support a more comprehensive understanding of dietary intake as a whole. It is a powerful approach to understand diet and its association to health, and to inform food-based recommendations that can be translated to the general population (5). While several studies have described empirically derived dietary patterns in young children (6–8), whether early dietary patterns (in the first 2 years of life) track or predict dietary patterns in later childhood has not been extensively investigated.

The present work aims to describe major dietary patterns prospectively collected at ages 1, 2, 3, 4, 5 and 8y, and their tracking through that period in a cohort of young children from 5 European countries (Germany, Belgium, Italy, Poland and Spain). This work aims as well determining what are the predictors of adherence to specific dietary patterns at ages 2 and 8 years.

METHODS

Study design and population

This is a longitudinal analysis of dietary data prospectively collected in children taking part from 1 to 8 years in the Childhood Obesity Project (EU CHOP), conducted in Germany, Belgium, Italy, Poland and Spain. More details of the project and results have been previously published (10,11). Briefly, the EU CHOP is the follow-up of an originally double blind randomized clinical trial in which infants were recruited (October 2002 - July 2004) from birth to maximum their first 8 weeks of life (median age 14 days (IQR=3-30days)) in neonatal care units of study centers. Inclusion criteria were being born healthy, term, from a singleton pregnancy, with a normal weight for gestational age, and to a mother without health problems nor medication that could influence intrauterine growth. The aim of EU CHOP was to determine whether infant formula with lower protein content during the first year of life reduces later obesity risk. Children were randomly assigned to receive either an infant or a follow-on formula during the first year of life with higher or lower protein contents (maximum and minimum ranges of the recommended protein content at the time that the intervention was conducted). A group of breastfed infants was also recruited for comparison, as a reference of healthy growth and weight gain.

Outcome measures

Dietary Intake Assessment

Three-day estimated and weighed food diaries were completed by the child's parent or caregiver at the age of 1, 2, 3, 4, 5, 6 and 8y. The diaries were reviewed by trained dieticians during the study visits and parents were asked to provide further details when required. Details about standard operating procedures to assess dietary intake within the project have been published elsewhere (9,12).

To support dietary pattern analyses, 7444 individual foods and beverages reported in the food diaries were allocated to 105 groups and then further collapsed to 29 major food groups based on their

nutrient profile and industrial processing. The 29 major food groups (e.g. white meat) and single foods

included in it (i.e. poultry) are reported as Online Supporting Material (Supplemental Table 1). Foods consumed at very low levels were allocated to major food groups to avoid skewed distributions, e.g. whole grain cereals were included in a major group of grains; pulses were included in the vegetables group; and fatty fish was merged with lean fish in a major group. Foods that were rarely consumed and had little to no variance were not included in the dietary pattern analyses (i.e. nuts, processed fish and soft drinks (13). Meats were classified according to fat content: i.e. bovine, pork and lamb were included in the "red meat" food item, while poultry and horse were classified in the "white and low fat meat". Further details about food items included in each of the 29 food groups could be found as Online Supporting Material (Supplemental Table 1).

Covariates

Country (Germany, Belgium, Italy, Poland and Spain), gender (male, female), mother's and father's education level (Low, Medium, High), mother's and/or father's having a different country of origin than the study centre (foreigner, not foreigner), birth order (1, 2, 3, 4, etc.), being single mother (single, not single), mother's and father's BMI (kg/m²), being breastfed vs. formula fed, formula intervention (high protein vs. low protein) and maternal age at child's birth (years) were collected with interviews at baseline. Those covariates were considered as possible predictors of dietary patterns.

Statistics

Dietary Pattern Analysis

Exploratory factor analysis was conducted to extract dietary patterns at 1, 2, 3, 4, 5, 6 and 8y of age. Briefly, this is a statistical method that reduces a large set of predictor variables (intakes of 29 major food groups, g/day), according to correlations between them, into a smaller number of factors (dietary patterns). A factor is the result of successive linear combinations of the predictors. This statistical method is exploratory and extracts dietary patterns that explain as much variation in predictors as

possible, without considering any previous knowledge or hypothesis (5). In the present study, exploratory factor analysis was conducted with a varimax rotation resulting in independent dietary patterns. The factors (dietary patterns) extracted were those that accomplished the following criteria: had a Kaiser Meyer Olkin (KMO) test for sample adequacy ≥0.6, had an eigenvalue >1, and the explained predictors' variation were not below the elbow in the scree plot (19).

Through the exploratory factor analyses, we provide the amount of variation in predictors explained by each pattern and the factor loadings for each food group for each identified pattern. Factor loadings indicate whether intake of the food group is positively or negatively associated with each dietary pattern and the magnitude of association. Those food groups with a factor loading greater than an absolute value of 0.20 were considered the most important food items associated with each pattern. For each dietary pattern, at each timepoint, each study participant received a standardized z-score (mean=0; SD=1) indicating how closely their intake resembled the dietary pattern, relative to the rest of the cohort. The dietary pattern z-score was calculated using a linear combination of the weighted, standardized food intakes (using scoring weights for each food group estimated by the exploratory factor analysis).

Applied dietary pattern scores

Although our exploratory factor analyses identified qualitatively consistent dietary patterns over time, there were some differences in factor loadings between one and other timepoints (Supplemental Table 2). In order to track dietary pattern z-scores for exactly the same patterns over time, we calculated applied dietary pattern z-scores. These were estimated by applying the scoring coefficients for each dietary pattern identified at 2 years of age, to all subsequent dietary intakes i.e. using the 2y patterns as a template for later ages. This means that all children were scored at 2, 3, 4, 5, 6 and 8y of age for the dietary patterns observed at 2 y of age.

Tracking Analysis

The transition from complementary foods to family food is usually completed between one and two years of age, and our factor analyses showed that dietary patterns were most consistent from 2 years of age onwards. For these reasons, we analyzed dietary pattern tracking from 2 to 8 years of age.

Tracking coefficients were estimated for each dietary pattern using generalized estimation equation (GEE) models regressing the pattern z-score at 2 years of age on all subsequent pattern z-scores, as suggested by Twisk (20), with a correlation matrix M-dependent (M=1). The tracking or stability in dietary patterns analyses were performed with the applied dietary patterns z-scores, in order to track exactly the same patterns over time. In brief, this analysis outputs a coefficient that ranges between 0 and 1, which correlates dietary pattern applied scores at all ages for each child. Tracking was considered low if the stability coefficient was <0.4, moderate if coefficient was 0.4 to 0.6, and strong when it was >0.6 (21,22).

Although the tracking coefficient describes the longitudinal linear association between dietary pattern scores at a population level, it describes average tracking. It does not specify for example, whether the highest or the lowest scores for a pattern track more strongly. This is important, since higher scores for a specific pattern would indicate a more or less healthy dietary intake. Therefore at each age, children were classified into quartiles using their applied dietary pattern z-scores. The likelihood of staying in the same extreme quartile at 2 and 8 years of age was calculated. For example, the percentage of children in the highest quartile at 2y, remaining in the same extreme quartile at 8y, for each dietary pattern. Binary logistic regression models were used to calculate the odds of being in the same extreme quartile at 2 and 8 years of age, compared to being in any other quartile, adjusting only for country.

Linear regression models were used to assess the direct and joint effect of those covariates mentioned above on each applied dietary pattern score at 2 and 8y. Effect of individual variables (as for example mother's education level, or birth order) on patterns at 2 and 8y were assessed by separated linear regression models with enter method (only including country adjustment). Variables with a significant effect (p<0.05) on applied dietary patterns scores at 2 or 8y in simple models, were then included in the final multivariate linear regression models for confounding. Therefore, in multivariate linear regression models, all variables with a significant effect on applied dietary patterns scores in simple linear regression models were included. In addition, in the multivariate linear regression models to assess the predictors of dietary patterns scores at 8y, the dietary patterns scores at 2y were included as possible predictors as well.

- Statistical significance was accepted at the level p<0.05
- All the statistical analyses were performed using SPSS 23.0 (IBM Corp., Armonk, NY, USA).

166 Ethics

The study was performed following the principles of the Helsinki Declaration (23). The study was submitted to and approved by local ethical committees. Parents or caregivers received written information and gave signed consent for their child to participate in the study at all different study periods (baseline to 2y, 3 to 6 and 7 to 8).

RESULTS

One-thousand six-hundred seventy-eight healthy term infants were recruited in neonatal services of study centers. One-thousand one-hundred infants were still in the study at 1y of life. From these 1100 infants, 827 reported dietary intake data, and 633 were included in the exploratory factor analysis, as 194 were breastfed and could not be added for analyses at that timepoint (as breastmilk was not

quantified). Flow chart of participants, their country of residence, and sex during the study period are reported in **Supplemental Figure 1**. All food diaries from children who were not unwell at the time of the food recording were included for analyses. At all ages, > 85% of the diaries had 3 days recorded, >7% had 2 days, <4.5% had only 1 day recorded.

The characteristics of study participants at recruitment and main timepoints are reported in **Supplemental Table 3.** The baseline family characteristics of the study population at main study timepoints (1y, 2y and 8y) are reported in **Supplemental Table 4**. In general, children of more educated parents were more likely to stay in the study.

Supplemental Table 2 shows the food factor loadings for each identified dietary pattern and the proportion of variation explained by those patterns at each time point. At 1 year of age, two major dietary patterns were identified. The first pattern was labelled as Core Foods Dietary Pattern (CORE); food groups having the strongest positive loadings were vegetables, potatoes, fish, olive oil, white and red meat, while ready-to-eat infant products, fruit juices, added sugars and confectionary had negative loadings. The second pattern was characterized by high positive loadings for saturated spreads (such as butter), added sugars, and cakes, biscuits and sweet pastries, and by negative loadings for olive oil and slightly for fish. Therefore, the second pattern was labelled as "Poor Quality Fats and Added Sugar" (F&S).

At ages 2, 3, 4, 5 and 8y, three major patterns were consistently identified: two had similarities to the CORE and F&S found at 1 year, and a new third pattern was identified. Although there were differences in factor loadings from one timepoint to another, common characteristics of the pattern labelled as CORE across timepoints were: it was always characterized by positive loadings for vegetables, fruits and olive oil and the majority of timepoints it was characterized by positive intakes of fish. Although some timepoints white and red meat loaded positively on that pattern, processed meats never did so. That CORE pattern was never characterized by positive intakes of added sugars, cakes, confectionary

nor fruit juices, and unless one timepoint, saturated spreads (such us butter) never loaded for that pattern. One difference in the CORE foods pattern from 1 to 2 years was that cow's milk ceased to load for that pattern at 2 years and 8 years and negatively loaded at 3 and 4 years.

Similarly, although loading factors differed between ages, the foods types loading for the pattern were similar across ages for the F&S pattern. This pattern was characterized consistently by the intake of saturated spreads (such us butter), added sugars and other sugar rich products such cakes or confectionary, or fruit juices. However, olive oil and fish were almost always loading negatively for that pattern F&S. Although the F&S pattern at 2y was roughly similar to that at 1y, cow's milk changed from having a positive loading at 1y to a negative loading at 2y and 3years, and ceased to load from 4y onwards.

The third new pattern found only from 2 years onwards was characterized as almost all timepoints by positive loadings for milk, flavored milks, fish, eggs, white meat, processed meat, potatoes and olive oil, and negative loadings for fresh fruits. Only at two and three years, vegetables loaded positively on that pattern. This third pattern was labelled as "High Protein sources dietary pattern" (PROT). At 6y, no factors were extracted since KMO for sample adequacy was <0.6.

The dietary pattern tracking coefficients from 2 to 8y were: 0.50 (95% CI: 0.43, 0.57) for CORE, 0.83 (95% CI: 0.76, 0.89) for F&S, and 0.53 (95% CI: 0.47, 0.59) for PROT. **Figure 1** shows the percentage of children in each dietary pattern quartile (A for CORE, B for F&S and C for PROT) at 2 and 8 years of age.

Of those children in the highest quartile for the F&S pattern at 2y, 72% remained in this quartile at 8y (Figure 1, B), translating to an OR= 3.6 (95% CI: 1.5, 8.4) for remaining in the top quartile, compared to being in any other quartile at 8 y of age. Of those children in the highest quartile for the CORE (Figure 1, A) and PROT (Figure 1, C) patterns at 2y, 45% and 36% remained in the same quartile at 8y,

respectively. This translated to an OR=2.01 (95% CI: 1.08, 3.8) for the CORE pattern, and a non-significant odds to remain in the highest PROT pattern (OR= 0.80 (0.40, 1.57) p=0.510)

For those children in the lowest quartiles for the CORE, F&S and PROT patterns at 2 y of age, 42%, 47.5% and 50% remained in the lowest quartile at age 8y, respectively. The adjusted odds to remain in the lowest quartiles of the dietary patterns from 2 to 8y were not statistically significant neither for CORE (OR= 1.60 (0.73, 3.46) p=0.329), nor F&S (OR=1.11 (0.58, 2.09) p=0.758) nor PROT (OR=1.23 (0.59, 2.56) p=0.573).

Table 1 shows predictors of dietary patterns scores at 2 and 8 years of age. Only country explained the score for CORE at 2y. However, at 8y, CORE scores were positively associated with the CORE score at 2y, country and higher maternal education.

F&S dietary pattern scores at 2y were explained by country and were positively associated with birth order of the child under study. Second born children had significantly higher F&S scores than first born children. F&S scores at 8y were positively associated with F&S score at 2y and country of origin, only.

A high maternal education level was inversely associated with PROT dietary pattern score at 2y. In contrast to the CORE and F&S patterns, the PROT pattern score at 8y was not associated with score for the same pattern at 2y. At 8y of age, the PROT scores were explained by country of origin and were inversely associated with having parents born in a different country than that of the study center and a high maternal education level.

DISCUSSION

In this longitudinal study of European children from five countries, we identified three major dietary patterns which appear to establish between one and two years of age and track into mid-childhood.

Of these, a dietary pattern characterized by added sugar, unhealthy fats and poor consumption of fish and olive oil tracked most strongly in this cohort; children having the highest scores for that pattern at 2y, were 3 times more likely to have the highest scores for that pattern at age 8. Similarly, children with the highest scores for a dietary pattern based on healthy core foods at 2y, were twice as likely to continue having at age 8y, a diet still characterized by unprocessed healthy foods such as vegetables, fruits, olive oil and fruits. Those findings indicate that early interventions to encourage healthy eating habits are imperative.

To our knowledge, this is one of few studies tracking empirical dietary patterns from infancy and across childhood (up to 8y of age). From one year of age, two distinct dietary patterns were prevalent: a "Core Foods Dietary Pattern" and a "Poor Quality Fats and Added Sugar" pattern. From two years, a third dietary pattern appeared beside the first two: a "High Protein Sources" dietary pattern characterized by a high consumption of animal products. This third pattern, was prevalent from 2 to 8 years, however, children with the highest scores for that pattern at 2y, had no increased risk to have the highest scores at age 8y.

Other studies have reported empirical dietary patterns in very young children. At six and twelve months, two dietary patterns ("infant guidelines" and "adult foods") were identified using principal components analysis in the Southampton cohort (24). These patterns are comparable to those identified in the current EU cohort study: the "infant guidelines" pattern was characterized by vegetables, fruits, meat and home cooked meals (similarly to the pattern we labelled as "Core foods"); the "adult foods" pattern was characterized by consumption of bread, snacks, biscuits and processed fruits (similar to our "Poor Quality Fats and Added Sugar" pattern that included a high proportion of discretionary or non-core foods). At 14 months of age, a "Health conscious" and a "Western-like" dietary pattern were identified (7) in 2420 toddlers from the Generation R cohort. These dietary

patterns were also similar to the CORE and F&S patterns observed in our study, however, it is unknown whether those patterns tracked to later ages.

Similarly, in more than 9000 children from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, three dietary patterns were identified at 2y of age using principal components analysis: "family foods" correlated with a traditional British family diet (e.g. meat, fish, puddings, potatoes and vegetables); "sweet and easy" associated with foods high in sugar and foods requiring little preparation (as sweets, crisps and soups); and "health conscious" associated with fruit, vegetables, eggs, nuts and juices (25). Despite some differences, these dietary patterns had similarities with ours: "family foods" similar to CORE and "sweet and easy" to F&S. Beyond three years of age, three other dietary patterns were observed as: "processed", 'traditional' and 'health conscious'. These patterns showed moderate stability (with Pearson correlations varying from 0.35 to 0.46) from 3y onwards (26). The different patterns found at 2y and from 3y onwards could be due to the use of a different dietary assessment tool, or a true change in diet in young childhood, as was observed between 1 and 2 years of age in our study

Between 1 and 2y of age, a transition period typically occurs, during which the infant progresses to family foods and this was reflected in changing dietary patterns in this cohort. Our findings are in general agreement with previously published work, which reports changes in dietary patterns between one and two years of age. This was also shown by an analysis conducted in approximately 500 Australian toddlers from two cohorts, the NOURISH and South Australian Infant Dietary Intake (SAIDI) study, identified two dietary patterns at 14 and 24 months. At 14 months, a "core foods" dietary pattern and a "mixture of core and non-core foods" were observed. However, at 2y, they were able to more clearly distinguish two dietary patterns, named as "core foods" and "non-core foods" (28).

A worth noting finding is that the less healthy pattern, observed from 1y, had the strongest tracking from 2 to 8y. In ~1000 two year old children from the EDEN cohort, two dietary patterns were identified cross-sectionally, and were named as "Processed and fast foods" and "Guidelines" dietary patterns. At 5 years of age, they identified different dietary patterns: a "Processed and fast foods" pattern and a "Protein-rich and diversified" pattern. While these dietary patterns were reported to moderately track from two to five years (8), the "Processed and fast foods" dietary pattern showed the strongest tracking (r= 0.35) which concurs with the F&S pattern tracking most strongly in our study.

There were several factors predisposing to higher or lower pattern scores in this European cohort of young children. The country of origin was a consistent predictor of dietary patterns scoring: children from Spain and Italy had significantly higher CORE scores at 2 and 8y, Poland had the highest scores for F&S pattern at 2 and 8y, and Spain had significantly higher scores for PROT pattern at both ages. In general, mother's education level predisposed to a healthier dietary patterns, characterized by lower scores for the "Protein sources" pattern at 2 and 8y, and higher scores for the "Core foods" pattern at 8y, independently of the country of origin. This finding was consistent with results from the RAINE study, in which greater maternal education level was associated with a healthier dietary pattern (27). Similarly, in the ALSPAC cohort, mother's educational attainment was associated with higher scores of healthier dietary patterns extracted through different methods (4). Similarly, two Danish observational cohorts observed an influence of the family characteristics on infants' dietary patterns at 9 months of age: infants from families with lower social class and higher BMI, already showed lower scores in a "health conscious" dietary pattern (6).

Interestingly, our multivariate analyses showed that having an older sibling predisposes to a higher F&S score at 2y but not at 8y. This may be due to parents introducing discretionary foods that are typically given to older children, to their younger siblings, at an earlier age e.g. cakes, biscuits and

confectionery. However, this association was diminished at 8y of age, suggesting that other factors may have a greater influence on dietary intakes at older ages.

As this was a longitudinal study, a potential limitation of this analysis is the decreasing number of children participating in each follow up over time, which could introduce some bias due to non-random loss to follow up. Such attrition could introduce bias, through for example, a greater proportion of families with a higher education level and greater interest in diet and health remaining in the study at 8y of age, than other families. Our longitudinal analyses included all data available at each time point, rather than restricting the analysis only to those children who completed every follow up. Another potential limitation is that the study sample was not distributed equally in all the countries. As expected, the country of origin was a strong predictor of dietary pattern scores, since every child is scored for all of the patterns, the differences by country do not prevent from being associated with health and disease conditions.

There are several strengths in the present work. Firstly, the dietary data were collected in five different European countries using 3 day food diaries following standardized methodology, which provides finer detail on dietary intakes than food frequency questionnaires. Secondly, we observed quite consistent dietary patterns in our study population from age 1 year upwards, supporting the fact that the dietary patterns were not a product of chance. Furthermore, we performed a confirmatory analyses to ensure we tracked exactly the same dietary patterns from 2 to 8 y. The methods we used for tracking dietary patterns, has some advantages compared to those used in previously published articles: while others used simple correlations between dietary patterns scores at two timepoints, we used generalized estimating equations, to calculate tracking coefficients using data from over 5 timepoints. Finally, our tracking analysis of extreme dietary pattern quartiles provides important additional information for developing targeted interventions.

The importance of the present findings, rely on the fact that early diet has been associated with later obesity and related disorders (29). A recent systematic review concluded that dietary patterns that are high in energy-dense, high-fat and low-fiber foods predispose young people to later overweight and obesity (30). Dietary patterns, which hold a comprehensive way of understanding diet overall (rather than single nutrients or foods), are a powerful way of analyzing diet and its effects on health.

It is worth highlighting as well, that children within the studied age range did not have full autonomy on food choice, as the parents or caregivers usually exert a control. However, the fact that parents influence children's diet in either a beneficial or detrimental way, it remains to be studied whether this influence remains later in life, when subjects will have more decision autonomy.

All of the previous studies, together with findings from our present work, support the need for early educational interventions in the complimentary feeding stage, when children are being introduced to adult foods, to establish healthy eating habits early. It is worth mentioning that educational interventions should not only focus on introduction of core foods such as vegetables and fruits, but should also focus on the avoidance of discretionary, low quality foods at early ages.

In summary, we conclude that dietary patterns may be established during a transition period between one and two years of age, and then track to mid-childhood. Specifically, unhealthy dietary patterns (characterized by added sugars, unhealthy fats and poor consumption of vegetables, fruits, fish and olive oil) track most strongly into midchildhood in this European cohort. These findings highlight the importance of establishing policies of educational programs during this transition period to reinforce healthy dietary habits. Specifically, families with mothers of low educational attainment may need additional support to avoid the introduction of Poor Quality Fats and Added Sugar rich products in

their child's diet. Further analyses in this cohort will reveal whether dietary patterns are associated with later obesity and metabolic disease risk.

ACKNOWLEDGMENTS

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Authors' contributions: VL, RC-M, JE, BK, GLA, WO made substantial contributions to conception and design; VL, VG, RC-M, JE, NF, MZ-J, BK, EV, AR, DG, PS, DR, MM made substantial contributions to acquisition of data; VL, GLA, VG, MW made substantial contributions to analysis and interpretation of data, VL drafted the article, GLA, VG, RC-M, JE, NF, MZ-J, BK, EV, AR, DG, PS, DR, MM, WO and MW revised it critically for important intellectual content. All the authors approved of the final version to be published. CHOP study group: V. Luque, R. Closa-Monasterolo, J. Escribano, N. Ferré, M. Gispert-Llauradó, C. Rubio-Torrents, M. Zaragoza-Jordana (Pediatrics, Nutrition and Development Research Unit, Universitat Rovira i Virgili, IISPV, Reus, Spain); J. Beyer, M. Fritsch, G. Haile, U. Handel, I. Hannibal, B. Koletzko, S. Kreichauf, I. Pawellek, S. Schiess, S. Verwied-Jorky, R. von Kries, M. Weber (Children's University Hospital, University of Munich Medical Center, Munich, Germany); A. Dobrzańska, D. Gruszfeld, R. Janas, A. Wierzbicka, P. Socha, A. Stolarczyk, J. Socha (Children's Memorial Health Institute, Warsaw, Poland); C. Carlier, E. Dain, P. Goyens, J.N. Van Hees, J. Hoyos, J.P. Langhendries, F. Martin, P. Poncelet, A. Xhonneux (ULB, Bruxelles, Belgium, and CHC St. Vincent, Liège-Rocourt, Belgium); E. Perrin (Danone Research Centre for Specialised Nutrition, Schiphol, The Netherlands), and C. Agostoni, M. Giovannini, A. Re Dionigi, E. Riva, S. Scaglioni, F. Vecchi, E. Verducci (University of

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FOOD GROUPS FOR FACTOR ANALYSIS	FOOD ITEMS
Refined and whole grains	Bread and rolls, rice, pasta, noodles, flours, infantile cereals, breakfast cereals
Processed cereal products	Convenience food of pasta
	(filled pasta as tortellini, industrially processed and pizza)
Eggs	Eggs
All fresh fruits	Apple, pear, peach, apricot, plum, nectarine, cherries,
	strawberry, berries, pineapple, avocado, banana, watermelon,
	melon, kiwi, orange, etc.
Processed fruit products	Compotes, fruit concentrated products, convenience food of fruits
Leafy green and salads	Spinach, celery, parsley, lettuce, endives, cabbage, lettuce
Vegetables	Cruciferous (cabbage, cauliflower, broccoli),
Ü	Fruiting vegetables (eggplant, cucumber, pepper, tomato),
	Leguminous vegetables (beans green, peas green),
	Others (artichoke, asparagus, onion,), pulses
Soups and vegetable dishes	Soups, vegetable dishes
Ready-to-eat infant foods	Pureed Infant fruits, vegetables, infant meals
Savory snacks	Chips, crisps, fried potatoes
Potatoes	Potatoes (boiled/ steamed/ smashed), tapioca, starchy plants
Cow's milk & regular yoghurt	Milk and curded milk, soya beverage, milk and cheese dishes
Flavored milk products	Yogurts with added sugars, milk deserts, milk shakes
Processed milk products	Ice cream, cream, puddings
Hard cheese	Hard cheese (hard, semi-hard)
Soft cheese	Soft cheese, cream cheese
Fruit juice	Fruit juices, fruit jelly
Teas	Teas
Olive oil	Olive oil and olives
Saturated spreads	Margarine, butter, vegetable fats
Other oils and oily sauces	Other oils and mayonnaise
Other sauces	Tomato sauce, mustard, soya sauce
Confectionery	Confectionery, sweets, marzipan, nougat, chocolate, chocolate,
	pralines, sweet products
Added sugars	Sugar, honey, sweet spreads, cocoa, jam
Cakes, biscuits, sweet pastries	Tarts, cakes, pastries, biscuits, crackers, crepes
Lean, fatty fish and sea food	Cod, red fish, whiting, flatfish, flounder, plaice, sole, herrings,
	mackerel, tuna fish, salmon, trout, crustaceans, shell fish, etc.
Red meat	Beef, veal, pork, lamb, offal
White and low fat meat	Poultry, Horse, goat, rabbit meat, winged game
Processed meat	Animal products, sausages, ham, bacon, cured meat, meat
	products

Supplemental Table 2. Factor loadings for all dietary patterns, exploratory factor analyses at

children's ages 1 to 3 years.

emaren suges I to s years.	1	year	2 years			3 years		
KMO sampling adequacy	0.7 0.6			0.6				
% VARIANCE EXPLAINED	15.2% 13.1%		13.1%			14.3%		
	PAT CORE	TERNS F&S	CORE	PATTERNS F&S	S PROT	PATTERNS CORE F&S PROT		
% variation for each pattern FOOD GROUPS	9.0%	6.2%	3.8%	5.1%	4.1%	3.5%	6.6%	4.2%
Fresh fruits	0.29	0.16	0.25	0.11	-0.12	0.34	0.01	0.01
Fresh leafy greens & salads	0.24	0.05	0.16	-0.01	-0.04	0.19	-0.02	0.01
Fresh vegetables	0.75	-0.07	0.34	0.14	0.27	0.28	0.18	0.26
Soups	-0.13	-0.03	-0.15	0.07	0.03	-0.05	0.32	0.02
Potatoes	0.76	0.10	0.36	0.28	0.35	0.04	0.27	0.21
Grains (refined & whole meal)	-0.17	0.11	-0.02	0.17	0.03	0.05	0.17	0.02
Processed cereal products	0.00	-0.01	0.02	-0.18	-0.21	0.21	-0.13	-0.34
Cow's milk & regular yoghurt	0.17	0.16	-0.09	-0.38	0.09	-0.25	-0.35	-0.03
Flavored milks	0.16	0.03	-0.06	-0.13	0.52	-0.26	-0.18	0.20
Processed milk products	-0.07	-0.35	0.09	-0.04	-0.21	0.09	-0.03	-0.07
Hard cheese	-0.12	-0.57	0.12	-0.15	-0.22	0.20	-0.12	0.02
Soft cheese	-0.15	0.17	0.06	0.44	-0.05	0.04	0.40	-0.06
Fish	0.51	-0.17	0.27	-0.27	0.34	0.02	-0.33	0.36
Eggs	-0.06	0.07	0.07	0.12	0.21	0.06	0.12	0.38
White meat	0.48	-0.01	0.20	-0.13	0.36	-0.06	-0.13	0.35
Red meat	0.32	0.02	0.30	-0.03	0.03	0.11	-0.02	0.15
Processed meat	0.02	0.08	-0.06	-0.01	0.37	-0.35	-0.20	0.35
Processed fruit ¹	-0.23	-0.19	-0.08	-0.08	0.05			
Ready-to-eat IF ¹	-0.56	0.10	-0.26	0.06	-0.02			
Chips and savory snacks	-0.00	0.01	0.04	-0.09	0.24	-0.01	0.00	0.06
Olive oil & olives	0.30	-0.82	0.62	-0.48	0.16	0.48	-0.52	0.44
Saturated spreads	-0.05	0.46	-0.08	0.55	0.04	-0.07	0.71	0.02
Other oils and oily sauces	0.01	0.17	0.04	0.07	0.11	-0.19	-0.10	0.26
Other sauces (not oil based)	0.00	0.18	-0.10	0.00	-0.03	-0.12	0.17	0.17
Added sugars	0.02	0.24	-0.12	0.31	0.02	-0.20	0.20	0.07
Cakes, biscuits, sweet pastries	0.08	0.31	0.01	0.03	-0.05	-0.06	-0.01	0.02
Confectionary	-0.02	0.11	-0.12	0.17	0.00	-0.11	0.27	-0.05
Fruit juices	-0.24	0.16	0.01	0.28	0.03	-0.07	0.23	0.01
Teas	-0.24	0.16	-0.12	0.27	-0.14	0.07	0.19	-0.11

Table 2'. Factor loadings for dietary patterns extracted from exploratory factor analyses at children's ages 4 to 8 years (*Cont.*).

ages 4 to 8 years (Cont.).		4 yea	rs		5 years			8 years	
KMO sampling adequacy	0.6			0.6			0.6		
% VARIANCE EXPLAINED		13.5% 14.2%				16.1%			
,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,,		PATTER			PATTER	NS	PATTERNS		
FOOD GROUPS	CORE	F&S	PROT	CORE	F&S	PROT	CORE	F&S	PROT
% variation for each pattern	3.1%	6.0%	4.4%	6.2%	3.1%	5.0%	5.3%	5.3%	5.5%
Fresh fruits	0.25	-0.20	-0.20	0.17	0.05	-0.16	0.40	-0.10	-0.18
Fresh leafy greens & salads	0.10	-0.14	-0.04	0.20	-0.07	-0.11	0.15	-0.16	-0.09
Fresh vegetables	0.55	-0.15	0.05	0.33	0.41	0.05	0.57	0.09	-0.05
Soups	-0.01	0.28	0.12	-0.27	-0.01	0.08	-0.14	0.04	-0.13
Potatoes	0.19	0.13	0.28	-0.01	0.29	0.05	0.10	0.44	0.05
Grains (refined & whole meal)	0.17	0.04	-0.00	-0.13	0.10	0.10	0.21	-0.24	-0.06
Processed cereal products	-0.16	-0.12	-0.19	0.18	-0.19	-0.23	-0.03	-0.21	-0.11
Cow's milk & regular yoghurt	-0.17	-0.11	0.31	0.05	-0.07	0.35	0.10	0.01	0.38
Flavored milks	-0.10	0.06	0.47	-0.11	0.05	0.40	-0.11	0.10	0.35
Processed milk products	-0.00	-0.04	-0.12	-0.00	0.02	-0.14	-0.22	0.01	-0.04
Hard cheese	-0.06	-0.13	-0.10	0.26	-0.07	-0.02	0.08	-0.09	0.08
Soft cheese	0.31	0.24	-0.12	-0.16	0.42	-0.27	0.09	0.30	-0.31
Fish	0.07	-0.33	0.33	0.29	0.03	0.33	0.26	-0.18	0.26
Eggs	0.12	0.05	0.25	0.01	0.24	0.24	0.27	0.31	0.05
White meat	0.04	-0.15	0.27	0.19	0.07	0.36	0.17	0.03	0.32
Red meat	0.12	-0.22	0.05	0.16	-0.02	0.01	0.00	0.09	0.22
Processed meat	-0.09	-0.03	0.38	-0.14	0.13	0.43	-0.04	0.03	0.25
Processed fruit ¹									
Ready-to-eat IF ¹									
Chips and savory snacks	-0.04	-0.01	-0.19	0.13	-0.03	-0.12	0.01	-0.12	0.24
Olive oil & olives	0.17	-0.71	0.26	0.78	0.09	0.38	0.59	-0.24	0.57
Saturated spreads	0.26	0.59	-0.09	-0.46	0.43	-0.27	-0.02	0.69	-0.37
Other oils and oily sauces	-0.12	0.06	0.35	-0.06	-0.02	0.25	-0.15	0.01	0.42
Other sauces (not oil based)	-0.14	0.07	0.01	-0.29	-0.17	-0.03	-0.31	0.01	-0.04
Added sugars	0.04	0.33	0.06	-0.18	0.13	-0.03	-0.12	0.45	0.05
Cakes, biscuits, sweet pastries	-0.17	0.03	-0.04	-0.04	-0.09	-0.13	-0.24	-0.19	0.01
Confectionary	0.00	0.29	0.04	-0.23	0.13	-0.10	-0.14	-0.00	-0.01
Fruit juices	-0.14	0.25	-0.02	-0.29	-0.03	-0.06	-0.19	0.28	-0.12
Teas	-0.03	0.11	-0.17	-0.05	-0.01	-0.19	-0.11	0.01	-0.20

KMO: Kaiser-Meyer-Olkin Test for suitability of the study sample for factor analysis; CORE: Core Foods Dietary Pattern, F&S: Poor Quality Fats and Added Sugar Dietary Pattern, PROT: High Protein Sources Dietary Pattern. Bold letters for all food factor loadings ≥0.2.¹Processed fruits and ready-to-eat infant products were consumed only in early infancy, as they were commercial products for infants.

Supplemental Table 3. Participants' characteristics at birth and ages 1, 2 and 8 years, for the study sample available (still in study and with dietary data reported) at 1, 2 and 8 years. Values are means and ±SDs.

	1 year	2 years	8 years
Birth characteristics	n = 633	n = 702	n = 392
Gestational age (weeks)	39.8 [±1.2]	39.8 [±1.2]	39.9 [±1.2]
Birth weight (kg)	3.3 [±0.3]	3.3 [±0.3]	3.3 [±0.3]
Birth length (cm)	50.7 [±2.6]	50.7 [±2.6]	50.6 [±2.6]
Head circumference at birth (cm)	34.2 [±1.3]	34.4 [±1.3]	34.2 [±1.4]
Anthropometry in childhood	n = 624	n = 728	n = 397
Weight (kg)	9.9 [±1.1]	12.4 [±1.4]	28.6 [±6.1]
Length (cm)	75.7 [±2.6]	88.0 [±3.2]	
Height (m)			1.30 [±0.05]
Head circumference (cm)	46.2 [±1.3]	48.6 [±1.5]	52.2 [±1.4]
Body Mass Index (kg/m²)	17.2 [±1.5]	16.2 [±1.3]	16.9 [±2.6]
Body Mass Index (z-score)	1.51 [±0.91]	0.34 [±0.93]	0.60 [±1.19]
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Supplemental Table 4. Baseline family characteristics of the study sample available (still in study

and with dietary data reported) at ages 1, 2 and 8 years.

	1 year	2 years	8 years
	n = 633	n = 747	n = 399
Mother's education level, n	631	745	398
Low, n (%)	172 (27.3)	150 (20.1)	60 (15.1)
Medium, n (%)	338 (53.6)	379 (50.9)	200 (50.3)
High, n (%)	121 (19.2)	216 (29)	138 (34.7)‡, α
Father's education level, n	621	739	398
Low, n (%)	192 (30.9)	175 (23.4)	82 (20.6)
Medium, n (%)	332 (53.5)	373 (50.5)	198 (49.7)
High, n (%)	97 (15.6)	191 (25.8)	118 (29.6)
Both parents foreigner, n (%)	20 (3.2)	20 (2.7)	4 (1.0)*, α
Single mother, n (%)	36 (5.7)	28 (3.8)	12 (3.0)
Birth order,	633	747	399
First child, n (%)	129 (66.5)	441 (59.0)	229 (57.4)
Second child, n (%)	55 (28.4)	237 (31.7)	138 (34.6)
More than second child, n (%)	10 (5.2)	69 (9.2)	32 (8.0)
Mother's BMI (kg/m²)	23.9 [±4.4]	23.4 [±4.2]	23.5 [±4.1]
Father's BMI (kg/m²)	26.0 [±3.7]	26.1 [±3.7]	26.4 [±3.5]

No significant differences in Chi^2 test for distributions at 24 months irrespective to 12 months. ‡ p-value< 0.001, † p-value= 0.006, *p-value= 0.011 compared to 1 year, α p-value= 0.042 compared to 2 years. ANOVA and posthoc bonferroni comparisons for mother's and father's BMI showed no statistical differences between timepoints. BMI = Body Mass Index, values are means ± SDs.

Table 1. Predictors of Dietary Pattern scores at children's ages 2 and 8 years

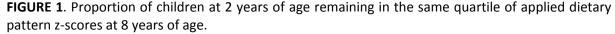
Pred	ictors of Dietar	y Pattern	s scores at age 2	years		
	Core Food	ds patteri	n score at 2y			
		В	95% CI	p-value	R ²	Model
						p-value
Country ¹	Germany	-0.93	(-1.10, -0.75)	<0.001	23.5%	p<0.001
	Belgium	-0.70	(-0.89, -0.50)	<0.001		
	Italy	0.09	(-0.06, 0.23)	0.23		
	Poland	-0.31	(-0.48, -0.15)	<0.001	7	
Poor C	Quality Fats and	Added S	ugar pattern sco	re at 2y		
		В	95% CI	p-value	R ²	Model
				•		p-value
Mother's education level ²	Medium	0.01	(-0.010, 0.12)	0.83	62.3%	p<0.001
	High	-0.07	(-0.21, 0.07)	0.32		
Father's education level	Medium	-0.07	(-0.18, 0.04)	0.20		
	High	-0.13	(-0.27, 0.01)	0.07		
Birth order (compared to first	child)					
Second child	in the family	0.09	(0.01, 0.12)	0.04		
Third (or >) child	in the family	0.10	(-0.05, 0.25)	0.19		
Country	Germany	0.94	(0.81, 1.08)	<0.001		
	Belgium	0.97	(0.81, 1.19)	<0.001		
	Italy	0.04	(-0.07, 0.15)	0.47		
	Poland	1.69	(1.57, 1.82)	<0.001		
•	Protein sour	ces patte	ern score at 2y			
_		В	95% CI	p-value	R ²	Model
						p-value
Mother's education level	Medium	-0.07	(-0.18, 0.04)	0.20	49.0%	p<0.001
	High	-0.16	(-0.29, -0.03)	0.014		
Country	Germany	-1.19	(-1.33, -1.05)	<0.001		
	Belgium	-0.96	(-1.12, -0.80)	<0.001		
	Italy	-1.40	(-1.52, -1.29)	<0.001		
	Poland	-0.78	(-0.91, -0.64)	<0.001		

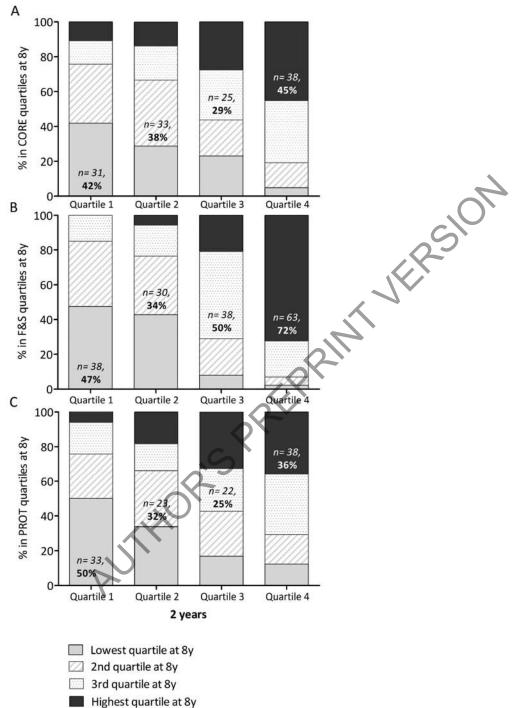
Table 1'. Predictors of Dietary Pattern scores at children's ages 2 and 8 years (*Cont.*).

Predictors of Dietary Patterns scores at age 8 years									
Core Foods pattern score at 8 years									
		В	95% CI	p-	R^2	Model			
				value		p-value			
Core foods pattern score at 2y	/	0.23	(0.10, 0.36)	0.001	37.6%	p<0.001			
Mother's education level	Medium	0.17	(-0.11, 0.45)	0.23					
	High	0.37	(0.07, 0.67)	0.014					
Country	Germany	-1.36	(-1.68, -1.03)	<0.001	7				
	Belgium	-1.39	(-1.80, 0.99)	<0.001					
	Italy	0.10	(-0.17, 0.37)	0.46					
	Poland	-0.61	(-0.87, -0.34)	<0.001					
Poor Qua	lity Fats and A	dded Sug	ar pattern score	at 8 years					
		В	95% CI	p-	R ²	Model			
		C		value		p-value			
Poor Quality Fats and Added S	Gugar	0.26	(0.11, 0.41)	0.001	65.8%	p<0.001			
pattern score at 2y		7							
Both parents foreign ³	6,	-0.29	(-1.23, 0.65)	0.54					
Mother's BMI [kg/m²]	3,5	-0.02	(-0.04, 0.03)	0.10					
Country	Germany	1.29	(1.00, 1.57)	<0.001					
	Belgium	1.18	(0.85, 1.51)	<0.001					
	Italy	0.24	(0.02, 0.45)	0.030					
80	Poland	1.70	(1.38, 2.02)	<0.001					
	Protein sourc	es patteri	n score at 8 years						
		В	95% CI	p-	R ²	Model			
				value		p-value			
Mother's education level	Medium	-0.17	(-0.40, 0.05)	0.14	39.2%	p<0.001			
	High	-0.26	(-0.51, -0.02)	0.031					
Both parents foreign ³		-1.53	(-2.36, -0.71)	<0.001					
Country	Germany	-1.17	(-1.42, -0.93)	<0.001					
	Belgium	-0.74	(-1.06, -0.43)	<0.001					
	Italy	-1.12	(-1.34, -0.91)	<0.001					
	Poland	-0.13	(-0.34, 0.09)	0.24					

Table 1. Each multivariate model separated by a line. Tested predictors in multivariate models were country, gender, mother's and father's education level (*low* for no completed studies and elementary, *medium* for professional studies and intermediate cycles, *high* for university degrees, master and doctorate), mother's and father's BMI, being single mother, mother's age at child's birth, birth order, ³both parents foreign = both parents having a different country of origin than that of the study center, being breastfed vs. formula fed and formula intervention. As variables with effect on patterns at 8y, were also considered dietary patterns scores at 2 years. Effect of all variables were first tested in simple models adjusted by country; only those with a significant effect (p<0.05) in simple models were introduced in the multivariate models. ¹All countries compared to Spain as reference; ²compared to low level of education.

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CORE: Core Foods Dietary Pattern, F&S: Poor Quality Fats and Added Sugar Dietary Pattern, PROT: High Protein Sources Dietary Pattern. Figure shows that 72% of the children in the highest quartile of F&S pattern at 2y remained in this extreme quartile at 8y (OR= 3.6 (95% CI: 1.5, 8.4) for remaining in the top quartile, compared to being in any other quartile at 8 y of age); 45% of the children in the highest quartile for the CORE pattern at 2y remained at the same highest quartile at 8y (OR=2.01 (95% CI: 1.08, 3.8)); 36% of the children in the highest PROT pattern quartile at 2y remained in the same quartile at 8y, with a non-significant odds to remain in the highest PROT pattern. The adjusted odds to remain in the lowest quartiles of the dietary patterns from 2 to 8y were not statistically significant.