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Original article

Dietary patterns acquired in early life are associated with cardiometabolic markers at school age

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SUMMARY

Background & aims: it has previously been described that dietary patterns established early in life tracked to late childhood. The aim of the present work was to analyse the association of dietary patterns that tracked from 2 to 8y with cardiometabolic markers at 8y of age.
Methods: The 3 identified patterns at 2y (that previous analyses showed to track to age 8y) were: "$Core_{DP}$", loaded for vegetables, fruits, fish, olive oil, etc.; "$F\&S_{DP}$", loaded by poor-quality fats and sugars; and "$Protein_{DP}$", mainly loaded by animal protein sources. Cardiometabolic markers at 8y were systolic blood pressure (SBP), insulin resistance (HOMA-IR), and triglycerides, and BMI z-score. To examine whether the association of diet with the outcomes was the result of a direct effect of diet at either two or 8y, or synergy between them, we used structural equation models.
Results: the associations between the patterns and the health outcomes were: $Core_{DP}$ was inversely associated with SBP and HOMA-IR; $Protein_{DP}$ was directly associated with HOMA-IR and SBP; and adherence to $F\&S_{DP}$ was directly associated with triglycerides and SBP. The associations between the patterns and the health outcomes were independent of BMI and were the result of a direct effect of diet at 2y, an indirect effect of diet at 2y through diet at 8y or a combination between both pathways.
Conclusion: dietary patterns acquired in early life, persisting to later childhood, were associated with cardiometabolic markers at school age independently of BMI.

1. Introduction

The risk of obesity and associated non-communicable diseases such as diabetes, cardiovascular disorders, and cancer is modifiable in part by having healthy dietary habits [1]. Dietary patterns associated with increased risk such as high sodium intake and low intake of fruits and wholemeal grains were linked to 11 million deaths in 2017 [2]. Dietary habits are established in early life when infants and young children are exposed to tastes and textures and develop new eating habits. Dietary habits established in early childhood tend to track to later ages and may modulate eating habits up to adulthood and older ages [3–6]. Evidence has accumulated that diet in early life, during critical windows of developmental plasticity, has a long-term impact on later health and disease risks [7].

Several methods have been used to analyse dietary habits concerning health. Analysing the intakes of single foods or food groups provides valuable information. However, the analyses of dietary patterns often allow for a more comprehensive analysis of dietary intake and its association with health [8]. Whereas a Western

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dietary pattern, consisting of low intakes of vegetables, fruits, fish, whole grains, and high intakes of red and processed meats, sugary drinks, refined grains, and fried potatoes, for instance, has been associated with increased cardiovascular disease risk markers in adolescents [9] and adults [10]. Although there is compelling evidence on the association between diet and non-communicable diseases in adulthood, little is known about the developmental origins of cardiometabolic health in childhood. In the EU Childhood Obesity study population, we identified three dietary patterns at two years that tracked to age 8y [5]; one was characterized by poor-quality fats and sugars, the second was mainly characterized by animal protein sources, and the third one was loaded by core foods more characteristic of a Mediterranean type diet (such as vegetables, fruits, fish, olive oil, etc.) [5].

The aim of the present work was to analyse the association between those dietary patterns (that were identified to track from 2 to 8y) with cardiometabolic markers at 8y of age. We hypothesized that children with lower adherence to a dietary pattern containing fruits and vegetables, and higher adherence to patterns mainly characterized by poor-quality fats and sugars, and foods from animal origin, would exhibit changes in cardiometabolic markers from early ages.

2. Material & methods

2.1. Study design and population

The CHildhood Obesity Project (EU CHOP) trial was conducted in Germany, Belgium, Italy, Poland, and Spain [11,12], with a double-blind randomized dietary intervention trial during the first year of life. The trial aimed to explore whether feeding an infant formula with reduced protein content during the first year of life could lower the body mass index (BMI) at 2 years of age and reduce later obesity risk. Children were randomly assigned to receive either an infant or a follow-on formula up to age one year with higher or lower protein contents, and a group of breastfed infants was also observed as a non-randomized reference group. Overall, 1670 infants were recruited at birth. Children were followed with multiple visits until school age. Here we analyse data of children ($n = 399$) with nutritional data at 2 and 8 years of age with health outcome measures taken at the 8-year follow-up visit (see the Flow Diagram as supplementary online material).

2.2. Health outcome measures

The main health outcomes were anthropometry, blood pressure, and blood sample parameters as surrogate markers of cardiometabolic health, detailed below.

2.3. Anthropometry

Weight was measured in underwear on a SECA 702/703 digital scale (precision ± 10g). Height was measured with a digital stadiometer SECA 242 (precision ±1 mm). Standardized procedures for a child's height measurement were in place in all sites [11,12]. Weight (Kg) and height (cm) were measured in duplicate and the mean was used for further analyses. Body mass index (BMI) was calculated as BMI [kg/m^2] = weight [kg]/height^2 (m). Weight, height, and BMI z-scores were calculated using the WHO references [13].

2.4. Blood pressure

Systolic (SBP) and diastolic blood (DBP) pressure [mmHg] was measured using a Digital tensiometer Dinamap ProCare 100/200 applying standardized procedure. Blood pressure was measured after at least 15 min from arrival to the centre, after at least 5 min resting, in duplicate on the left arm supported by horizontal support, slightly elevated (i.e. close to heart level). Both measurements were separated by a slot time of 5 min, and the mean was used for statistical analyses. Systolic and diastolic blood pressure were standardized as percentiles by body height using the references from the American Academy of Pediatrics from 2017 [14].

2.5. Blood sample analyses

Fasted venous blood samples were taken to measure total cholesterol (total-CHOL), Low Density Lipoprotein cholesterol (LDL-c) [mg/dL], High density lipoprotein cholesterol (HDL-c) [mg/dL], triglycerides [mg/dL] and glucose [mg/dl] at the clinical chemistry laboratories of the study centres with routine methods used for clinical diagnostics [15]. Total-CHOL, HDL-c, triglycerides, and glucose were analysed by either enzymatic or indirect potentiometry methods; LDL-c was calculated by the Friedewald equation [16]. Insulin [μIU/ml] was quantified in one central lab in one batch using an immunoradiometric assay (DiaSource, Nivelles, Belgium) [17] after sample storage at −80 °C. The homeostasis model assessment for insulin resistance index (HOMA-IR) was calculated as a proxy for insulin resistance [18,19].

2.6. Cardiometabolic risk score

Children were classified according to their cardiometabolic risk parameters at age 8y. Abdominal obesity was defined as Waist-to-height ratio (WHtR) ≥0.55; according to references in European children from the IDEFICS study [20,21], elevated HOMA-IR was classified as ≥90th percentile, low HDL cholesterol (≤10th percentile), high triglycerides ≥90th percentile, and high systolic and/or diastolic blood pressure ≥90th percentile according to the American Academy of Pediatrics from 2017. As this was a population of mainly healthy children, subjects were classified as metabolically unhealthy if had 2 or more altered parameters.

2.7. Predictors of health outcome measures

Predictors were dietary patterns adjusted by sociodemographic and biological confounders, detailed below.

2.8. Assessment of dietary patterns

Three-day estimated and weighed food diaries were completed by the child's parent or caregiver at 2y and 8y. Details on the standardized procedures we applied for assessing dietary intakes have been previously published [22,23]. Foods were converted into nutrients by using the BLS II food composition table [24] in dedicated software and local food composition databases [25–28]. To perform dietary pattern analyses, 7444 individual foods and beverages reported in the food diaries were allocated into 105 groups and then further collapsed into 27 major food groups, based on their nutrient profile and their degree of processing. Further details on the food groups, the exploratory factor analyses, and the extracted dietary patterns in the CHOP study were previously published [5]. Briefly, identified dietary patterns at 2y were: a “Core Foods Pattern” ($Core_{DP}$) which was characterized by higher intakes of fruit, vegetables, potatoes, fish, white and red meat, and olive oil. A “Poor-Quality Fats and Sugars dietary pattern” ($F\&S_{DP}$) was positively associated with intakes of potatoes, soft cheese, saturated spreads, fruit juices, and teas and negatively associated with intakes of fish and olive oil. And finally, a “Protein Sources Dietary Pattern” ($Protein_{DP}$) that was correlated with intakes of vegetables, potatoes, white meat, red meat, processed fish, eggs, chips and snacks, and

flavoured milk. Fig. 1 shows factor loadings, which reflect associations between food groups and dietary patterns at 2y. Study participants received a z-score indicating adherence to each dietary pattern based on reported food intake [5]. To consider tracking of dietary patterns from 2 to 8 years, we applied the scoring coefficients for each dietary pattern identified at 2y of age, to dietary intakes at 8y (as it would be external predefined dietary patterns). Thus, adherence to the $Core_{DP}$, $F\&S_{DP}$, and $Protein_{DP}$ at age 8y was fully comparable to the child's diet at age 2y. This approach allowed comparing the effect of the adherence to a given dietary pattern at 2 and 8 years on health outcomes at the endpoint 8y. The main predictors of dietary patterns at 2y were parents' education level and country of origin and, for the $F\&S_{DP}$ having an older sibling [5]. The main predictors of dietary patterns at 8y were parents' education level and adherence to the same pattern at 2y.

2.9. Covariates

Covariates used in linear regression models as possible confounders for the effect of dietary patterns on the outcome measures were country of origin (Germany, Belgium, Italy, Poland, and Spain), maternal smoking during pregnancy at any time (yes vs. no), feeding during the first year of life (low protein vs. high protein formula or breastfeeding for at least 3 months), maternal education (high, medium or low), mean energy intake (kcal/day) at 8y and for outcome measures other than BMI, BMI was included as a covariate (i.e. on systolic blood pressure, HOMA-IR).

2.10. Statistics

Descriptive results are shown as means and standard deviations (SD). The frequency of categorical variables such as gender or cardiometabolic risk categories is shown as n (%). The association of dietary pattern z-scores with nutrient intakes and cardiometabolic markers (biochemical parameters and blood pressure) is shown using Pearson correlation coefficients.

To analyse the relationships between dietary patterns and the main outcome measures at 8y (BMI z-score, systolic and diastolic blood pressure and base 10 logarithmic transformed HOMA-IR), we used linear regression models including the three dietary patterns scores at 8y or dietary patterns scores at 2y and 8y, so they were mutually adjusted. These two types of linear regression models considered all possible confounders (country of origin, maternal smoking during pregnancy, feeding during the first year of life, maternal education, energy intake at 8y and BMI). All the linear regression models were built introducing as entering method in a first step the main predictors (dietary patterns) which were included together in the model mutually adjusting, as adherence to a single pattern does not describe the full diet of a subject; afterward, to avoid redundancy of variables the countries of origin and all possible covariates were introduced in a second step as step forward method. Thereby, dietary patterns effects and other covariates with a significant effect on the main outcome measure were retained in the model.

To explore whether associations of diets with the health outcome parameters (parameters that showed a significant association in linear regression analyses) were the result of a direct effect of diet either at 2y or 8 years or synergy between them (since diet at 2y influenced diet at later ages), we performed mediation analyses (through structural equation models). These path models were constructed having the three dietary patterns at 2y (with analyses of covariance between them, so they were mutually adjusted in the model) as predictors of the health outcome at 8y directly or indirectly through the score for the same dietary pattern at age 8y. Error terms were added to the health outcome and intermediate variables.

To quantify whether dietary patterns were associated with an unhealthy cardiometabolic phenotype, we performed a binary logistic regression analysis on variables with an effect on high blood pressure, high HOMA-IR and unhealthy cardiometabolic score ≥ 2. The model included repeated measures of dietary patterns at 2 and 8y, country, gender, feeding type during the first year of life, child's BMI at 8y, maternal education and maternal smoking during pregnancy.

Statistical significance was accepted at the level $p < 0.05$. The statistical analyses were performed using SPSS 27.0 and the Amos SPSS extension for version 27.0 (IBM Corp., Armonk, NY, USA).

2.11. Ethics

The study was performed following the principles of the Helsinki Declaration [29]. The study documents were submitted to and reviewed by the local ethical committees. Parents or caregivers received written information and gave signed consent for their

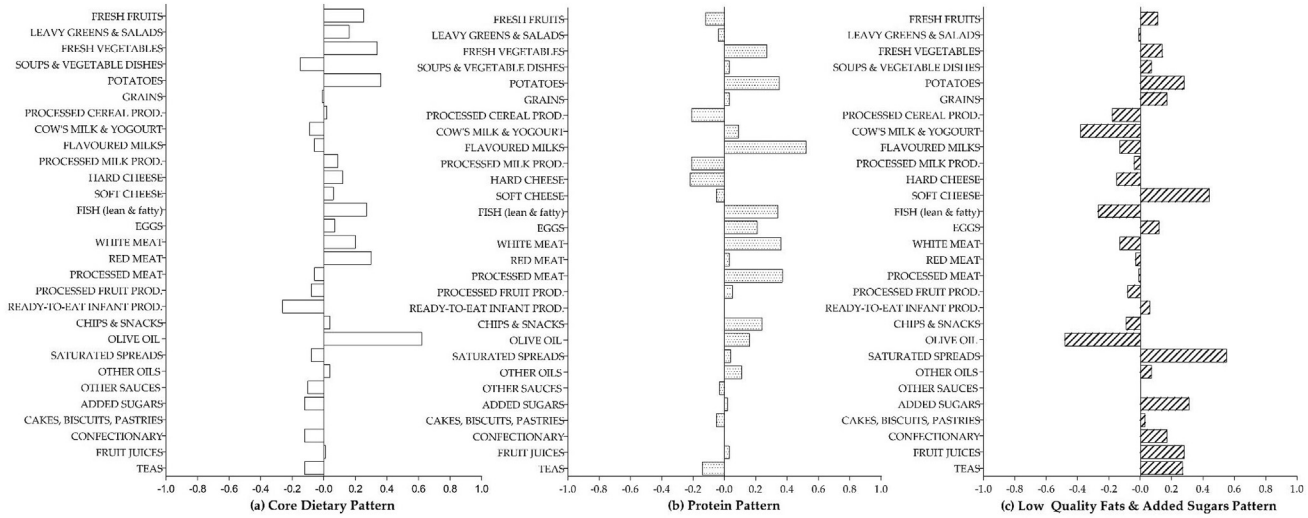

Fig. 1. Loading factors for dietary patterns at ages 2y (adapted from Luque et al. [5]) **(a)** Loading factors for the Core Foods Dietary Pattern (**b**) Loading Factors for the Protein Pattern **(c)** Loading Factors for the Poor-Quality Fats and Sugars Pattern.

child to participate in the study at recruitment and successive follow-up.

3. Results

At age 2y (final endpoint from the original clinical trial), 747 children completed the anthropometrical and dietary evaluation. At 8y, 653 children attended the visit, from whom 399 dietary intake was reported. This subset of 399 children had similar baseline characteristics (sex proportion, feeding type in early infancy, birth weight, rate of maternal smoking during pregnancy, maternal BMI before pregnancy) than the overall sample of children recruited at birth. A flow chart figure of participation in blood sampling and food recording at each time point is shown as supplementary online material. The sample at 8y differed from the overall sample in maternal education level (25.3% had a low education level and 24.5% a high education level in the overall sample, vs. 15.0% and 34.7% in the 8y sample). From these, 386 children had a full set of data including physical examination, report of dietary intake through 3 days food diaries at 2 and 8y and blood pressure measured at age 8y. Among these, 274 underwent a blood sample analysis in fasted conditions. The description of anthropometrical and cardiometabolic markers (biochemical analyses and SBP and DBP at age 8y) are shown in Table 1.

Table 1
Characteristics of the study sample.

	n	(%)
Sample size, n=399		
Boys/girls	192/207	(48.1/51.9)
Country		
Germany	60	(15)
Belgium	30	(7.5)
Italy	75	(18.8)
Poland	83	(20.8)
Spain	151	(37.8)
Infant feeding (1st year of life)		
Low protein formula	136	(34.1)
High protein formula	126	(31.6)
Maternal feeding (≥3months)	137	(34.3)
Mother's education level		
Low	60	(15.1)
Medium	200	(50.3)
High	138	(34.7)
	Mean	**(SD)**
Anthropometry, n=399		
Weight (kg) at 2y	12.46	(1.43)
Height (cm) at 2y	88.1	(3.12)
Body mass index (z-score) at 2y	0.27	(0.96)
Weight (kg) at 8y	28.64	(6.11)
Height (cm) at 8y	129.6	(5.7)
Body mass index (z-score) at 8y	0.46	(1.21)
Blood sample analysis at 8y, n= 274		
Total cholesterol (mg/dl)	167	(28)
HDL cholesterol (mg/dl)	59	(15)
LDL cholesterol (mg/dl)	96	(25)
Triglycerides (mg/dl)	59	(26)
Glucose (mg/dl)	83	(8)
Insulin (uIU/ml)	8.9	(1.8)
HOMA-IR	1.84	(0.75)
Blood Pressure at 8y, n=386		
Systolic Blood Pressure (mmHg)	100	(10)
Diastolic Blood Pressure (mmHg)	57	(7)
Systolic Blood Pressure (percentile)	57.9	(27.6)
Diastolic Blood Pressure (percentile)	44.5	(21.8)

3.1. Cardiometabolic risk assessment

Children were classified according to their cardiometabolic risk parameters at age 8y. Abdominal obesity (WHtR) was found in 5.3% of the cases (n = 31), HOMA-IR was ≥90th percentile in 45.8% cases (n = 183), low HDL cholesterol (≤10th percentile) was found in 7.8% of the cases (n = 33), high triglycerides in 2.6% of the subjects (n = 11) and high systolic and/or diastolic blood pressure according to the AAP references in 16.4% of the sample (n = 91).

3.2. Association between nutrient intakes and dietary patterns scores

Table 2 shows the simple (non-adjusted) Pearson correlations of patterns scores with energy and macronutrient intakes at 2y and 8y.

Roughly, the $Core_{DP}$ scores were directly associated with daily intakes of energy and it was the only dietary pattern directly associated with fibre intake at 8y. At 2y, the $F\&S_{DP}$ was not associated with energy intake, was inversely associated with proteins and fats, and positively with carbohydrates, fibre, and sugars. At age 8y, the associations of the $F\&S_{DP}$ scores with nutrients changed: it was mainly associated with energy and sugars and inversely to dietary fibre.

It is worth pointing out that the $F\&S_{DP}$ scores did not correlate with the amount of dietary fat intake although the dietary fat quality was poorer. The $Protein_{DP}$ scores were associated with energy intake, both at 2y and 8y. At 2y, $Protein_{DP}$ scores were associated with energy-adjusted proteins, and at 8y, $Protein_{DP}$ scores were directly associated with proteins and fats and inversely associated with sugar and fibre adjusted for energy.

3.3. Association and mediation analyses of the dietary patterns from 2 to 8y and cardiometabolic markers at age 8y

Surprisingly, a higher $Core_{DP}$ score at 8y was associated with a higher BMI. The effect was rather small and the overall linear regression model (including a protective effect of breastfeeding ($B = -0.310$, $p = 0.038$)), poorly explained the overall variance in BMI (Table 3).

The Structural Equation Models (Fig. 2) showed that adherence to each pattern at 2y was associated to scores for that patterns at 8y ($E = 0.40$, $p < 0.001$) for the $Core_{DP}$; $E = 0.40$, $p < 0{,}001$ for the $Protein_{DP}$ and $E = 0.68$, $<p0.001$ for $F\&S_{DP}$.

The linear regression model on serum triglycerides at 8y showed a significant direct association with the $F\&S_{DP}$ at 2y (Table 3). The mediation model depicted the same direct association of the $F\&S_{DP}$ score at 2y with serum triglycerides at age 8y ($p < 0.001$) (Fig. 2). There was no effect of dietary patterns on LDL-c nor total cholesterol, and models were not significant (r2 <1% and p-value = 0.727 and 0.954, respectively).

Interestingly, the $Core_{DP}$ scores at 8y were associated with a reduction of HOMA-IR in cross-sectional and longitudinal linear regression models (that were in turn adjusted by BMI) explaining the 34.2% and 36.5% of the HOMA-IR variability, respectively (Table 3). The mediation model on HOMA-IR revealed that the $Core_{DP}$ score at 2y had a direct significant effect and the $Core_{DP}$ score at 8y a non-significant trend to reduce HOMA-IR at age 8y (Fig. 2); furthermore, there was a direct and indirect significant association of the $Protein_{DP}$ at 2y on increasing HOMA-IR.

The linear regression models showed that a higher $Protein_{DP}$ score at 8y was associated with increased SBP and DBP at the same age (Table 3). The longitudinal linear regression models showed that higher scores for the $Protein_{DP}$ both at 2 and 8y were associated with significantly increased SBP at 8y (Table 3).

The Structural Equation Model showed that the association between the $Protein_{DP}$ at 2y with SBP at age 8y was indirect, through the score for the $Protein_{DP}$ at 8y. Similarly, higher $F\&S_{DP}$

Table 2
Pearson correlation coefficients between dietary patterns scores and nutrients intake.

	Core foods dietary pattern scores	Animal Protein Sources dietary pattern scores	Poor-Quality Fats and Sugars dietary pattern scores
2y			
Energy intake (kcal/day)	0.337‡	0.430‡	0.093‡
Protein intake (g/day)	0.424‡	0.468‡	−0.264‡
Protein intake (g/kcal)	0.268‡	0.368‡	−0.461‡
Fat intake (g/day)	0.346‡	0.370‡	−0.081‡
Fat intake (g/kcal)	0.158‡	0.071‡	−0.241‡
Carbohydrate intake (g/day)	0.174‡	0.240‡	0.287‡
Carbohydrate intake (g/kcal)	−0.209‡	−0.221‡	0.343‡
Dietary Fibre (g/day)	0.213†	0.205‡	0.233‡
Dietary Fibre (g/kcal)	0.047‡	−0.001‡	0.210‡
Sugars (g/day)	0.004	0.294‡	0.358‡
Sugars (g/kcal)	−0.267‡	0.043†	0.352‡
8y			
Energy intake (kcal/day)	0.339‡	0.297‡	−0.010
Protein intake (g/day)	0.509‡	0.509‡	−0.307‡
Protein intake (g/kcal)	0.324‡	0.382‡	−0.378†
Fat intake (g/day)	0.410†	0.368‡	−0.174‡
Fat intake (g/kcal)	0.283‡	0.263‡	−0.254‡
Carbohydrate intake (g/day)	0.069†	0.003†	0.186‡
Carbohydrate intake (g/kcal)	−0.317‡	−0.375‡	0.279‡
Dietary Fibre (g/day)	0.233‡	−0.06*	0.012‡
Dietary Fibre (g/kcal)	0.032	−0.260‡	0.023
Sugars (g/day)	−0.087†	0.175‡	0.312‡
Sugars (g/kcal)	−0.309‡	0.027	0.365‡

*p < 0.05, †p < 0.01, ‡p < 0.001.

Table 3
Linear regression models on the effect of dietary patterns during childhood on health outcomes at 8y.

	BMI (z-score)		HOMA-IR (log 10)		Triglycerides (log 10)		Systolic Blood Pressure (percentile)		Diastolic Blood Pressure (percentile)	
	B (95% CI)	P value	B (95% CI)	P value	B (95% CI)	P value	B (95% CI)	P value	B (95% CI)	P value
Cross-sectional models: effect of diet at 8y on health outcomes at the same age										
$Core_{DP}$ at 8y	0.19 (0.06, 0.32)	0.004	−0.02 (−0.04, −0.00)	0.022	−0.01 (−0.03, 0.01)	0.276	−1.87 (−4.61, 0.88)	0.183	−0.621 (−3.02, 1.78)	0.611
$Protein_{DP}$ at 8y	0.06 (−0.08, 0.21)	0.405	0.01 (−0.01, 0.04)	0.291	−0.02 (−0.04, 0.01)	0.062	7.04 (3.95, 10.13)	<0.001	4.14 (1.58, 6.70)	0.002
$F\&S_{DP}$ at 8y	−0.01 (−0.13, 0.11)	0.828	−0.01 (−0.02, 0.01)	0.477	−0.01 (−0.03, 0.04)	0.638	−0.88 (−4.36, 2.59)	0.618	−1.18 (−4.19, 1.83)	0.442
	R^2 = 6.9%[a]		R^2 = 34.2%		R^2 = 17.2%[b]		R^2 = 27.7%[c]		R^2 = 21.0%[d]	
Longitudinal models: effect of diet at 2 and 8y on health outcomes at the same age										
$Core_{DP}$ at 2y	−0.01 (−0.19, 0.17)	0.896	−0.01 (−0.04, 0.02)	0.687	−0.02 (−0.01, 0.04)	0.294	−3.21 (−6.94, 0.51)	0.091	−0.80 (−4.00, 2.41)	0.626
$Protein_{DP}$ at 2y	−0.07 (−0.25, 0.12)	0.483	−0.02 (−0.01, 0.06)	0.181	−0.01 (−0.04,-0.02)	0.459	3.98 (0.23, 7.73)	0.038	3.02 (−0.20, 6.23)	0.066
$F\&S_{DP}$ at 2y	0.17 (−0.05, 0.38)	0.128	−0.01 (−0.04, 0.03)	0.669	0.05 (0.01, 0.08)	0.006	6.92 (2.39, 11.46)	0.003	2.75 (−1.14, 6.64)	0.165
$Core_{DP}$ at 8y	0.21 (0.06, 0.35)	0.006	−0.02 (−0.05, −0.00)	0.043	−0.01 (−0.03, 0.01)	0.379	−1.04 (−4.13, 2.05)	0.509	−1.74 (−4.37, 0.90)	0.209
$Protein_{DP}$ at 8y	0.13 (−0.04, 0.30)	0.131	0.01 (−0.02, 0.04)	0.412	−0.02 (−0.05, 0.01)	0.130	5.81 (2.25, 9.38)	0.001	2.18 (−0.87, 5.23)	0.160
$F\&S_{DP}$ at 8y	−0.10 (−0.27, 0.07)	0.230	−0.00 (−0.03, 0.03)	0.916	−0.06 (−0.10, 0.04)	0.186	0.73 (−4.30, 2.83)	0.686	0.79 (−2.27, 3.85)	0.613
	R^2 = 7.9%[e]		R^2 = 36.5%[f]		R^2 = 17.0%[g]		R^2 = 30.9%[h]		R^2 = 17.2%	

BMI: Body mass index, HOMA-IR: Homeostasis Insulin Resistance Index (base 10 logarithm). All models adjusted by country, energy intake at 8y, sex, diet during the first year of life, maternal education level, maternal smoking during pregnancy, and BMI z-score (except when it was the outcome measure). $Core_{DP}$: Core foods dietary pattern; $Protein_{DP}$: Animal protein sources pattern; $F\&S_{DP}$: Poor-quality fats and added sugars pattern; The R^2 for the goodness of fit is provided for each model. Confounders with effect on the outcome variable.

[a] Breastfed ≥4 months (B = −0.31, p = 0.038), smoking during pregnancy (B = 0.37, p = 0.007).
[b] Mother's education level (medium B = −0.08, p = 0.003, high b = −0.06, p = 0.030, vs. low).
[c] Child's BMI z-score (B = 7.96, p < 0.001), smoking during pregnancy (B = 9.48, p < 0.001).
[d] Breastfed ≥4 months (B = −5.66, p = 0.009);[e] smoking during pregnancy (B = 0.43, p = 0.002).
[f] BMI z-score (B = 0.07, p < 0.001).
[g] Mother's education level (medium B = −0.06, p = 0.005 vs. low).
[h] BMI z-score (B = 7.80, p < 0.001), smoking during pregnancy (B = 8.01, p = 0.005), higher protein formula during the first year (B = −6.59, p = 0.020).

scores at 2y were associated with significantly increased SBP at 8y (Table 3); and $F\&S_{DP}$ scores at 2y had a direct significant association with SBP at 8y (E = 0.26, p < 0.001), but not an indirect association through the pattern at 8y (Fig. 2). The Core pattern at 2y had a direct significant inverse association with SBP (E = −0.11, p = 0.034) but not an indirect association through the same pattern at 8y. Structural Equation Models on BMI and DBP did not reveal any significant effect (models not presented).

$F\&S_{DP}$ scores at 2y were associated with increased odds of 2.1 to have a high systolic or diastolic blood pressure at age 8 years (Table 4), and $Protein_{DP}$ scores showed a trend to increased odds (1.68, p = 0.056) of elevated HOMA-IR at age 8 years.

Negelkerke R2 for Blood Pressure = 20.3%; for HOMA-IR = 33.8%; for Cardiometabolic Risk scOre = 23.7%.Models adjusted by country, gender, maternal education, feeding during the first year of life, child's BMI at 8y.

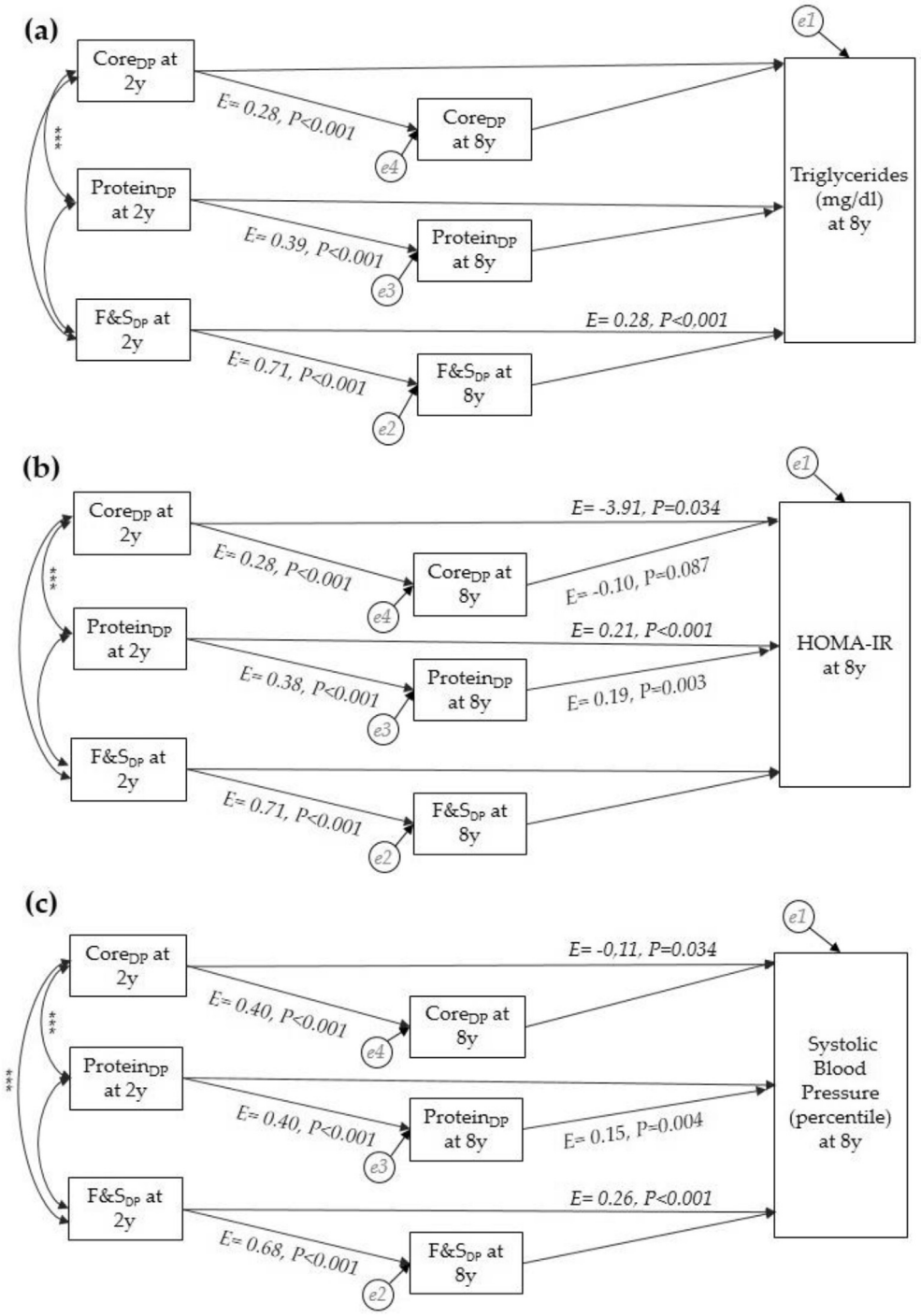

Fig. 2. Structural Equation Models to predict direct and indirect effects of dietary patterns at 2 and 8y on (a) Triglycerides, (b) HOMA-IR as a proxy for insulin resistance, and (c) Systolic Blood Pressure. Core$_{DP}$: Core foods dietary pattern; Protein$_{DP}$: Animal protein sources pattern; F&S$_{DP}$: Poor-quality fats and added sugars pattern; ***covariances between dietary patterns at 2y were significant at the $p < 0.001$ level. (a) The F&S$_{DP}$ at 2y was directly associated with triglycerides at 8y. (b) The Core$_{DP}$ had a direct effect on HOMA-IR and a non-significant indirect trend to reduce it; besides, the Protein$_{DP}$ at 2y had a direct and an indirect effect on HOMA-IR at 8y. (c) The Core$_{DP}$ and the F&S$_{DP}$ had direct effects on systolic blood pressure at 8y, and Protein$_{DP}$ at 2y had an indirect effect on systolic blood pressure at 8y.

4. Discussion

This study shows that dietary patterns identified from 2y of life and tracking to 8y are associated with cardiometabolic health markers in children as young as 8y.

The results revealed that children adhering to a dietary pattern mainly based on animal sources with poor intake of fruits and vegetables from 2y to 8y of age had a higher systolic and diastolic blood pressure and HOMA-IR. However, a unique dietary pattern did not define the whole diet of the subjects. Similarly to the

$Protein_{DP}$, scoring higher for a dietary pattern characterized by fats of poor-quality and added sugars at 2y, was associated as well with increased systolic blood pressure and triglycerides at age 8y. Furthermore, scoring high at 2y for a dietary pattern characterized by the consumption of vegetables, fruits, olive oil, fish, and white meat (the $Core_{DP}$) was associated with lower systolic blood pressure and a trend to lower HOMA-IR at age 8y. Dietary patterns were not associated to LDL cholesterol and showed weak effects on DBP.

Focusing on the effect of animal protein intake on blood pressure in children, several studies have attempted to elucidate a possible association. A systematic review published in 2015 found inconclusive evidence from studies reporting a direct association, an inverse association, or no association between dietary protein and blood pressure in children [30]. The authors discussed the possibility that inconsistencies between studies could result from different types of protein (i.e. vegetable vs. animal) having different health effects, and that the full diet (but not only proteins) needs to be taken into account. A strength of our study is that our analyses did not only consider protein intake but rather an overall dietary pattern. Our results suggest the idea that a diet rich in animal protein (accompanied by animal fats) and poor in plant protein may increase blood pressure in children. We cannot confirm that animal protein itself causally increased blood pressure in children, as the associations found are the results of a combination of several dietary factors. Maternal smoking during pregnancy was associated with increases, and being fed with a higher protein formula during the first year was associated with reductions, of systolic blood pressure in children, consistently with previous results and hypotheses [31–35].

One further key finding of our study is that although the dietary patterns representing a poorer diet quality (independently of overall energy intake) may start to exert negative effects on cardiometabolic health (such as blood pressure, HOMA-IR, and triglycerides) at a very young age, those effects were not induced by an increase in BMI.

In children, no consistent associations between dietary patterns and BMI or overweight have been reported at young ages. A review of the evidence on the association between childhood dietary patterns and later obesity risk, found less consistent results in young children than in adolescents, leading to the conclusion that dietary patterns that were high in energy-dense, high-fat and low-foods predisposed young people to later overweight and obesity [36]. A possible reason for this difference between children and adolescents could be that other behavioural factors and physical activity may play a more important role in weight gain at such young ages. The fact that there was no significant effect of the dietary patterns on BMI in our study sample, does not exclude a possible increased obesity risk later in life. Furthermore, in these young children, while adherence to the $Core_{DP}$ was associated with higher energy intake, adherence to the $F\&S_{DP}$ was not, supporting that the associations found between the dietary patterns and cardiometabolic health are related to the quality of the diet, but not to the overall energy intake, and are not mediated by increased BMI.

It is worth highlighting that cardiometabolic health markers at 8y were associated with diet at 2y and 8y, but dietary patterns at 8y were in turn the result (among others) of dietary patterns learned until 2y [5]. It is unlikely that dietary patterns at a single moment (e.g. 8y) may affect cardiometabolic health at the same timepoint. To assume such association as valid, we should consider that dietary patterns at 8y may represent the usual diet from a longer period than actually could exert effects on health. The path models have served to unravel both direct and indirect effects of the diet at 2y on the health outcomes at 8y. These results suggest a possible synergistic effect of early dietary pattern development on subsequent cardiometabolic markers. This means, that early diet could have two synergistic pathways to act on later health: by metabolic programming of diet during the first 1000 days of life and by the acquisition of unhealthy dietary patterns that would continue affecting cardiometabolic health later in life (Fig. 3). Considering that blood pressure in childhood trend to track to adulthood [37], the adequate development of dietary habits during the first 1000 days of life would be a powerful target of prevention of non-communicable diseases, especially if dietary patterns track to adult ages.

A strength and a weakness of the present study is the fact that it has been conducted in a population of mainly healthy children, where the frequency of unhealthy metabolic phenotype was low. Even in this case, we were able to demonstrate an association between unhealthy dietary patterns and cardiometabolic health consequences.

The strengths of this work are the longitudinal methodology in a multicentre study including five European countries with a long follow-up from toddler to school age where dietary patterns tracking from infancy to later childhood has been previously identified [5]. Possible limitations are the sample size of subjects who had a complete set of data at 8y and the observational nature of the study, which does not allow firm conclusions on causality. Another possible limitation is that dietary patterns were extracted from 3 days food records. Although this is considered the most precise method to quantify young children's intakes, it would be desirable to have a record from a longer period (e.g. 7 days). However, overwhelming tasks to parents were foreseen as a risk of withdrawal from the study. The high frequency for HOMA-IR might be related to low references from the IDEFICS study. Although one could realize that these references may overestimate insulin resistance in children, we used this reference for consistency with the other parameters (categorized according to the IDEFICS criteria) and because the usually accepted value of >3.16 [38] may be too high in pre-pubescent children. We were not able to show causality to a pathological condition in a relatively small cohort study, in young children from the general population, but only associations with increased markers of cardiometabolic phenotype.

Table 4
Binary logistic regression analysis on dietary patterns predicting a cardiometabolic risk score ≥2 parameters.

	Systolic or Diastolic Blood Pressure ≥90th percentile		HOMA-IR ≥90th percentile		Cardiometabolic risk score ≥2	
Dietary patterns	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
$Core_{DP}$ at 2y	0.73 (0.45, 1.17)	0.186	0.69 (0.40, 1.19)	0.183	0.89 (0.48, 1.65)	0.712
$Protein_{DP}$ at 2y	1.23 (0.76, 1.98)	0.401	1.59 (0.92, 2.78)	0.100	1.82 (0.90, 3.69)	0.095
$F\&S_{DP}$ at 2y	2.1 (1.2, 3.6)	0.008	0.75 (0.38, 1.48)	0.407	1.37 (0.74, 2.54)	0.309
$Core_{DP}$ at 8y	0.80 (0.53, 1.21)	0.294	0.83 (0.53, 1.28)	0.395	0.88 (0.52, 1.47)	0.616
$Protein_{DP}$ at 8y	0.96 (0.64, 1.52)	0.948	1.68 (0.99, 2.84)	0.056	1.03 (0.59, 1.82)	0.909
$F\&S_{DP}$ at 8y	0.86 (0.55, 1.32)	0.856	1.22 (0.74, 2.01)	0.429	0.880 (0.51, 1.54)	0.653

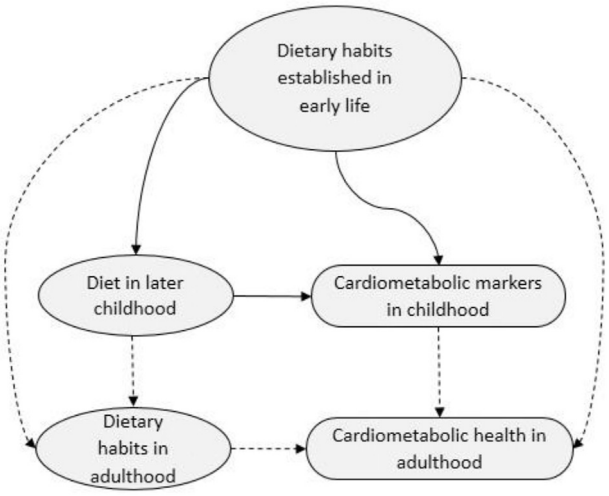

Fig. 3. The synergistic effect of early diet hypothesis. Early acquisition of unhealthy dietary patterns could act on adult health through different pathways: a "metabolic programming pathway" and the fact that dietary patterns acquisition could track to later ages, which could cause a synergistic deleterious effect on cardiometabolic health later on. This figure shows in solid lines, the pathways demonstrated by this work and a previous one [5], and the dotted lines indicate possible pathways of affecting adult health (not been demonstrated yet).

In conclusion, dietary patterns acquired in early life and persisting to later childhood were associated with markers of cardiometabolic health at school age, in a population of mainly healthy children. These results reinforce that unhealthy diets should be avoided from the very beginning of life independently of the children's BMI. Appropriate development of dietary habits from the first stages of life should be a target for the prevention of non-communicable diseases in adulthood.

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Authors' contributions

Conceptualization, V.L.; data curation, V.G. M.Z., M.T.; formal analysis, V.L.; methodology, V.L., G.L.A.; investigation, V.L., R.C., V.G., M.Z., N.F., B.K., E.V., D.G., A.X., J.E.; resources, R.C., J.E., B.K.; writing—original draft preparation, V.L.; writing—review & editing all authors; supervision, J.E., R.C., G.A., V.G., B.K. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. Supplementary data

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