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Original article

Metabolic profiles of ultra-processed food consumption and their role in obesity risk in British children

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SUMMARY

Background & aims: Higher consumption of ultra-processed foods (UPF) has been associated with childhood obesity, but underlying mechanisms remain unclear. We investigated plasma nuclear magnetic resonance metabolic profiles of higher UPF consumption and their role in obesity risk in the British ALSPAC cohort.

Methods: We performed cross-sectional and prospective metabolome wide association analyses of UPF, calculated from food diaries using the NOVA classification. In cross-sectional analysis, we tested the association between UPF consumption and metabolic profile at 7 years (N = 4528), and in the prospective analysis we tested the association between UPF consumption at 13 years and metabolic profile at 17 years (N = 3086). Effects of UPF-associated metabolites at 7 years on subsequent fat mass accumulation were assessed using growth curve models.

Results: At 7 years, UPF was associated with 115 metabolic traits including lower levels of branched-chain and aromatic amino acids and higher levels of citrate, glutamine, and monounsaturated fatty acids, which were also associated with greater fat mass accumulation. Reported intake of nutrients mediated associations with most metabolites, except for citrate.

Conclusions: UPF consumption among British children is associated with perturbation of multiple metabolic traits, many of which contribute to child obesity risk.

1. Introduction

The availability and consumption of ultra-processed foods (UPFs) has grown rapidly in Europe and globally since the 1970s [1,2]. Recent studies indicate that over 60% of total calories consumed among children in the United Kingdom and in the United States are from UPFs [3–5] and their consumption is now increasing most rapidly in low- and middle-income countries [6]. UPFs undergo substantial industrial processing, contain highly-processed/purified ingredients and additives [7] and often have higher energy density, higher content in free sugars and salt, saturated and trans-fats, and lower fibre and micronutrient content, compared to minimally processed foods and freshly prepared

Abbreviations: UPF, Ultra-Processed Food; ALSPAC, Avon Longitudinal Study Of Parents And Children; NMR, Nuclear Magnetic Resonance Spectroscopy; BMI, Body Mass Index; NSSEC, National Statistics Socio-Economic Classification; MWAS, Metabolome-Wide Association Study; ENT, Effective Number Of Tests; eGFR, Estimate Of Glomerular Filtration Rate; MUFA, Monounsaturated Fatty Acid; SFA, Saturated Fatty Acid; PLS, Partial Least Squares; AAA, Aromatic Amino Acids; BCAA, Branched-Chain Amino Acids; PUFA, Polyunsaturated Fatty Acids; DHA, Docosahexaenoic Acid; LDL, Low-Density Lipoproteins; HDL, High-Density Lipoproteins; IDL, Intermediate Density Lipoproteins; VLDL, Very Low-Density Lipoproteins; PM, Proportion Mediated; T2D, Type 2 Diabetes; CVD, Cardiovascular Diseases.

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meals [8]. The worldwide shift toward a dramatic increase in the consumption of UPFs appears partly responsible for the global obesity epidemic [9] and may contribute to an increased risk of cardiometabolic diseases.

Studies in adults have reported associations between higher UPF consumption and elevated risks of weight gain [10], obesity [10,11], type 2 diabetes (T2D) [12,13], cardiovascular diseases (CVD) [14], cancer [15] and mortality [16]. In children, higher UPF consumption has been associated with greater increases in adiposity from childhood to early adulthood in the British Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort [3] and with C-peptide, a marker of insulin resistance, in the pan-European HELIX study [17]. However, it is still unclear if the adverse health effect of UPFs result only from the poorer nutritional profiles of these foods or if there are additional mechanisms related to ultra-processing itself, such as satiating properties or the presence of artificial compounds [18]: While a British cohort study of adults found that effects of UPF on cardiometabolic risk was mediated by and dependent on nutritional quality of the diet [14], a recent randomised control trial among adults demonstrated greater weight gain in adults consuming an UPF diet compared to a nutrient and energy-matched minimally processed diet [19].

Metabolic phenotyping (metabolomics) can provide a high-level resolution picture of biological signatures underlying complex traits and exposures and is increasingly used to provide objective assessments of diet [20]. Previous research on food-related metabolic profiles has largely focused on specific individual food groups [21–25] while dietary patterns are far less studied [20,26–28]. Recent studies show that the Mediterranean pattern diet [16,29] and Western pattern diets [30] have a significant impact on the serum metabolome. Only one study has investigated metabolic profiles related to UPF consumption, applying nuclear magnetic resonance spectroscopy (NMR) in urine to identify a panel of six metabolites indicative of UPF consumption in European children [17]. NMR metabolomics provides highly reproducible and quantified simultaneous measurements of a wide range of low molecular weight molecules and in blood assesses metabolites related to lipid metabolism and transport, fluid balance, glycolysis, liver function and inflammation [31]. Description of metabolic profiles associated with UPFs may elucidate the mechanisms linking diet, obesity, and disease development, even during childhood. Understanding of the molecular pathways underlying weight gain may facilitate interventions that prevent their initiation or interrupt their progression prior to clinical disease.

Here, we investigate the role of UPF intake, assessed using the NOVA classification [32], on the NMR plasma metabolome in children, participating in the ALSPAC study. This study has three aims: a) to describe the metabolic profiles associated with UPF consumption, b) to understand the role of specific nutrients in mediating the metabolic profiles of UPF and c) to assess the role of these profiles in predicting subsequent adiposity trajectories.

2. Subjects and methods

2.1. Study population

The study population included participants from the ALSPAC cohort. Initially, ALSPAC recruited women living in Avon, in United Kingdom, with an expected delivery date between 1 April 1991 and 31 December 1992 [33,34]. Participants have been followed up with questionnaires and clinical measurements at regular time intervals, providing lifestyle, behavioural and biological data. The ALSPAC study website contains details of all available data through a search tool (http://www.bris.ac.uk/alspac/researchers/our-data/). Children were included in the cross-sectional analysis if they had food diary and metabolic profiling data at 7 years. Children were included in the prospective analysis if they had food diary data available at 13 years and metabolic profiling data at 17 years. A flowchart of individuals that were considered eligible for the analysis is shown in supplementary material 1 (Figure S1).

The study has obtained ethical approval from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees in accordance with the Human Tissue Act [34]. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

2.2. Metabolomics

Metabolomic profiling was carried out using ^{1}H NMR spectroscopy on plasma samples cohort for the ages of 7 years (cross-sectional analysis) and 17 years (prospective analysis). Samples collected at age 7 years were non-fasted and samples collected at 17 years were fasted for 6 h. This molecular signature of systemic metabolism consists of 232 metabolic traits. The NMR platform provided quantification of 14 lipoprotein subclasses (particle concentration, lipid concentrations and composition), fatty acids and fatty acid composition, ketone bodies, amino acids, gluconeogenesis-related metabolic traits and gluconeogenesis and glycolysis related metabolites [35,36]. Details related of the NMR platform have been published previously [35].

2.3. Dietary assessment

At the age of 7 and 13 years, parents and the child with parental help, respectively were invited to record all foods and drinks consumed over 3 days in a structured diary: preferably 1 weekend day and 2 weekdays [37,38]. Then during a clinic visit, nutrition fieldworkers examined food records and interviewed the participants to clarify any uncertainties on portion size, cooking methods and leftovers. Dietary data were reviewed by a nutritionist and intakes were coded using the DIDO (Diet In, Data Out) computer program and were linked to the fifth edition of McCance and Widdowson's British food composition tables [39]. Additional up-to-date nutrient information was obtained from the National Diet and Nutrition Survey database and manufacturers' information and nutrients from dietary supplements were not included in the analysis.

Foods and beverages were classified based on the four Nova classification groups [32] and has been fully described in a previous analysis in ALSPAC [3]. Briefly, the groups in Nova are: 1) “unprocessed or minimally processed foods” such as fruits, vegetables, legumes, eggs, meat, fish, or milk, 2) “processed culinary ingredients” such as salt, sugar, vegetable oils, 3) “processed foods” such as canned or bottled vegetables or legumes preserved in brine, whole fruit preserved in syrup, tinned fish preserved in oil, most freshly baked bread and 4) “UPF” that are formulations of ingredients, mostly of exclusive industrial use, typically created by industrial technologies and processes [40] such as soft drinks, mass produced packaged breads and buns, cookies, pastries, cakes and cake mixes, pre-prepared meat, cheese, pasta and pizza dishes (supplementary material 1, Figure S2). To account for variations in total energy intake, we estimated the daily relative energy intake of UPF as a percentage of total energy intake as a continuous variable [41] similar to previous work published with ALSPAC data [3] and other datasets [42,43].

2.4. Covariates

Maternal education was collected from a self-reported questionnaire at 32 weeks of gestation, classified into three categories: (i) low: Certificate of Secondary Education, Vocational or Ordinary- (O-) level, educational qualifications generally obtained at 17 years of age; (ii) intermediate: Advanced- (A-) level (subject-specific qualifications generally obtained at age 18 years and required for university entry); (iii) high: university degree and above. Family income at 7 years was classified into three categories: low <£200 per week, medium £200-£399 per week and high >£400. Average time spent watching TV was available for the ages 7 and 17 years and was classified into 3 categories based on the time spent watching TV during the day: low (<1 h), average (1–2 h) and high (>3 h). A smoker at home was defined as at least one parent smoking when the child was 7 years old. Physical activity at 7 years was classified into 3 categories: (i) every day, (ii) 2–6 time a week and (iii) once a week, less than once a week and never. Physical activity in adolescence was estimated based on the recording of the Actigraph 7164 accelerometer at the age of 15 years and classed into 3 categories based on the quantiles of daily activity monitored by the accelerometers. Finally, pre-pregnancy maternal body mass index (BMI) was calculated using maternal pre-pregnancy self-reported weight and height measurements and the National Statistics Socio-economic classification (NSSEC) index of the mother during pregnancy was obtained from the Office for National Statistics.

2.5. Data imputation

To maximize power and potentially reduce bias in our analysis, we applied a multivariable multiple imputation procedure to

Table 1
Demographic, anthropometric and clinical outcome variables. Values are given in mean (standard deviation, SD) for continuous variables or percent (%) for categorical variables.

	Early Childhood		Adolescence	
	(N = 4528)	p-value[a]	(N = 3086)	p-value[a]
Child age at metabolomic measurements (yrs)		**0.0005**		**0.0006**
Mean (SD)	7.48 (0.17)		16.80 (1.08)	
Sex of child		0.76		0.30
Male	2361 (52.1%)		1509 (48.9%)	
Female	2167 (47.9%)		1577 (51.1%)	
Ethnicity		0.08		0.05
White	4391 (97.0%)		2980 (96.6%)	
Non-white	137 (3.0%)		106 (3.4%)	
Child weight (kg)		0.88		0.53
Mean (SD)	25.6 (4.26)		65.2 (12.7)	
Child height (cm)		0.50		0.38
Mean (SD)	126 (5.30)		171 (9.09)	
Overweight/Obesity child(WHO classification)		0.20		0.93
Non-overweight/Obesity	3635 (80.3%)		2415 (78.3%)	
Overweight/Obesity	893 (19.7%)		671 (21.7%)	
Child watching TV on weekdays				
Less than 1 h	1098 (24.2%)		625 (20.3%)	
1–2 h	3041 (67.2%)	**2e-16**	1978 (64.1%)	**0.001**
3 or more hours	389 (8.6%)	**2e-16**	483 (15.7%)	**2.65e-06**
Child watching TV on weekends				
Less than 1 h	438 (9.7%)		471 (15.3%)	
1–2 h	2548 (56.3%)	**2e-16**	1689 (54.7%)	0.25
3 or more hours	1542 (34.1%)	**2e-16**	926 (30.0%)	**0.0009**
Child weekly activity (7 years)/MVPA weekly activity (15 years)				
Every day/Over 4th quintile	515 (11.4%)		419 (13.6%)	
2–6 times a week/Over 2nd quintile	2383 (52.6%)	**0.0026**	2420 (78.4%)	0.92
Once a week or les/Underr2nd quintile	1630 (36.0%)	**0.0029**	318 (10.3%)	0.18
UPF consumption, (% of the total daily consumed food kcal) at 7 and 13 years of age				
Mean (SD)	61.1 (11.6)		57.8 (13.5)	
Maternal age at pregnancy (years)		0.10		**0.005**
Mean (SD)	29.3 (4.44)		29.5 (4.37)	
Family income				
Low <£199 per week	479 (10.6%)		302 (9.8%)	
Medium £200-£399 per week	1740 (38.4%)	0.94	1152 (37.3%)	0.51
high >£400 per week	2309 (51.0%)	**9.01e-07**	1632 (52.9%)	**8.01e-05**
Smoker at home		**0.01**		**0.0001**
No	3604 (79.6%)		2512 (81.4%)	
Yes	924 (20.4%)		574 (18.6%)	
Maternal education				
Low (O level/vocational/CSE/No education qualifications)	2519 (56.6%)		1557 (50.5%)	
Medium (A level)	1231 (27.2%)	**5.12e-15**	924 (29.9%)	**2.2e-16**
High (degree)	113 (17.2%)	**2e-16**	605 (19.6%)	**2.2e-16**
Maternal pregnancy BMI (kg/m2)		**8.47e-10**		**0.005**
Mean (SD)	22.8 (3.52)		22.8 (3.65)	
Maternal NSSEC during pregnancy[b]				
Low	1504 (42.2%)		923 (29.9%)	
Medium	1562 (44.9%)	**5.13e-15**	1476 (47.8%)	**1.41e-05**
High	882 (11.9%)	**< 2e-16**	687 (22.3%)	**0.001**

[a] p-value for the association with UPF consumption (Nova4%) in bivariate analyses (Analysis of Variance or Pearson's correlation tests).
[b] National Statistics Socio-economic Classification.

impute the missing covariates (ranging 1.2%–35.4%). Imputation was carried out based on the multiple chained equations method with the R package "mice" [44] with the assumption that data were missing at random. First, we performed 100 imputations by 1000 chains of regression and then we applied Rubin's rule [45] for combining the separate estimates and standard errors from the analytical models performed on each of the 100 imputed datasets.

2.6. Statistical analysis

2.6.1. Metabolome-Wide Association Study

A Metabolome-Wide Association Study (MWAS) was conducted to investigate the association between metabolomic profile and UPF using multiple linear regression and applying separate cross-sectional and longitudinal analyses. The dependent variable of the model was the metabolite concentration level in plasma blood, and the exposure variable of the model was ultra-processed food consumption as a percentage of total energy intake. In the cross-sectional analysis, we investigated the association between UPF consumption at 7 years and each metabolite concentration at 7 years, and in the prospective analysis the association between UPF consumption at 13 years and metabolite concentration at 17 years. All metabolite concentrations were log-transformed to obtain approximately normal distributions and subsequently scaled to standard deviation units. The models were adjusted for sex of child, child age and BMI at the age of the metabolomic data availability, maternal age during pregnancy, maternal education level, pre-pregnancy maternal BMI, family income, average time spent watching TV, any smoker at home, and physical activity. Covariates were identified a priori in literature and were chosen based on a bivariate analysis of their correlation with outcomes (Analysis of Variance) at $P < 0.05$. Bonferroni correction for effective number of tests (ENT) [46] was applied throughout to account for multiple test comparisons ($\alpha' = \alpha/\mathrm{ENT}$ where $\alpha = 0.05$ and $\mathrm{ENT}_{\mathrm{childhood}} = 15$ and $\mathrm{ENT}_{\mathrm{adolescence}} = 12$ for metabolic profiling data at 7 and 17 years, respectively) (supplementary material 1, Bonferroni correction methods).

To assess the robustness and consistency of our MWAS analysis in both ages, we ran a sensitivity analysis by: 1) sex; 2) household income (high and non-high income levels); 3) maternal education (high and non-high level education); 4) relative energy intake based on the median value (< and ≥ median value); 5) individuals with and without overweight/obesity. We categorized the participants with or without overweight, based on WHO sex- and age-adjusted BMI z-scores (World Health Organization, 2008) (supplementary material 1); 6) Removal children with the lowest 10% of a creatinine clearance-based estimate of glomerular filtration rate (eGFR) (46). eGFR is an indicator of kidney function in children, which and may influence metabolic profiles. Additionally, we conducted a stepwise regression approach to explore the effect size of four different sets of covariates in both ages. We ran four different models: Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, and BMI; Model 3 was adjusted for age, sex and activity related variables (daily activity and average time spent watching TV); Model 4 was adjusted for age, sex and socioeconomic/lifestyle-related variables (maternal education level, pre-pregnancy maternal BMI, family income, any smoker at home), and maternal NSSEC during pregnancy.

2.7. Mediation analysis of nutrients

Mediation analysis was conducted to assess whether the effect of UPF relative intake at 7 years on metabolic traits at 7 years is mediated by specific nutrients, since the nutritional content of UPF is known to be heterogeneous [47]. The following nutrients were calculated from food diaries and assessed in separate mediation models: protein (g); free sugars (g); total sugar (g); fat intake (g); monounsaturated fatty acid (MUFA) (g); saturated fatty acid (SFA) (g); polyunsaturated fatty acid (g); daily omega-3 fatty acid (g) from fish only; daily carbohydrate (g); dietary cholesterol (mg); vitamin C (mg); vitamin B6 (mg); vitamin B9 (folate) (ug); vitamin B12 (ug); potassium (mg); and sodium (mg).

The mediation analysis was carried out using the R package "medflex" [48]. We applied linear regression models and used an imputation approach [49] to co-estimate the natural direct effect (not mediated by nutrient) and the natural indirect effect (mediated by nutrient) of the daily UPF intake at 7 years on metabolic traits. Mediator and outcome models were adjusted for age and sex of child, child BMI, maternal age during pregnancy, maternal education level, pre-pregnancy maternal BMI, family income, average time spent watching TV, any smoker at home, physical activity, and NSSEC as fixed effects. Confidence intervals (95%) were calculated by a nonparametric bootstrap method with 1000 replications.

2.7.1. Growth curve models

Linear growth curve models were used to investigate the longitudinal associations between baseline quartiles of the metabolic profile at 7 years and fat mass measured at ages of 9, 12, 15 and 17 years of age. These were adjusted for age and sex of child, child BMI, maternal age during pregnancy, maternal education level, pre-pregnancy maternal BMI, family income, average time spent watching TV, any smoker at home, and physical activity as fixed effects, and age at measurement of fat mass was fitted using random effect for each participant. We included an interaction term between age and metabolic trait level to examine the differences in mean growth trajectories among metabolic trait quantiles (25%, 50%, 75%). We assessed non-linearity by fitting a quadratic age term in the fixed and random parts of the growth models. Since baseline adiposity exerts strong effects on the metabolome (often in opposing directions to associations observed with UPF [50]), we stratified by non-overweight and overweight (WHO definition) to further control for baseline adiposity.

Partial least squares (PLS) regression models were constructed to describe the overall association between the plasma metabolome and UPF consumption at age 7 years, where the ^{1}H NMR metabolic profiles served as the descriptor matrix and UPF% was the response variable. PLS predictive models were built for the study population, the subgroups with overweight and those without overweight against UPF relative consumption to control for effects of baseline adiposity. We assessed the robustness of the PLS model using a k-fold cross validation ($k = 10$ segments) for the 232 centred and scaled metabolic traits. The optimum component number was selected based on the predicted residual error sum of squares.

3. Results

3.1. Study population

The characteristics of the study population are summarized in Table 1. A total of 4528 children were included in the cross-sectional analysis and 3086 children were included in the prospective analysis (supplementary material 1, Figure S1). The proportion of UPF out of the total food consumed daily (in kcal) was 61.1% on average at the age of 7 years and 57.8% at age of 13 years (Table 1). Higher UPF consumption was associated with child age, greater TV watching and lower physical activity by the child, lower maternal age and higher maternal BMI, presence of smoker at home, and lower family income, maternal education, and socio-economic class (Table 1).

3.2. Metabolome-Wide associations with UPF consumption

In the cross-sectional analysis, 115 metabolic traits (out of 232 traits tested) were associated with UPF intake assessed at 7 years, after multiple testing correction (Figs. 1 and 2, supplementary material 1, Table S1). UPF was negatively associated with tyrosine and phenylalanine (aromatic amino acids, AAA) and isoleucine, leucine, and valine (branched-chain amino acids (BCAA)). Of these metabolites, valine showed the strongest associations. UPF intake was positively associated with glutamine, citrate, and creatinine. UPF intake was associated with lower total saturated and poly-unsaturated fatty acids (PUFA), including conjugated linoleic acid and docosahexaenoic acid (DHA). The ratio of MUFA to total fatty acids was increased with UPF consumption. In contrast, the ratios of polyunsaturated and omega-3 fatty acids to total fatty acids were decreased, with the strongest effects seen for the omega-3 fatty acids. Total choline, phosphatidylcholine and other cholines, and phosphoglycerides and triglycerides in low-density lipoproteins (LDL), were significantly decreased with higher UPF consumption. Total, free, esterified and remnant cholesterol, and total cholesterol levels in all high-density lipoproteins (HDL) fractions and LDL fractions were decreased with UPF intake (Figs. 1 and 2). Among the lipoprotein measures, apolipoprotein A-I was significantly decreased, and significant decreases were observed for large and very large HDL, small, medium, and large LDL, intermediate density lipoproteins (IDL) particles. Additionally, there were non-

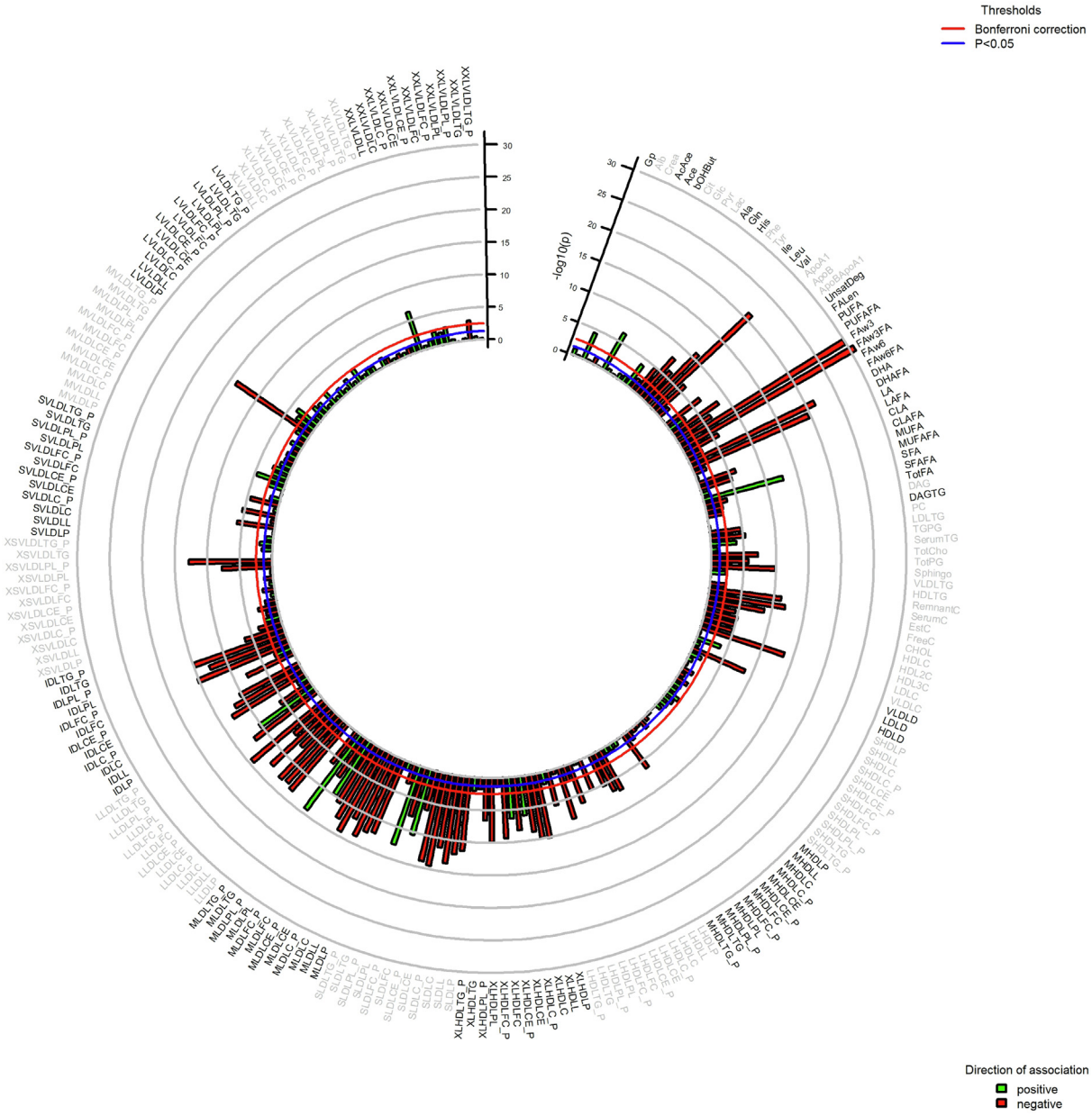

Fig. 1. Circus plot showing the associations between UPF % intake and child plasma blood metabolome in cross-sectional analysis. The circle shows the log p-value (−log10 (p-value)) of the metabolic traits at the age of 7 years. The red line refers to the Bonferroni corrected p-value while the blue line refers to P < 0.05. Positive associations are shown in green and negative in red. All the models were adjusted for age and sex of child, child body mass index (BMI), maternal age during pregnancy, maternal education level, pre-pregnancy maternal BMI, family income, average time spent watching TV, any smoker at home, physical activity and NSSEC during pregnancy. Abbreviations are available in supplementary material 2 (Table S1). p-values are available in supplementary material 2 (Table S2).

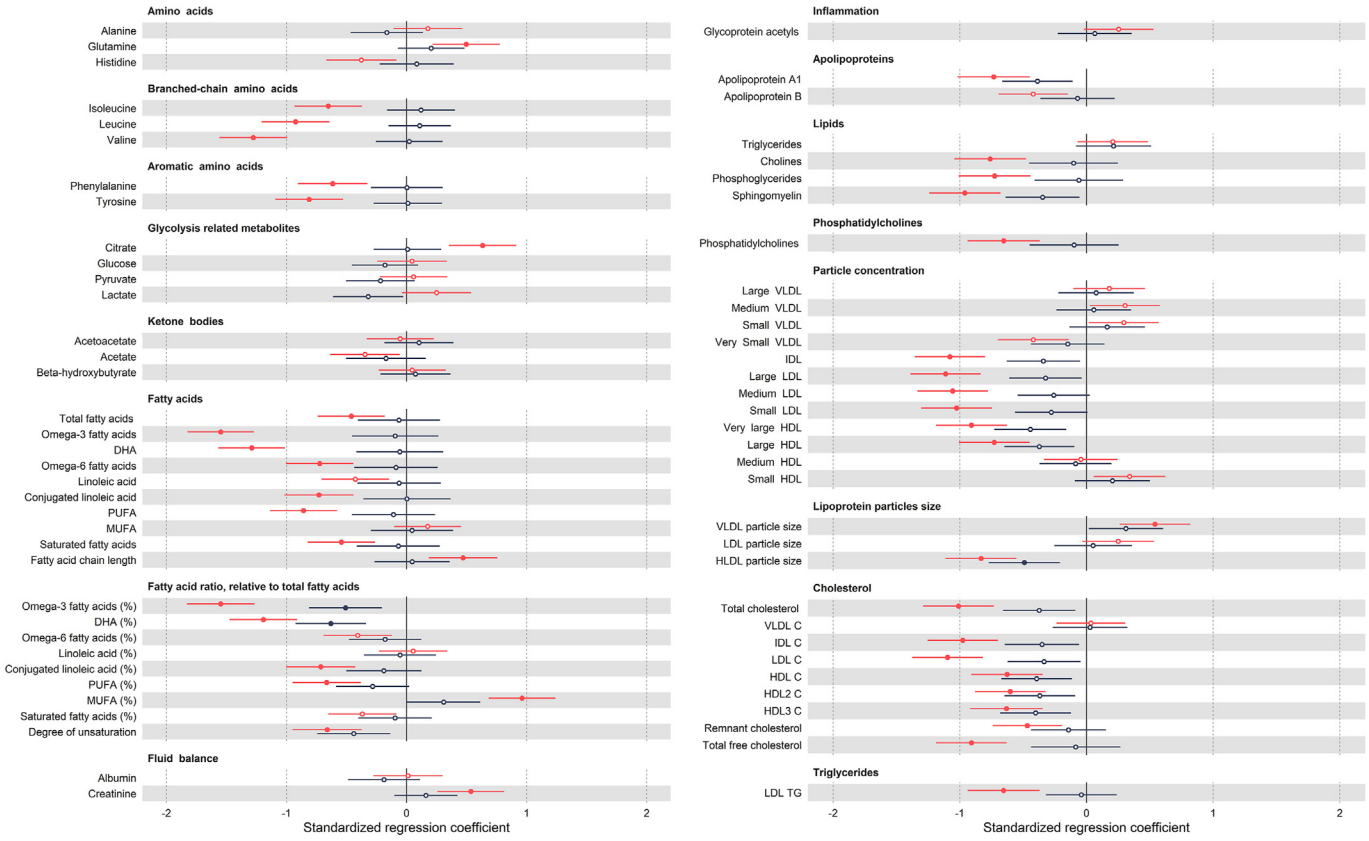

Fig. 2. Metabolite associations between UPF % intake and plasma blood metabolome at 7 years (cross-sectional analysis, red lines) and 17 years (prospective analysis, black lines). Lipoprotein subclasses and ratios are not displayed to improve readability. Standardized regression coefficients (95% confidence interval, CI) of the association between UPF relative intake energy and 69 selected metabolic features at the age of 7 (N = 4528) and 17 years (N = 3086). Models were adjusted for age and sex of child, child body mass index (BMI), maternal age during pregnancy, maternal education level, pre-pregnancy maternal BMI, family income, average time spent watching TV, any smoker at home, physical activity and NSSEC during pregnancy. The filled dot declares significant association after Bonferroni correction (effective number of tests (ENT, $n_{7years} = 15$ and $n_{16years} = 12$). Where C indicates cholesterol; DHA, docosahexaenoic acid; HDL, high-density lipoprotein; IDL, intermediate-density lipoproteins; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TG, triglycerides and VLDL, very low-density lipoprotein.

significant increases in concentrations of small HDL, small, medium, large, very large and extremely large very low-density lipoproteins (VLDL) (Figs. 1 and 2).

The prospective analysis showed that 37 metabolic traits at 17 years were associated with UPF consumption assessed at 13 years after multiple-testing correction (Fig. 2, supplementary material 1, Figure S3 and supplementary material 2, Table S1). UPF consumption was negatively associated with the ratios of DHA and omega-3 fatty acids to total fatty acids, the concentration of very large HDL particles, free and total cholesterol, phospholipids and total lipids in large and very large HDL, and the ratio of free cholesterol to total lipids in medium and large HDL and IDL.

Table 2
Diet nutrients associations with UPF. Coefficients and 95% confidence intervals of associations between UPF % intake (per total energy) and macronutrient intake at the age of 7 years (N = 4528).

Macronutrient intake	Unit change in nutrient per % increase in UPF consumption (95% Confidence Interval)	p-value
Saturated fatty acid intake (g)	−0.0023 (-0.0026,-0.0019)	5.53E-19
Monounsaturated fatty acid intake (g)	−0.0005 (-0.0009,0.0000)	5.46E-02
Polyunsaturated fatty acid intake (g)	0.0036 (0.0029,0.0044)	1.38E-21
Protein intake (g)	−0.0029 (-0.0031,-0.0027)	6.73E-16
Dietary cholesterol intake (mg)	−0.0006 (-0.0006,-0.0005)	8.11E-18
Carbohydrate intake (g)	0.0003 (0.0002,0.0003)	3.22E-20
Daily n-3 fatty acid intake (g) from fish	−0.1389 (-0.1717,-0.1062)	1.00E-16
Fat intake (g)	−0.0004 (-0.0006,-0.0003)	1.70E-07
Total sugar intake (g)	0.0001 (0.00003,0.0002)	8.12E-03
Free sugar intake (g)	0.0009 (0.0008,0.0010)	2.10E-18
Vitamin C intake (mg)	−0.0001 (-0.0001,-0.00003)	2.49E-03
Vitamin B6 intake (mg)	−0.0189 (-0.0240,-0.0139)	3.00E-13
Vitamin B9 (Folate) intake (mg)	−0.0003 (-0.0003,-0.0002)	1.64E-13
Vitamin B12 intake (mg)	−0.0138 (-0.0152,-0.0124)	6.00E-14
Sodium intake (mg)	0.00002 (0.00001,0.00003)	1.28E-05
Potassium intake (mg)	−0.000065 (-0.00007,-0.00006)	−7.00E-05

There was considerable consistency in the metabolomic signatures in the cross-sectional and prospective analyses, with over 86% of the examined metabolic traits having the same direction of association with UPF relative intake (Fig. 2, supplementary material 2, Table S1).

The correlations among metabolic traits at both ages are shown in supplementary material 1 (supplementary material 1, Figure S4-S5).

3.3. Mediation analysis by nutrient intake

UPF consumption was associated with lower calculated intake of proteins and fat, particularly saturated fat, cholesterol, and micronutrients including folate and B and C vitamins, and higher levels of sugars and carbohydrate at age 7 years (Table 2, supplementary material 1, Figure S4). Natural indirect effects (mediating pathway by nutrient) and total effects (of UPF consumption on metabolite) in childhood are shown in Fig. 3 and supplementary material 2, Table S2. Significant mediation by protein intake in the association with UPF was observed for multiple metabolites including for creatinine (proportion mediated (PM) : 13.4%), phenylalanine (PM: 26%), tyrosine (PM: 25%), leucine (PM: 32%), valine (PM: 26%) and isoleucine (PM: 28%), total cholesterol (PM: 24%), DHA (PM: 12.5%), PUFA (PM: 19%), cholines (PM: 21%), phosphoglycerides (PM: 20%), large HDL (PM: 30.5%) and very large HDL

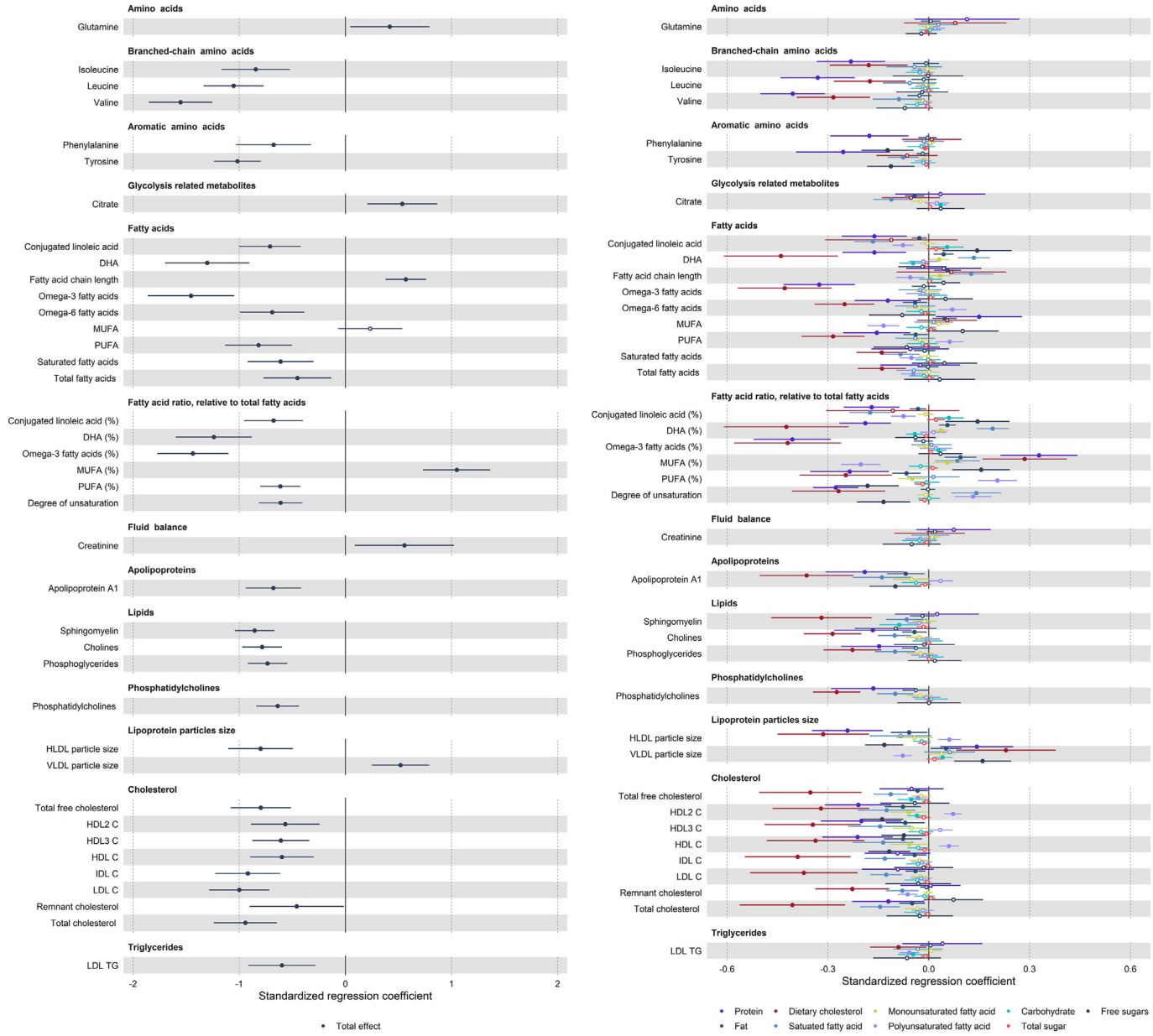

Fig. 3. Standardized regression coefficients for the relationship between UPF % intake and metabolite level as mediated by dietary nutrients at age 7 years. Regression coefficients in standard deviation increment in metabolic traits and 95% confidence intervals for natural indirect effect and total effect (dark blue line) from single mediation analysis for the relationship between UPF relative intake and metabolome at 7 years of age mediated by dietary nutrients. The model was adjusted for age and sex of child, child body mass index (BMI), maternal age during pregnancy, maternal education level, pre-pregnancy maternal BMI, family income, average time spent watching TV, any smoker at home, physical activity and NSSEC during pregnancy. The black and coloured lines are the totals effect and natural indirect effect, respectively. The filled dot declares significant associations after Bonferroni correction. C indicates cholesterol; CI, confidence interval; DHA, docosahexaenoic acid; HDL, high-density lipoprotein; IDL, intermediate-density lipoproteins; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TG, triglycerides; and VLDL, very low-density lipoprotein.

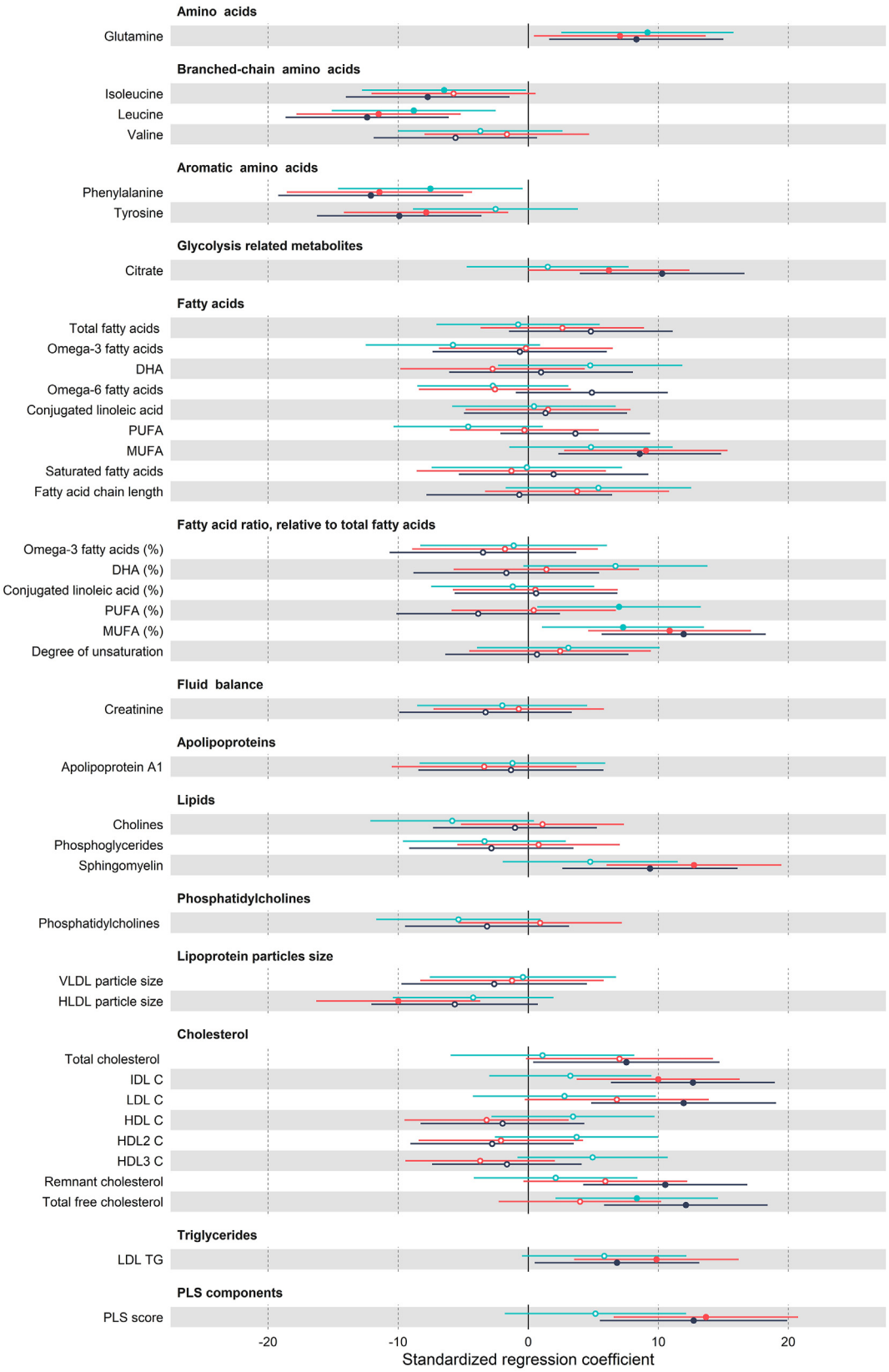

Fig. 4. Longitudinal associations between fat mass and interaction term between age and metabolome among 4357 ALSPAC children of normal weight at 7 years. Regression coefficients (95%CI) of the interaction term that examines the difference in average growth trajectories of fat mass compared with baseline metabolite level quartile reference group. The green, red and black line are the 2nd, 3rd and 4th quartiles, respectively. The models were employed individual-specific random intercept, random slope using age and quadratic age as the underlying timescale, and further adjusted for age and sex of child, child body mass index (BMI), maternal age during pregnancy, maternal education level, pre-pregnancy maternal BMI, family income, average time spent watching TV, any smoker at home, physical activity and NSSEC during pregnancy. C indicates cholesterol; CI, confidence

(PM: 26.9%). SFA intake mediated associations with blood levels of tyrosine (PM: 8%), valine (PM: 6%), SFA (PM: 13%), MUFA to total fatty acids ratio (PM: 8%), cholines (PM: 13%), phospholipids and glycerides (PGs) (PM: 14%), cholesterol (PM: 27%) and lipoprotein concentrations (PM: from 10 to 15%). Cholesterol intake mediated associations with total serum cholesterol (PM: 43.1%), BCAAs (PM: 16–21%), DHA (PM: 34%), PUFA (PM: 35%), SFA (PM:23%), cholines (PM: 37%), PGs (PM: 31%), and lipoproteins (PM: 18–47%). Little mediation was observed with calculated carbohydrate or sugar intake. Omega-3 fatty acid intake from fish mediated associations with omega-3 to total fatty acids ratio and DHA by 12% and 16% respectively (supplementary material 1, Figure S6). Vitamin B12 mediated 22% of the association between UPF and glutamine (supplementary material 1, Figure S6) and between 3 - 4% of UPF associations with the leucine, valine, and tyrosine. Notably, there was no evidence that the effects of UPF relative intake on citrate were mediated by any of the tested mediators. Mediation by micronutrients is shown in supplementary material 1, Figure S6.

3.4. Metabolite associations with fat mass trajectories

To understand the role of plasma metabolic profiles in adiposity trajectories, linear growth curve models were used to investigate the longitudinal associations between baseline quartiles of metabolic features at 7 years and fat mass measured at ages of 9, 12, 15 and 17 years of age year for quartiles of concentrations of metabolites associated with UPF-consumption, compared to the lowest quartile, in the normal weight population at 7 years (Fig. 4). Increases in fat mass per year were significantly greater for children with the highest metabolite quartiles for glutamine, citrate, MUFA, MUFA as ratio to total fatty acids, LDL triglycerides, IDL, small, medium, and large LDL and total cholesterol. Increases in fat mass per year were significantly lower for children with the highest metabolite quartiles for isoleucine, leucine, phenylalanine, and tyrosine. Evidence for a dose response across quartiles was observed for isoleucine, leucine, phenylalanine, tyrosine, citrate, MUFA as ratio to total fatty acids, IDL and medium LDL. Considering the reported association between UPF consumption and greater fat mass accumulation per year in this population [3] and the metabolomic profiles of UPF consumption (Figs. 1 and 2), the growth curve models support a role of glutamine, citrate, isoleucine, leucine, phenylalanine, tyrosine, MUFA as ratio to total fatty acids in the association between UPF consumption and fat mass accumulation.

Moreover, a PLS model of UPF consumption at 7 years was constructed using 232 metabolites at a baseline of 7 years of age to provide a multivariate characterisation of the metabolic profile of UPF consumption (supplementary material 1, Figure S7-S10). Children with the third and fourth highest quartiles of the PLS score (i.e. a metabolic profile most associated with UPF consumption) show significantly greater fat mass accumulation per year compared with children in the lowest quartile of PLS score (Fig. 4).

Results from the linear growth models for all metabolites are provided in supplementary material 2, Table S3, including for the total and overweight populations, with and without adjustment for UPF intake.

3.5. Sensitivity analysis (MWAS)

The sensitivity analysis of the effect of UPF relative intake on the metabolic traits suggests that our findings are robust. In our comparisons, 89% and 78% of the associations between UPF relative intake and metabolic traits retained their effect direction in stratified analyses at 7 and 17 years of age, respectively (supplementary material 2, Table S4 and S5). Findings from the MWAS remained consistent while adjusting for covariates in multiple steps. In all cases over 90% of the associations retained their effect direction compared to the fully adjusted model (supplementary material 2, Table S6 and S7).

4. Discussion

In a large British population-based birth cohort, we have described a metabolic profile of UPF consumption in plasma. In the analysis of diet and metabolic profile, both assessed at age 7 years, we observed with greater UPF intake relative to total energy consumed, decreased plasma levels of AAA and BCAAs and increases in citrate, creatinine and glutamine, lower levels of fatty acids with a decreased proportion of omega-3 and PUFA and increased proportion of monounsaturated and longer chain length fatty acids. Lipids including cholines, phosphoglycerides, sphingomyelins and total cholesterol were reduced, and there was a shift in lipoprotein distribution towards lower levels of higher density lipoproteins, characterised by reduced size of HDL and increased size of VLDL. Mediation analysis indicated that citrate and glutamine are associated with UPF, independently of tested macronutrients. In the longitudinal analysis of UPF consumption at age 13 years and fasted metabolic profiles at 17 years, reflective of longer-term changes, we similarly observed reductions in omega-3 fatty acids and DHA as a proportion of total fatty acids and lower levels of very large HDL and a smaller HDL particle size. Growth curve models indicated a role for glutamine, citrate, isoleucine, leucine, phenylalanine, tyrosine, and MUFA as ratio to total fatty acids measured at 7 years in the association between UPF consumption and subsequent fat mass accumulation.

The metabolic effects of a diet rich in UPF may contribute to reported links between UPF consumption and cardiometabolic disease [10–14], since some of the observed metabolic changes are established risk factors of cardiometabolic disease. Lower circulating levels of DHA, omega-3 and omega-6 fatty acids and higher levels of MUFA have been linked to higher CVD risk [51–53], while lower HDL-C and small HDL size are considered to reflect atherogenic dyslipidaemia and be associated with intra-abdominal adipose tissue accumulation and peripheral insulin resistance [54]. Recent studies demonstrated the potential direct role of HDL-C on glucose metabolism through modification of glucose uptake in skeletal muscle and promotion of pancreatic β-cell insulin secretion [55,56], with epidemiological studies suggesting that low levels of HDL-C are associated with T2D risk [57–59]. Glutamine was associated with a 6-year incidence of high carotid intima–media thickness (a marker of subclinical atherosclerosis) [59], while longer chain saturated acids were also reported to be elevated in prediabetes and T2D [60].

However, we noted reductions in circulating levels of BCAAs and AAAs, both of which are well established to be raised in obesity and to be predictive of insulin resistance/T2D [61,62] and CVD respectively [63]. The observed increases associated with metabolic ill-health result from endogenous shifts in metabolism through for instance activity of the BCKD complex, which lowers BCAA catabolism and clearance [62]. Mediation analysis indicated that the lower blood levels observed in this study in association with UPF,

interval; DHA, docosahexaenoic acid; HDL, high-density lipoprotein; IDL, intermediate-density lipoproteins; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids; PLS, partial least square; PUFA, polyunsaturated fatty acids; and TG, triglycerides. Q2-Q4 represents lowest to highest quintile of baseline metabolome at 7 years. Baseline refers to the age of 7 year.

result at least in part, from lower intake of protein containing foods, rather than physiological mechanisms. Lower levels of valine and tyrosine were also found to be associated with UPF in urine from European children [17]. The same study found no association of these metabolites with the KIDMED score, an indicator of diet quality, suggesting they may be specific to UPFs. Numerous intervention studies and animal studies have shown that a higher intake of BCAAs has beneficial signalling effects, with positive effects on parameters including body composition, glycemia and satiety [62,64]. Proposed mechanisms for these positive effects include direct effects on hypothalamic and brainstem processes involved in satiety [64]. Lower blood BCAA levels could therefore influence later propensity for overweight through mechanisms such as control of food intake, contributing to effects of UPF on weight gain. This was supported, particularly for leucine and isoleucine but also the AAAs, in the growth curve analysis. Indeed, experimental studies on food consumption indicate that UPFs have low satiety potential and induce high glycaemic responses [18,65].

Among the ALSPAC children, UPF consumption at 7 years was associated with lower reported intake of saturated fats and cholesterol, in contrast to studies in Brazil [66] and Chile [67] that have reported higher levels of saturated fats with UPF intake. This was confirmed by our metabolic profiling that showed lower circulating levels of these lipids in association with UPF consumption, with most of the effect of UPF on total plasma cholesterol mediated by reported cholesterol intake. This difference in the ALSPAC population may be explained by higher intakes of saturated fats generally and the weaker relationship between UPF and saturated fat in the UK (particularly at higher UPF intake levels) compared to less developed countries [68]. There is scientific consensus that general diet should not exceed 10% of energy from saturated fats and that SFA increase plasma levels of atherogenic LDL cholesterol when substituted in the diet for carbohydrates or unsaturated fatty acids. However there is still uncertainty regarding whether reduction of SFA intake directly affects cardio-metabolic disease risk and the role of food matrix [69]. The growth curve analysis indicated that both higher plasma LDL cholesterol and MUFA, but not SFA, were associated with fat mass accumulation. We observed both lowered HDL and LDL cholesterol (traditionally considered “good” and “bad” cholesterol respectively), however cholesterol in the VLDL fraction, reported to have the strongest effects on CVD risk [51] remained unchanged. Our analyses suggest that SFA, and indeed cholesterol, do not contribute to the association between UPF and adiposity in ALSPAC children and support the view that the overall diet should be emphasised in recommendations to reduce SFA intake. Although the NMR metabolic profiling method is limited in identifying individual fatty acids, we observed a significantly higher average chain length of fatty acids indicating differences in species of circulating fatty acids among high UPF consumers. This may indicate reduced levels of short chain fatty acids, an indicator of gut dysbiosis associated with less varied diets [70], which is an emerging risk factor of metabolic ill health [71]. Furthermore, we lacked information on trans fats, which are produced by industrial hydrogenation of unsaturated fatty acids, and are often present at high levels in UPF [67].

The mediation analysis did not show any evidence for the role of specific macronutrients in the associations between UPF and citrate and glutamine. Glutamine is not an essential amino acid so is likely less impacted by reduced protein intake as for the BCAAs and AAAs. However, some of the effect of UPF on glutamine levels was mediated by lower intake of vitamin B12. Urinary glutamine has recently been reported to be negatively correlated with intake of multiple B vitamins [72], which is presumably related to the role of B vitamins as cofactors in many metabolic processes including amino acid metabolism. Increases in citrate with higher UPF consumption may be related to the observed increases in glutamine as the amino acid is a precursor of 2-oxoglutarate in the tricarboxylic acid cycle. Also, increases in plasma citrate may directly relate to its use as a food additive in UPF. Citrate is the most widely used additive in the food industry, as it is a very efficient food flavouring agent and preservative [73]. While the molecule is considered inert by regulatory agencies, little is known about the metabolic fate of excess citrate intake. Emerging evidence in mouse studies have shown citrate supplementation increases fasting glycaemia, glucose intolerance and the expression of pro-inflammatory cytokines in adipose and liver tissues [74,75]. The growth curve analysis showed that higher levels of both citrate and glutamine at 7 years were associated with greater fat mass accumulation.

Our study has some limitations. There may be potential misclassification of diet by the Nova classification, and calculated nutrient intakes, but this is likely minimized given that detailed food descriptions were provided through diaries used in the ALSPAC cohort. The availability of multiple food diaries also lowers measurement bias, and most participants (92%) completed 2 or more days, and potential dietary misreporting was examined based on the ratio of energy intake to estimated energy expenditure with effects on growth trajectories remaining closely consistent after the exclusion of under- and over-reporters [3]. It should be acknowledged that there is considerable debate regarding what factors determine the level of food processing and multiple classifications systems exist for food processing [76]. However, the NOVA classification remains the most applicable system for observational studies and Nova-classified UPFs have been widely and consistently associated with adiposity and cardiometabolic outcomes, generally independently of nutritional profiles [77]. Dietary assessments were conducted in the early 2000s which may reduce relevance to current dietary habits, however British children at that time were already consuming high levels of UPFs, at similar levels to today [78]. We only included assessment of diet at two timepoints, providing single snapshots of exposure which may not have been sufficient to characterise long-term intakes particularly in the prospective analysis, although only modest changes in UPF consumption were observed from 7 to 13 years of age [3]. Similarly, the metabolome was only assessed at two timepoints which may not have captured long-term alterations. Furthermore, the fasting-status of samples for metabolomic assessment differed in the cross-sectional and prospective analyses, reducing comparability. However, non-fasted samples are an advantage in the cross-sectional analysis as it improves detection of biomarkers of diet reported over the previous days, while the use of fasted samples in the prospective analysis may be an advantage for detection of longer-term changes. Although we adjusted for a range of socioeconomic and lifestyle factors, we cannot exclude the possibility that factors that were not available for analysis may influence our results in this observational study. For instance, we did not have information available on dietary supplement usage, which may be associated with UPF consumption. To our knowledge, this is the largest cohort study to investigate the effect of UPF consumption on child NMR metabolic profiles with information on small molecules and lipoprotein subclasses using cross-sectional and longitudinal data. The study is characterized by a large sample size from a British population, sufficient to control for base-line adiposity levels for assessment of effects of metabolites on subsequent adiposity trajectories. The targeted metabolomic approach is both a strength and limitation: while it provides a broad range of identified features, the number of molecules assessed is small compared to untargeted mass-spectrometry based metabolomics. Future work in this area may include the incorporation of complementary metabolomic methods, cross-

omics, in addition to evidence on the influence of the gut microbiota in host metabolism.

5. Conclusions

Higher level consumption of UPF during childhood is associated with altered metabolomic profiles among British children. Specific nutrient intake contributed to some of this UPF-associated metabolic profile. Higher levels of citrate, glutamine and MUFA and lower levels of BCAAs and AAAs may contribute to the association between UPF and fat mass accumulation in children. Our findings shed new light on metabolic effects of UPF consumption and suggest potential mechanisms underlying the harmful effects of UPF, which should be validated in causal studies.

Author contributions

The authors' responsibilities were as follows—EH and OR drafted the manuscript; EH, OR and PV conceived the study. EH performed most statistical analyses. KC applied the Nova classifications. All authors: critically reviewed and approved the final manuscript. OR and PV supervised the study and OR coordinated the study.

Conflict of interest

The authors declare no competing interests.

Data access

Data used for this submission can be made available on request to the ALSPAC Executive. The ALSPAC data management plan describes in detail the policy regarding data sharing, which is through a system of managed open access. Full instructions for applying for data access can be found here: http://www.bristol.ac.uk/alspac/researchers/access/.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2022.09.002.

References

[1] Monteiro CA, Cannon G, Moubarac J-C, Levy RB, Louzada MLC, Jaime PC. The UN Decade of Nutrition, the NOVA food classification and the trouble with ultra-processing. Publ Health Nutr 2018;21:5–17.
[2] Seferidi P, Scrinis G, Huybrechts I, Woods J, Vineis P, Millett C. The neglected environmental impacts of ultra-processed foods. The Lancet Planetary Health 2020;4:e437–8.
[3] Chang K, Khandpur N, Neri D, Touvier M, Huybrechts I, Millett C, et al. Association between childhood consumption of ultraprocessed food and adiposity trajectories in the Avon longitudinal study of parents and children birth cohort. JAMA pediatrics 2021:e211573. e.
[4] Onita BM, Azeredo CM, Jaime PC, Levy RB, Rauber F. Eating context and its association with ultra-processed food consumption by British children. Appetite 2021;157:105007.
[5] Neri D, Martinez-Steele E, Monteiro CA, Levy RB. Consumption of ultra-processed foods and its association with added sugar content in the diets of US children. NHANES 2009-2014. Pediatric obesity 2019;14:e12563.
[6] Who. WHO. UNICEF/WHO/The World Bank Group Joint Child Malnutrition Estimates: levels and trends in child malnutrition: key findings of the 2020 edition. 2020.
[7] Fardet A, Rock E. Ultra-processed foods: a new holistic paradigm? Trends Food Sci Technol 2019;93:174–84.
[8] Moubarac J. Ultra-processed food and drink products in Latin America: trends, impact on obesity, policy implications. Washington, DC, USA: Pan American Health Organization World Health Organization; 2015. p. 1–58.
[9] Chen X, Zhang Z, Yang H, Qiu P, Wang H, Wang F, et al. Consumption of ultra-processed foods and health outcomes: a systematic review of epidemiological studies. Nutr J 2020;19:1–10.
[10] Rauber F, Chang K, Vamos EP, da Costa Louzada ML, Monteiro CA, Millett C, et al. Ultra-processed food consumption and risk of obesity: a prospective cohort study of UK Biobank. Eur J Nutr 2020:1–12.
[11] Beslay M, Srour B, Méjean C, Allès B, Fiolet T, Debras C, et al. Ultra-processed food intake in association with BMI change and risk of overweight and obesity: a prospective analysis of the French NutriNet-Santé cohort. PLoS Med 2020;17:e1003256.
[12] Srour B, Fezeu LK, Kesse-Guyot E, Allès B, Debras C, Druesne-Pecollo N, et al. Ultraprocessed food consumption and risk of type 2 diabetes among participants of the NutriNet-Santé prospective cohort. JAMA Intern Med 2020;180:283–91.
[13] Levy RB, Rauber F, Chang K, Louzada MLdC, Monteiro CA, Millett C, et al. Ultra-processed food consumption and type 2 diabetes incidence: a prospective cohort study. Clinical Nutrition 2021;40:3608–14.
[14] Griffin J, Albaloul A, Kopytek A, Elliott P, Frost G. Effect of ultraprocessed food intake on cardiometabolic risk is mediated by diet quality: a cross-sectional study. BMJ Nutrition, Prevention & Health 2021. bmjnph-2020-000225.
[15] Fiolet T, Srour B, Sellem L, Kesse-Guyot E, Allès B, Méjean C, et al. Consumption of ultra-processed foods and cancer risk: results from NutriNet-Santé prospective cohort. bmj 2018:360–k322.
[16] Schnabel L, Kesse-Guyot E, Allès B, Touvier M, Srour B, Hercberg S, et al. Association between ultraprocessed food consumption and risk of mortality among middle-aged adults in France. JAMA Intern Med 2019;179:490–8.
[17] Stratakis N, Siskos AP, Papadopoulou E, Nguyen AN, Zhao Y, Margetaki K, et al. Urinary metabolic biomarkers of diet quality in European children are associated with metabolic health. eLife 2021;11(e71332). https://doi.org/10.7554/eLife.71332.
[18] Fardet A. Minimally processed foods are more satiating and less hyperglycemic than ultra-processed foods: a preliminary study with 98 ready-to-eat foods. Food Funct 2016;7:2338–46.
[19] Hall KD, Ayuketah A, Brychta R, Cai H, Cassimatis T, Chen KY, et al. Ultra-processed diets cause excess calorie intake and weight gain: an inpatient randomized controlled trial of ad libitum food intake. Cell Metabol 2019;30:67–77. e3.
[20] Garcia-Perez I, Posma JM, Gibson R, Chambers ES, Hansen TH, Vestergaard H, et al. Objective assessment of dietary patterns by use of metabolic phenotyping: a randomised, controlled, crossover trial. Lancet Diabetes Endocrinol 2017;5:184–95.
[21] Gibson R, Lau C-HE, Loo RL, Ebbels TM, Chekmeneva E, Dyer AR, et al. The association of fish consumption and its urinary metabolites with cardiovascular risk factors: the International Study of Macro-/Micronutrients and Blood Pressure (INTERMAP). The American journal of clinical nutrition 2020;111:280–90.
[22] Lau C-HE, Siskos AP, Maitre L, Robinson O, Athersuch TJ, Want EJ, et al. Determinants of the urinary and serum metabolome in children from six European populations. BMC Med 2018;16:1–19.
[23] Guertin KA, Moore SC, Sampson JN, Huang W-Y, Xiao Q, Stolzenberg-Solomon RZ, et al. Metabolomics in nutritional epidemiology: identifying metabolites associated with diet and quantifying their potential to uncover

diet-disease relations in populations. The American journal of clinical nutrition 2014;100:208–17.
[24] Playdon MC, Sampson JN, Cross AJ, Sinha R, Guertin KA, Moy KA, et al. Comparing metabolite profiles of habitual diet in serum and urine. The American journal of clinical nutrition 2016;104:776–89.
[25] Scalbert A, Brennan L, Manach C, Andres-Lacueva C, Dragsted LO, Draper J, et al. The food metabolome: a window over dietary exposure. The American journal of clinical nutrition 2014;99:1286–308.
[26] Collins C, McNamara AE, Brennan L. Role of metabolomics in identification of biomarkers related to food intake. Proc Nutr Soc 2019;78:189–96.
[27] Rebholz CM, Lichtenstein AH, Zheng Z, Appel LJ, Coresh J. Serum untargeted metabolomic profile of the Dietary Approaches to Stop Hypertension (DASH) dietary pattern. The American journal of clinical nutrition 2018;108:243–55.
[28] Martinez JA, Keun HC, Siskos AP. Effects of mediterranean diet on the metabolome. Mediterranean diet. Springer; 2016. p. 121–37.
[29] Li J, Guasch-Ferré M, Chung W, Ruiz-Canela M, Toledo E, Corella D, et al. The Mediterranean diet, plasma metabolome, and cardiovascular disease risk. Eur Heart J 2020;41:2645–56.
[30] Dhungana S, Carlson JE, Pathmasiri W, McRitchie S, Davis M, Sumner S, et al. Impact of a western diet on the ovarian and serum metabolome. Maturitas 2016;92:134–42.
[31] Tzoulaki I, Ebbels TM, Valdes A, Elliott P, Ioannidis JP. Design and analysis of metabolomics studies in epidemiologic research: a primer on-omic technologies. American journal of epidemiology 2014;180:129–39.
[32] Monteiro CA, Cannon G, Moubarac JC, Levy RB, Louzada MLC, Jaime PC. The UN Decade of Nutrition, the NOVA food classification and the trouble with ultra-processing. Publ Health Nutr 2018;21:5–17.
[33] Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, et al. Cohort profile: the Avon longitudinal study of parents and children: ALSPAC mothers cohort. Int J Epidemiol 2013;42:97–110.
[34] Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, et al. Cohort profile: the 'children of the 90s'—the index offspring of the Avon longitudinal study of parents and children. Int J Epidemiol 2013;42:111–27.
[35] Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 2009;134:1781–5.
[36] Kujala UM, Mäkinen V-P, Heinonen I, Soininen P, Kangas AJ, Leskinen TH, et al. Long-term leisure-time physical activity and serum metabolome. Circulation 2013;127:340–8.
[37] Cribb VL, Jones LR, Rogers IS, Ness AR, Emmett PM. Is maternal education level associated with diet in 10-year-old children? Publ Health Nutr 2011;14:2037–48.
[38] Glynn L, Emmett P, Rogers I, Team AS. Food and nutrient intakes of a population sample of 7-year-old children in the south-west of England in 1999/2000—what difference does gender make? Journal of Human Nutrition and Dietetics 2005;18:7–19.
[39] Holland B, Welch A, Unwin I, Buss D, Paul A, Southgate D. McCance and Widdowson's the composition of foods. Royal Society of Chemistry; 1991.
[40] Monteiro CA, Cannon G, Lawrence M, Costa Louzada Md, Pereira Machado P. Ultra-processed foods, diet quality, and health using the NOVA classification system. Rome: FAO; 2019.
[41] Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. The American journal of clinical nutrition 1997;65:1220S. 8S.
[42] Costa CdS, Assunção MCF, Loret de Mola C, Cardoso JdS, Matijasevich A, Barros AJ, et al. Role of ultra-processed food in fat mass index between 6 and 11 years of age: a cohort study. Int J Epidemiol 2021;50:256–65.
[43] Vedovato GM, Vilela S, Severo M, Rodrigues S, Lopes C, Oliveira A. Ultra-processed food consumption, appetitive traits and BMI in children: a prospective study. Br J Nutr 2021;125:1427–36.
[44] Buuren Sv, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. J Stat Software 2010:1–68.
[45] Rubin DB. Multiple imputation for nonresponse in surveys. John Wiley & Sons; 2004.
[46] Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genet Epidemiol: The Official Publication of the International Genetic Epidemiology Society 2008;32:361–9.
[47] Lorenzoni G, Di Benedetto R, Silano M, Gregori D. What is the nutritional composition of ultra-processed food marketed in Italy? Nutrients 2021;13:2364.
[48] Steen J, Loeys T, Moerkerke B, Vansteelandt S. Medflex: an R package for flexible mediation analysis using natural effect models. J Stat Software 2017;76.
[49] Vansteelandt S, Bekaert M, Lange T. Imputation strategies for the estimation of natural direct and indirect effects. Epidemiol Methods 2012;1:131–58.
[50] Handakas E, Lau CH, Alfano R, Chatzi VL, Plusquin M, Vineis P, et al. A systematic review of metabolomic studies of childhood obesity: state of the evidence for metabolic determinants and consequences. Obes Rev 2021:e13384.
[51] Würtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation 2015;131:774–85.
[52] Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. Ann Intern Med 2014;160:398–406.
[53] Wu JH, Lemaitre RN, King IB, Song X, Psaty BM, Siscovick DS, et al. Circulating omega-6 polyunsaturated fatty acids and total and cause-specific mortality: the Cardiovascular Health Study. Circulation 2014;130:1245–53.
[54] Arsenault BJ, Lemieux I, Després J-P, Gagnon P, Wareham NJ, Stroes ES, et al. HDL particle size and the risk of coronary heart disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. Atherosclerosis 2009;206:276–81.
[55] Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, et al. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation 2009;119:2103.
[56] Haase CL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. HDL cholesterol and risk of type 2 diabetes: a Mendelian randomization study. Diabetes 2015;64:3328–33.
[57] Schmidt MI, Duncan BB, Bang H, Pankow JS, Ballantyne CM, Golden SH, et al. Identifying individuals at high risk for diabetes: the Atherosclerosis Risk in Communities study. Diabetes Care 2005;28:2013–8.
[58] Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med 2007;167:1068–74.
[59] Würtz P, Mäkinen V-P, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, et al. Metabolic signatures of insulin resistance in 7,098 young adults. Diabetes 2012;61:1372–80.
[60] Li X, Xu Z, Lu X, Yang X, Yin P, Kong H, et al. Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry for metabonomics: Biomarker discovery for diabetes mellitus. Anal Chim Acta 2009;633:257–62.
[61] Guasch-Ferré M, Hruby A, Toledo E, Clish CB, Martínez-González MA, Salas-Salvadó J, et al. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Diabetes Care 2016;39:833–46.
[62] Pallares-Méndez R, Aguilar-Salinas CA, Cruz-Bautista I, del Bosque-Plata L. Metabolomics in diabetes, a review. Ann Med 2016;48:89–102.
[63] Prieto-Merino D, Tillin T, Ghorbani A, Artati A, Wang Q, Tiainen M, et al. Metabolite profiling and cardiovascular event risk. 2015.
[64] Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol 2014;10:723.
[65] Monteiro CA, Cannon G, Levy RB, Moubarac J-C, Louzada ML, Rauber F, et al. Ultra-processed foods: what they are and how to identify them. Publ Health Nutr 2019;22:936–41.
[66] Ribeiro GJS, de Araújo Pinto A. Consumption of ultra-processed foods in Brazilian children: an analysis of regional trends. J Pediatr Nurs 2021;61:e106–11. https://doi.org/10.1016/j.pedn.2021.06.006.
[67] Araya C, Corvalán C, Cediel G, Taillie L, Reyes M. Ultra-processed food consumption among Chilean Preschoolers is associated with diets promoting non-communicable diseases. Front Nutr 2021;8:127.
[68] Steele EM, Batis C, Cediel G, da Costa Louzada ML, Khandpur N, Machado P, et al. The burden of excessive saturated fatty acid intake attributed to ultra-processed food consumption: a study conducted with nationally representative cross-sectional studies from eight countries. J Nutr Sci 2021:10.
[69] Krauss RM, Kris-Etherton PM. Public health guidelines should recommend reducing saturated fat consumption as much as possible: debate consensus. Am J Clin Nutr 2020;112:25–6.
[70] Parada Venegas D, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, et al. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Front Immunol 2019;10:277.
[71] Chambers ES, Preston T, Frost G, Morrison DJ. Role of gut microbiota-generated short-chain fatty acids in metabolic and cardiovascular health. Current nutrition reports 2018;7:198–206.
[72] Posma JM, Garcia-Perez I, Frost G, Aljuraiban GS, Chan Q, Van Horn L, et al. Nutriome–metabolome relationships provide insights into dietary intake and metabolism. Nature food 2020;1:426–36.
[73] Evans G, de Challemaison B, Cox DN. Consumers' ratings of the natural and unnatural qualities of foods. Appetite 2010;54:557–63.
[74] Leandro JG, Espindola-Netto JM, Vianna MCF, Gomez LS, DeMaria TM, Marinho-Carvalho MM, et al. Exogenous citrate impairs glucose tolerance and promotes visceral adipose tissue inflammation in mice. Br J Nutr 2016;115:967–73.
[75] Branco JR, Esteves AM, Leandro JGB, Demaria TM, Godoi V, Marette A, et al. Dietary citrate acutely induces insulin resistance and markers of liver inflammation in mice. J Nutr Biochem 2021;98:108834.
[76] Sadler CR, Grassby T, Hart K, Raats M, Sokolović M, Timotijevic L. Processed food classification: Conceptualisation and challenges. Trends Food Sci Technol 2021;112:149–62.
[77] Dicken SJ, Batterham RL. The role of diet quality in mediating the association between ultra-processed food intake, obesity and health-related outcomes: a review of prospective cohort studies. Nutrients 2021;14:23.
[78] Rauber F, Martins C, Azeredo C, Leffa P, Louzada M, Levy R. Eating context and ultraprocessed food consumption among UK adolescents. Br J Nutr 2021:1–11.