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Science & Technology in childhood Obesity Policy

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D3.3: Report on urinary metabolomics assessment of diet

Author(s): Professor Gary Frost, Imperial College London

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Dissemination Level

PU	Public	☒
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Abbreviation	Definition
NCDs	Non-communicable diseases
WHO	World Health Organisation
HELIX	Human Early Life Exposome

Science and Technology in childhood Obesity Policy

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1 Summary

Background: The burden of non-communicable diseases, such as obesity, diabetes, coronary heart disease and cancer, can be reduced by the consumption of healthy diets. Accurate monitoring of changes in dietary patterns in response to food policy implementation is challenging. This is particularly true in children given the length of expose to the environment Metabolic profiling allows simultaneous measurement of hundreds of metabolites in urine, many of them influenced by food intake. We aim to use metabolite profiling to classify children according to dietary behaviour and compare this to food frequency questionnaire.

Methods: We have previously developed a dietary metabolomic scoring methodology. based on the World Health Organisation's healthy eating guidelines (increase fruits, vegetables, wholegrains, dietary fibre and decrease fats, sugars, and salt). Here we apply this technology to 1200 children the Helix cohort. We used ^{1}H-NMR spectroscopy previously collected in Helix to achieve this.

Findings: We were able to demonstrate variability in metabolomic score with the greater number of children's diets classified as unhealthy. We were unable to show any relationship between the metabolite score and the output from the FFQ or body weight. We did see a relationship between the metabolomic healthy eating score and percentage body fat.
Limitations: There is need for an independent nutritional biomarker to further understand relationship between the metabolomic dietary score and dietary intake
Interpretation: At the present time the metabolomic dietary score indicated that the great portion of children have an unhealth diet. We were unable to demonstrate a relationship between the metabolomic score and the FFQ output.

2 Introduction

Consumption of "western dietary patterns" (high in saturated fat, cholesterol, sodium, added sugars; low in fruit, vegetables, fibre) increases the risk of obesity and many non-communicable diseases (NCDs), including diabetes, coronary heart disease and cancers. Overall dietary "patterns" may be more informative about non-communicable disease risk than individual foods or nutrients. Many governments have introduced population-based policies aiming to improve dietary patterns and reduce disease burden. These policies have a common core (reflected in the WHO Global Strategy on Diet Physical Activity and Health6) of decreasing added sugar, sodium and total fat consumption, and increasing intakes of wholegrain cereals, fruits, vegetables and fibre. The North Karelia project demonstrated that such dietary change can contribute to decreased coronary disease at the population level.

A major limitation of nutritional science is the objective assessment of dietary intake in free-living populations. Monitoring dietary change in national surveys and large prospective studies relies on self-reported food intake using instruments such as food-frequency questionnaires, dietary recall and diet diaries, with prevalence of misreporting estimated at 30–88%. This is compounded by bias in dietary misreporting (with underreporting biased towards 'unhealthy' foods and over-reporting towards fruits and vegetables) contributing to inaccuracy and data misinterpretation. Underreporting dietary energy intake is exacerbated in obese individuals, a major concern considering the increasing prevalence of obesity globally.

Established dietary biomarkers such as urinary sodium, potassium and nitrogen track intake of specific nutrients only. Currently there is no independent, objective methodology for assessing overall dietary patterns in free-living populations. Recently metabolomic analysis has been applied to the Helix cohort with relationships between individual metabolites which are associated with food

components and food items in the FFQ analysis been demonstrated child urinary proline betaine and fruit intake, meat with creatine and taurine and TMAO with fish intake (Lau CE, et al. BMC Med. 2018 Nov 8;16(1):202. doi: 10.1186/s12916-018-1190-8). The Frost group has recently developed a metabolomic dietary assessment tool that in adults reports a health eating dietary pattern in adults (Garcia-Perez et al, Lancet, 2017). Here we apply this tool to the Helix dataset to understand the relationship between the metabolomic dietary score and the food frequency questionnaire.

3 Methodology

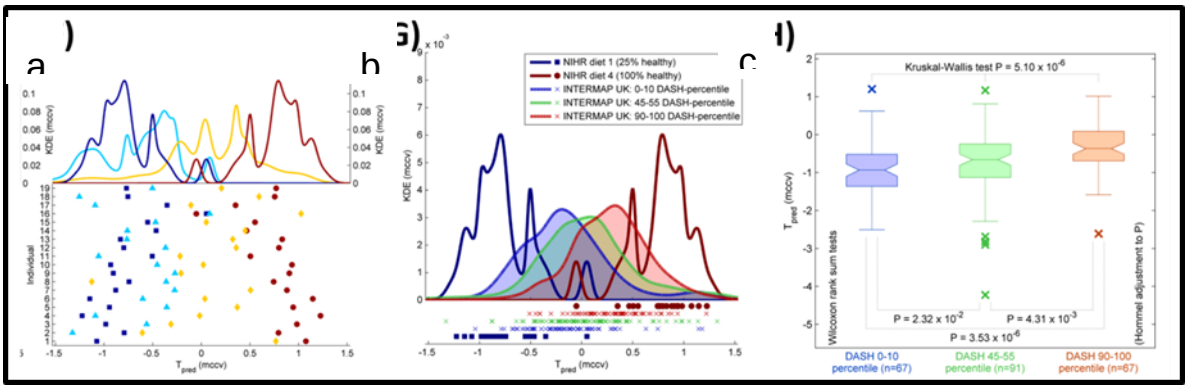
To develop this, we have analysed urinary metabolite profiles to objectively assess the overall diet quality (Garcia-Perez et al, Lancet, 2017) of 1,200 children from six European countries participating in the HELIX cohort. We have tested the resulting metabolite profile against the international FFQ that has been developed for Helix, to identify nutrient consumption and assess how self-report bias varies by country, age, parental education and socioeconomic status. Here we report the initial findings of the application on the metabolomic dietary assessment tool

Data set: The aim of the Human Early Life Exposome (HELIX) study was to measure and describe multiple environmental exposures during early life (pregnancy and childhood) in a prospective cohort and associate these exposures with molecular omics signatures and child health outcomes. The HELIX study represents a collaborative project across six established and ongoing longitudinal population-based birth cohort studies in six European countries (France, Greece, Lithuania, Norway, Spain and the UK). From these cohorts, simultaneous follow-up was used to develop a sub cohort of 1301 mother-child pairs where biomarkers, omics signatures and child health outcomes were measured at child age 6–11 years. Common, standardised protocols were used to collect biological samples, measure exposure biomarkers and omics signatures and assess child health across the six cohorts. Interviews with the mothers used a computer-aided version of a common standardised questionnaire developed for HELIX.

NMR Data from Helix: NMR spectroscopy was carried out on the two spot urine samples (one taken at bed time the other first void). In the case of this analysis only the first void urine samples were used. Method has been recently been published (Lau CE . BMC Med. 2018 Nov 8;16(1):202. doi: 10.1186/s12916-018-1190-8)

NMR Dietary assessment tool: This has been previously published Garcia-Perez et al, Lancet, 2017. In brief we have developed a metabolic profiling methodology which allows independent assessment of dietary profile. To achieve this, we developed metabolic profiling models from precisely known intakes in the study conducted in a metabolic research unit. Volunteers were exposed to diets which were 25%, 50%, 75% and 100% concomitant WHO healthy eating recommendations (Lancet Endocrinology and Diabetes 2017) for a four-day period on four occasions. Clear differences in the urinary metabolic profiles were observed comparing the 100% and 25% healthy diets.

The model constructed using urinary spectra from individuals in the extreme (healthy vs unhealthy) diet groups studied in a controlled environment. The model constructed) was used to predict the 24-h urinary metabolic profiles of volunteers after following 4-days of strict adherence to the intermediate diets (50% or 75% healthy). Samples clustered according to the healthiness of the diet, with a linear gradient from least to most healthy. Furthermore, the same model was subsequently used to predict healthy eating habits in free-living people whose diet was considerably more varied than those volunteers participating in the controlled nutritional trial (Figure 1 a,b and c)



3.1 Figure 1.

(a) Model of 25% DI (■) versus 100% DI (●),predicts the 50% healthy DI (▲) and 75% healthy DI (♦). The figure indicates there is a linear trend between the four DIs from unhealthy (negative T_{pred}) to healthy (positive T_{pred}). **(b)** Applicability of the model (A) in predicting healthy eating in three diverse groups from a free-living population (INTERMAP UK). Top shows the predicted distributions of the 25% (blue) and 100% healthy DI (red) and the three INTERMAP UK groups defined by low (×), middle (×) and high (×) DASH scores. **(c)** Boxplot of T_{pred} shows a linear trend across the three INTERMAP UK groups and that all differences between the three groups are statistically significant.

We calculated a predictive score, T_{pred}, from a Monte Carlo Cross-Validated model (1,000 iterations) of controlled clinical trial data. The T_{pred} is indicative of how a metabolite profile relates to the profiles of 100% and 25% diet that were consumed in a highly controlled environment that assured fully adherence to intervention diet. The T_{pred} ranges roughly from -3 to 3; a positive T_{pred} indicates the metabolite profiles resembles more the diet with higher concordance with WHO healthy eating guidelines, whereas a negative T_{pred} is reflective of less concordance with WHO guidelines. The Tpred score is then converted to a Healthy Index score by comparing the position of the individual Tpred to the ranking gained from the original study.

Statistical Analysis: After extracting variables of interest from the HELIX data set were run by either Kruskal-Wallis or Wilcoxon rank sum tests, as all of the potential predictors were categorical and the exposure variables were not normally distributed. The variables that yielded a p-value lower than 0·2 in bivariate analyses were selected to enter into the multiple linear regression models. To ensure normality of the distributions of the outcome variables, the univariate linear regression models and subsequent multiple linear regression models were built using loge-transformed variables.

4 Result

Description of the cohort

Table 1 below gives the descriptive data of the cohort analysis.

4.1 Table 1

Descriptive data of the Helix cohort presented as medium and interquartile range as the diet did not conform to a normal distribution.

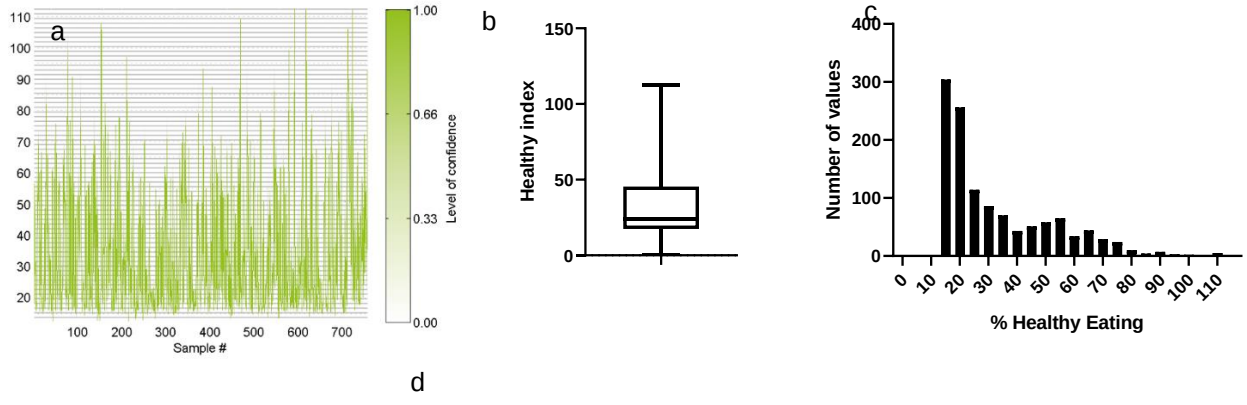
	Males		Females		Total	
	Medium	IQR	Medium	IQR	Medium	IQR
Number	655		537		1192	
Age yrs	7.5	2.4	7.8	2.3	7.4	6.5
BMI	16.3	2.8	16.7	2.8	16.3	2.7
FM	5.2	3.9	5.9	2.8	5.5	3.8
FFM	22.8*	6.9	16.4*	2.8	21.2	6.7
Kidmed	3	2	3	2	3	2

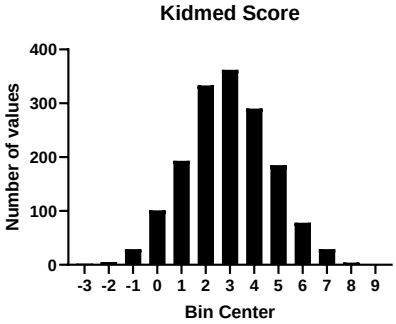
*significant difference between males and females P<0.01

4.2 Figure 2

Summary of metabolomic data expressed as a healthy index is shown below. Graph a is an example of the variability in metabolite Healthy Index score in 700 of the Helix children. Graph b is a summary box plot of the data. There is a large spread in the data with the medium falling below the 50% scale of the metabolomic healthy eating scale. Graph c clearly demonstrates that the Metabolomic healthy eating score is not normally distributed, but it does demonstrate that in larger number of children have a score below 50% update World Health Organization healthy eating targets. This is in contrast to the normal distribution of the Kidmed Score shown in Graph d. This difference in distribution we have observed in the number of adult cohorts (unpublished) where there is a discrepancy Between the self-reported diary score and that of the metabolite score. In other cohorts we have been able to demonstrate in those that report a healthy eating profile from self-reported dietary intake but a poor metabolite score have worse glycaemic control.

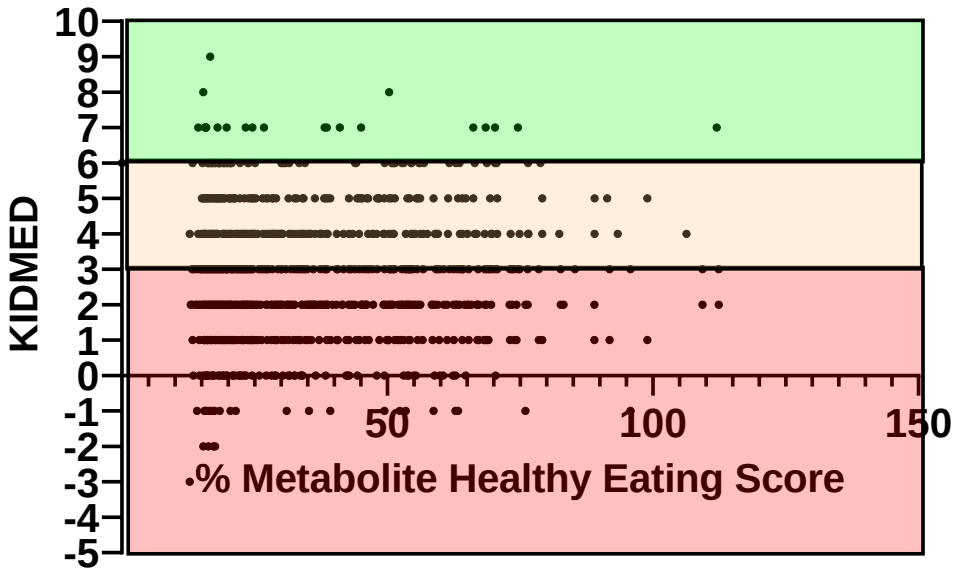
Figure 2 summary of metabolomic data expressed as a healthy index, Graph (a) is a representative plot of the individual variation in the metabolomic healthy eating score. Graph (b) shows the box and whisker of the metabolomic healthy eating score, Graph (c) shows the distribution of the metabolomic healthy eating scores, Graph(d) the distribution of the Kidmed Score





4.3 Figure 3

A scatter plot of the relationship between the % metabolite score and the Kidmed score. Red represent a Kidmed score associated with healthy eating, yellow a score which is moderate healthy eating and green is aligned with recommendation



Next, we compared the metabolomic healthy eating score to the Kidmed score. The Kidmed score had been derived from the FFQ data as a representative score of compliance to a healthy intake. Here we used Spearman's correlation. There was no significant relationship between the Kidmed score and the metabolomic healthy eating score. Table 2 shows a correlation matrix to explore initial relationship between Tpred and metabolomic healthy eating score (MHES) and anthropometry and Table 3 the relationship with measures from the FFQ. We demonstrate a weak correlation between the Tpred score both waist measure and waist and fat free mass but no relationship this the measures from the FFQ.

4.4 Table 2:

Correlation matrix using Spearman's correlation to investigate relationship between Tpred, metabolomic health eating score (MHES) and anthropometric measures

	Maternal BMI	Maternal Age	Age	Waist	Fatmas	Non fatmass	Kidmed score	FAS score	Tpred1	MHES
Maternal BMI	1.000	-0.032	-.293**	.163**	.251**	.234**	-.123**	-.139**	0.034	0.031
Maternal Age	-0.032	1.000	.118**	.051*	0.018	0.007	.119**	.138**	0.021	0.037
Age	-.293**	.118**	1.000	-0.006	-0.002	-0.004	.107**	.339**	-0.008	-0.012
Waist	.163**	.051*	-0.006	1.000	.525**	.489**	-0.010	-0.010	.065*	0.046
Fatmas	.251**	0.018	-0.002	.525**	1.000	.933**	-0.044	-0.036	0.056	0.040
Non fatmass	.234**	0.007	-0.004	.489**	.933**	1.000	-.063*	-.063*	.061*	0.047
Kidmed score	-.123**	.119**	.107**	-0.010	-0.044	-.063*	1.000	.094**	0.005	0.011
FAS score	-.139**	.138**	.339**	-0.010	-0.036	-.063*	.094**	1.000	-0.023	-0.033
Tpred1	0.034	0.021	-0.008	.065*	0.056	.061*	0.005	-0.023	1.000	.957**
MHES	0.031	0.037	-0.012	0.046	0.040	0.047	0.011	-0.033	.957**	1.000

*=<0.05 **=<0.01

4.5 Table 3.

Correlation matrix using Spearman's correlation to investigate relationship between Tpred and the metabolomic health eating score (MHES) and dietary measures from the FFQ

	Tpred1	MHES	Total Vegetable intake	Total Fruit intake	Total Meat intake	Sweet intake	Soda intake	Pulse intake	Chicken intake	KIDMED
Tpred1	1.000	.957**	0.018	-0.041	-0.054	-0.024	-0.001	0.013	-0.018	0.005
MHES	.957**	1.000	0.033	-0.036	-0.051	-0.016	-0.002	0.029	-0.009	0.011
Total Vegetable intake	0.018	0.033	1.000	.405**	.051*	0.015	-0.033	.060*	-.099**	.455**
Total Fruit intake	-0.041	-0.036	.405**	1.000	-0.006	.081**	-0.015	.072**	-.053*	.485**
Total Meat intake	-0.054	-0.051	.051*	-0.006	1.000	.058*	.091**	0.039	.441**	.053*
Sweet intake	-0.024	-0.016	0.015	.081**	.058*	1.000	.292**	-.109**	0.046	-.100**
Soda intake	-0.001	-0.002	-0.033	-0.015	.091**	.292**	1.000	-0.044	0.048	-.127**
Pulse intake	0.013	0.029	.060*	.072**	0.039	-.109**	-0.044	1.000	.191**	.289**
Chicken intake	-0.018	-0.009	-.099**	-.053*	.441**	0.046	0.048	.191**	1.000	-0.036
KIDMED	0.005	0.011	.455**	.485**	.053*	-.100**	-.127**	.289**	-0.036	1.000

*=<0.05 **=<0.01

To investigate if the relationship between the metabolite score was confounded by covariance, we understood a stepwise linear regression. We demonstrate no relationship between the Metabolite Healthy Eat score and the Kidimed score, the Kidmed dietary profile score, total fruit and vegetable intake, fat mass and zBMI score. There was no relationship between any of these measurement

4.6 Table 4

Regression analysis exploring the relationship between the percent metabolic healthy eating score (MHES) and the Kidmed dietary profile score, total fruit and vegetable intake (total V and F), fat mass and zBMI score

	Kidmed Score		Total V and F		Total Sugar		Fatmass		Z score BMI	
Model	B	Sig.	B	Sig.	B	Sig.	B	Sig.	B	Sig.
1	0.003	0.257	0.001	0.955	-0.009	0.331	0.001	0.346	0.001	0.668
2	0.003	0.245	0.001	0.966	-0.009	0.321	0.001	0.346	0.001	0.672
3	0.003	0.240	0.001	0.940	-0.009	0.322	0.001	0.339	0.001	0.632
4	0.003	0.208	0.003	0.876	-0.010	0.301	0.001	0.345	0.001	0.615
5	0.003	0.193	0.003	0.863	-0.010	0.289	0.001	0.355	0.001	0.622
6	0.004	0.171	0.003	0.848	-0.010	0.267	0.001	0.444	0.000	0.788

Model 1 Percent MHES

Model 2 Percent MHES, age

Model 3Percent MHES, age, sex

Model 4 Percent MHES, age, sex, FAS score

Model 5 Percent MHES, age, sex, FAS score, breast fed

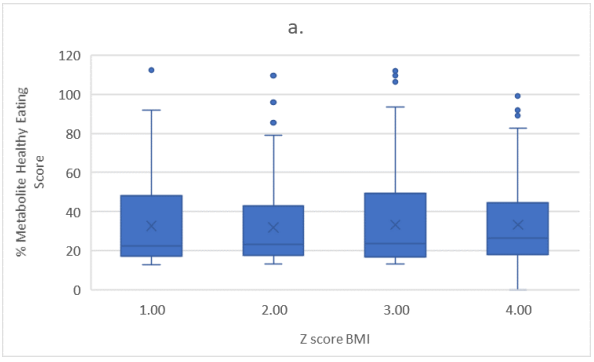
Model 6 Percent MHES, age, sex, FAS score, breast fed, maternal weight

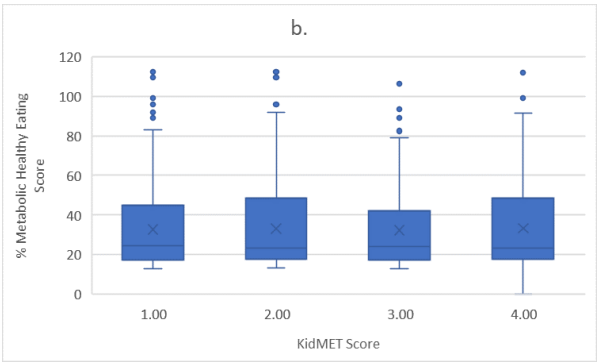
To explore if the relationship between the metabolite score metabolomic health eating score and the FFQ was affected by covariant we undertook step wise liner regression analysis these are presented in table 4. There is no significant difference between the groups on non-parametric ANOVA testing

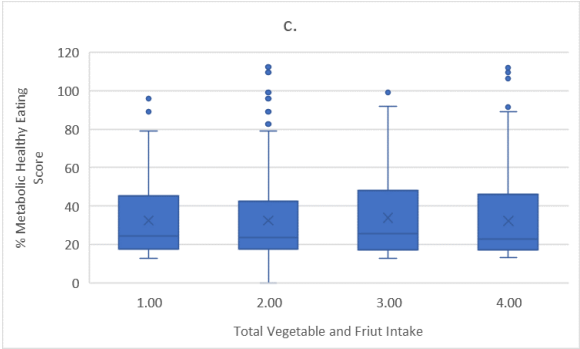
Quartile analysis did not demonstrate any relationship between the fruit and vegetable intake, Kidmed score, measures of body composition. The three graphs shown below in Figure 4 are examples of this analysis showing that there is no change across the quartiles

4.7 Figure 4

Quartile analysis presented as box and whisker plots of example variables in the HELIX data set and the percentage healthy eating score. a. Z score of BMI, b. Kidmed score, c. fruit and vegetable intake. The is no significant difference between ground on non parametric ANOV testing







5 Limitation

At this stage the major limitation is need for an independent nutritional biomarker to demonstrate the robustness of the metabolite scoring. This is under discussion with the team at the moment.

6 Discussion

We have been able to demonstrate that an NMR based metabolite score produces a model that varies across individual. The results from the Healthy Eating Metabolite Score suggests that the dietary profile is less healthy than that estimated by the Kidmed score from the FFQ. Previous studies using the HELEX data has demonstrated a relationship between individual food metabolites and dietary measures used in the FFQ (Lau CE, etal . BMC Med. 2018 Nov 8;16(1):202. doi: 10.1186/s12916-018-1190-8). At present it is not possible to demonstrate a relationship between the Metabolite Healthy Eating Score and the Kidmed score or the fruit and vegetable intake estimated from the FFQ. The reason for this lack of relationship is unknown given that that metabolomic dietary profiling has shown strong relationships to dietary intake in adults (Garcia etal 2017). There is a need to explore the possibility of using an independent nutritional biomarker to understand further the percentage healthy eating index. The analysis of the relationship of the Metabolite Dietary Score and the metadata is still ongoing.

7 Further work

This analysis needs to be complimented by a standard biological urinary nutyrition biomarker to assess the accuracy of the metabolite healthy eating score.