SUPPLEMENTARY MATERIAL

Metabolome biomarkers linking dietary fibre intake with its cardiometabolic effects: Results from the Danish Diet, Cancer and Health - Next Generations MAX study

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Index

Supplementary discussion

The phenolic lipid 2,6-DHBA is one the main metabolites that we found associated with dietary fibre. However, 2,6-DHBA has barely been reported as a biomarker related with dietary fibre consumption, and its specific sources are yet unknown. It has previously been described to be a marker related to the intake of oat and sugar beet fibre (1), and it has also been shown to be a heat stable phenolic compound present in white grape pomace (2). It is thought to be a microbial metabolite primarily derived from alkylresorcinols or lignans fermentation, although the specific microbial enzymes have not been identified in humans yet (3). In a US diet validation study 2,6-DHBA was found to be a reproducible biomarker associated with wholegrain with a reported ICC of 0.62 in evaluations 6 months apart (4). Moreover, following an untargeted plasma metabolomics approach, Johansson-Persson et al. (3) observed an increase in 2,6-DHBA, as well as in 2-aminophenol-sulfate, after a 5-week crossover dietary intervention with a high fibre diet consisting of oat bran, rye bran, and sugar beet fibre in 25 healthy adults. On the other hand, 2-aminophenol-sulfate was also described to be increased in urine in a randomized, controlled crossover trial with an 8-week dietary intervention comparing a wholegrain diet vs. a refined grain diet (5). Nonetheless, in our study, plasma 2,6-DHBA and 2-aminophenol levels were associated between them and with the consumption of wholegrain cereals. Therefore, we may suggest that 2,6-DHBA and 2-aminophenol may be somehow associated with dietary fibre coming from wholegrain cereals; although, we cannot out rule other dietary, host or gut microbial sources.

Indolepropionic acid, a tryptophan metabolite generated by the gut microbiome, has been reported to be associated with the intake of fruits (6), total dietary fibre (7), and higher adherence to different healthy dietary patterns, such as the Healthy Nordic Diet (8), and the French dietary recommendations (9). Indeed, it has been hypothesized that a high intake of dietary fibre could cause a higher production of indolepropionic acid through modifications of gut microbiota composition or activity (10) (11). Importantly, indolepropionic acid levels have been associated with lower likelihood of developing type 2 diabetes, better insulin secretion and low-grade inflammation (10). Given the close relationship between dietary fibre and intake of fruits and vegetables, our results on the association between indolepropionic and dietary fibre intake were mostly confirmatory(12). Nonetheless, herein we report for the first time a moderate reliability and consistency over time of plasma indolepropionic acid levels, with an ICC of 0.52 during a one-year period, underscoring its potential use in more and larger epidemiological studies.

Other metabolites associated with fibre intake in the present study were 3,4-DHBA, linoleoyl-carnitine, ethyl-glucuronide, and proline betaine. To our knowledge, 3,4-DHBA has not been reported as a biomarker associated specifically with dietary fibre. 3,4-DHBA is a phenolic compound widely distributed in many food plants such as olives, white grapes, blackberries and strawberries, but also in beans flours (13) and wholegrain buckwheat (14,15) Accordingly, in our

analysis, 3,4-DHBA was significantly associated with total fruits and legumes. 3,4-DHBA is considered a major metabolite of complex polyphenols, especially anthocyanins (16), and it is thought to be generated by colonic microbiota by B-ring fission of cyanidin-O-glycoside (17). It has been reported to have a strong in vitro and in vivo antioxidant and anti-inflammatory activity (18), to decrease the expression of lipogenic enzymes (19), and to increase glucose uptake in adipocytes (20) among others, but the link of this compound with dietary fibre intake and its metabolic effect is still unclear.

Proline betaine has been consistently proposed as a candidate biomarker for citrus intake (21). Indeed, it has shown to accurately predict orange juice intake measured in 24 h and fasting urine samples, and to provide predictions of citrus intake in good agreement with self-reported intakes (22). Unlike other betaines, proline betaine cannot be synthesized endogenously, and diet is the only source (23). Similar to indolepropionic acid, an observational study showed a positive association between plasma proline betaine levels and higher adherence to the Healthy Nordic Diet (8). In our study, the association between proline betaine and dietary fibre was not consistent across the different time point evaluations. One possible explanation is that in the present study consumption of citrus fruits had a mean contribution of 13% to total fruits intake. However, disregarding its association with dietary fibre, proline betaine levels may reflect a particular role of citrus fruits in host cardiometabolic health that deserves further studies. Linoleoyl-carnitine is another compound significantly associated with dietary fibre in this study. Little is known about its relationship with overall dietary fibre intake. Linoleoyl-carnitine is an acyl-carnitine derived from linoleic acid. Acyl-carnitines play an important role in the mitochondrial energy metabolism, but circulating acyl-carnitines have been speculated to be a marker of detoxification and removal of excess fatty acids (24). Their circulating concentration have also found to be influenced by diet and specific nutrients. Specifically, linoleoyl-carnitine have been associated with the intake of the omega-6 fatty acid linoleic acid, probably coming from vegetable oils used as salad dressings (25). However, in our analysis it was specifically associated with wholegrain cereals, legumes, and vegetables. Last, ethyl-glucuronide has been proven as a biomarker of alcohol consumption (26,27). This negative association could be explained by the fact that plant-foods rich in fibre are not usually linked with alcohol consumption, but rather foods of animal origin as observed in a French cohort (28).

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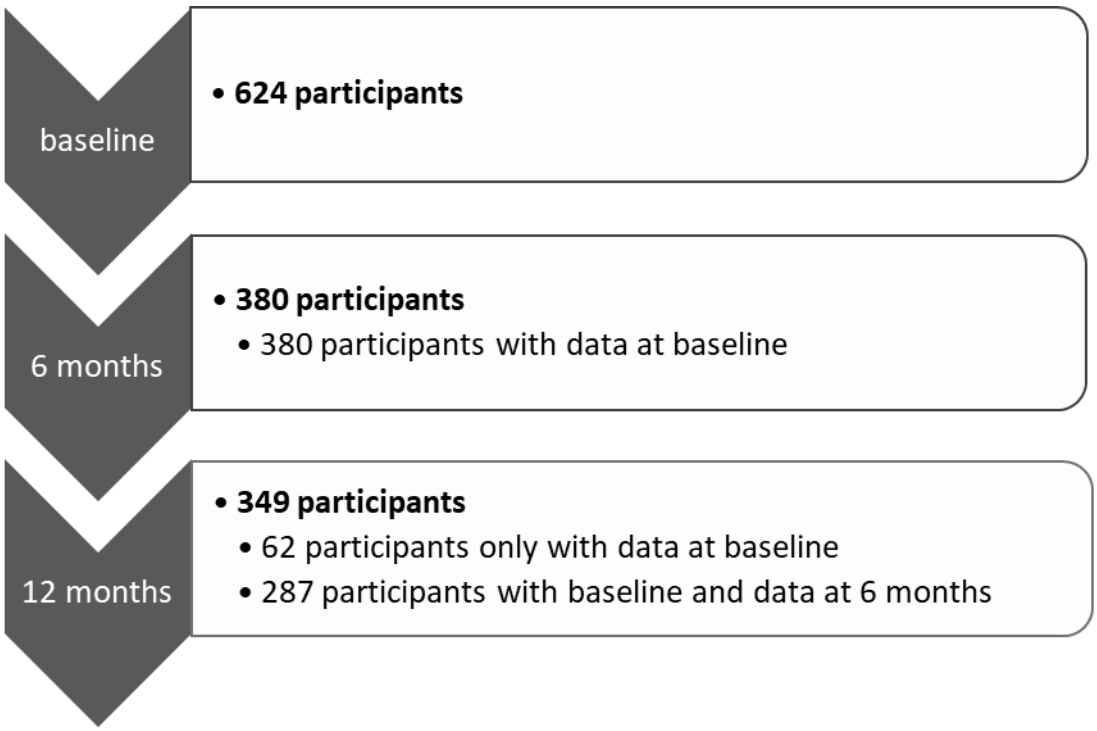
Supplementary Table 1. Dietary characteristics of the DCH-NG MAX study population according to tertiles of dietary fibre intake.

	All n= 624 k=1,353	Fibre <16g/day (T1) k= 452	Fibre: 16-25g/day (T2) k= 450	Fibre >25g/day (T3) k= 451
Dietary characteristics				
Energy (10^3 kcal/d)	2.1 ± 0.8	1.7 ± 0.6	2.1 ± 0.6	2.6 ± 0.9 *
Total fats (% of Energy)	35 ± 9	35 ± 11	36 ± 8	34 ± 8
SFA (% of Energy)	11 ± 5	12 ± 5	12 ± 4	10 ± 4 ***
MUFA (% of Energy)	12 ± 5	12 ± 5	13 ± 5	12 ± 4
PUFA (% of Energy)	6.0 ± 2.8	5.6 ± 3.1	6.0 ± 2.5	6.5 ± 2.5 ***
Total carbohydrates (% of Energy)	43 ± 10	41 ± 12	43 ± 9	45 ± 9 ***
Total sugars (% of Energy)	14 ± 8	14 ± 9	14 ± 7	14 ± 6
Protein (% of Energy)	17 ± 6	18 ± 6	17 ± 5	16 ± 5 ***
Dietary fibre (g/d)	21 ± 10	11 ± 4	20 ± 2	33 ± 8 ***
[g/1000 kcal]	[11 ± 5]	[7 ± 4]	[11 ± 4]	[14 ± 5] ***
Alcohol (g/d)	0 (0 - 13)	0 (0 - 18)	0 (0 – 14)	0 (0 – 14)
Sodium (g/d)	3.0 ± 1.7	2.4 ± 1.4	3.0 ± 1.4	3.7 ± 2. 1 **
Food intake				
Cereal whole grain (g/d)	115 (45-200)	45 (0-90)	122 (70-185)	204 (129-285) ***
Cereal refined (g/d)	45 (0-125)	45 (0-120)	50 (0-142)	40 (0-124)
Fruits (g/d)	150 (0-313)	100 (0-250)	148 (3-270)	210 (54-400) ***
Vegetables (g/d)	140 (30-290)	74 (0-170)	133 (20-265)	255 (130-411) ***
Legumes (g/d)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0) *
Nuts and seeds (g/d)	0 (0-10)	0 (0-0)	0 (0-8)	0 (0-30) ***

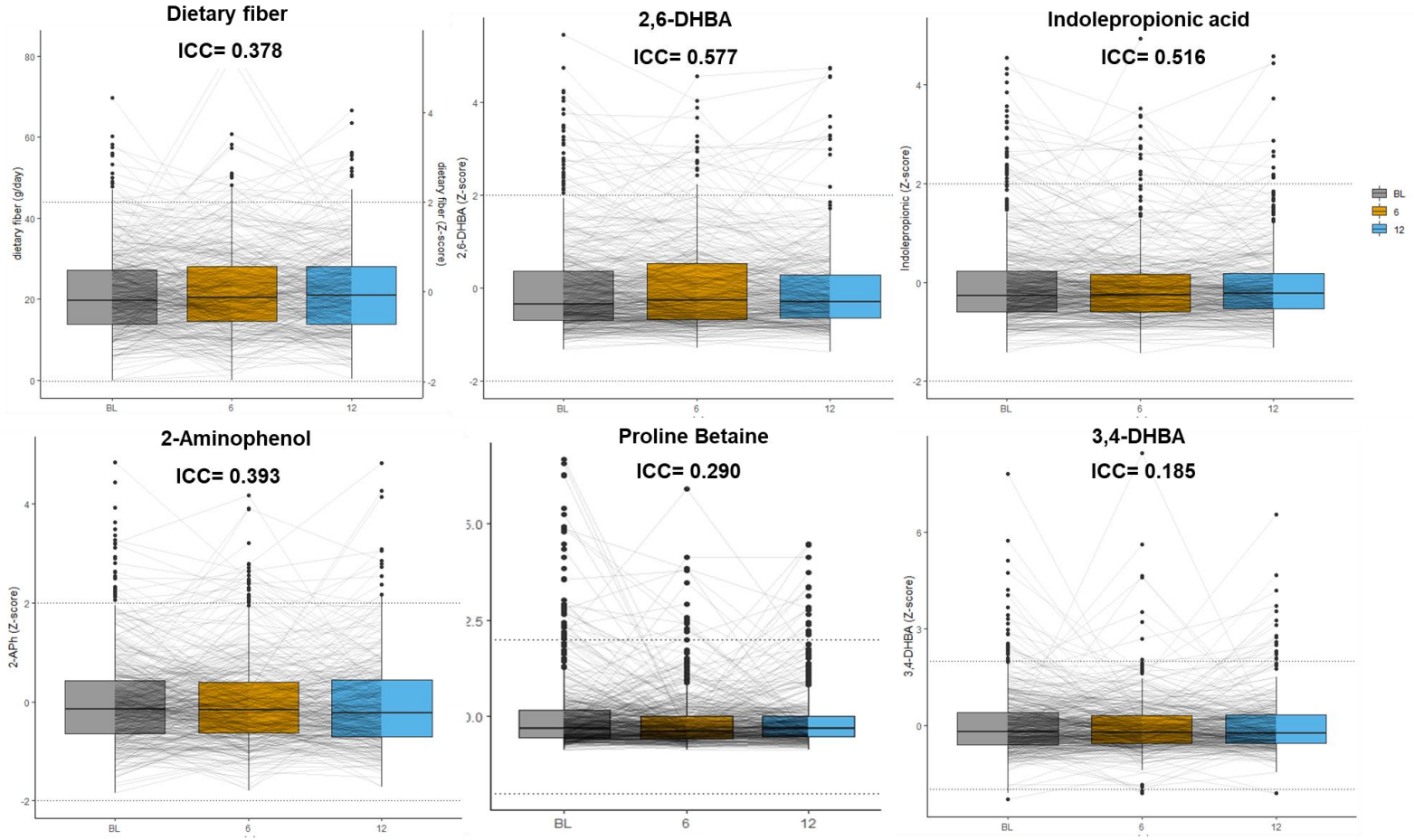
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Mean and standard deviation, or median (Q1-Q3) were used to describe continuous variables following a Gaussian or skewed distribution, respectively. *****p for trend<0.05, ** p for trend<0.01, *** p for trend<0.001 using age- and sex-adjusted generalized linear models.

Variables with skewed distribution were log-transformed before entering the analyses. n=participants, k=observations.

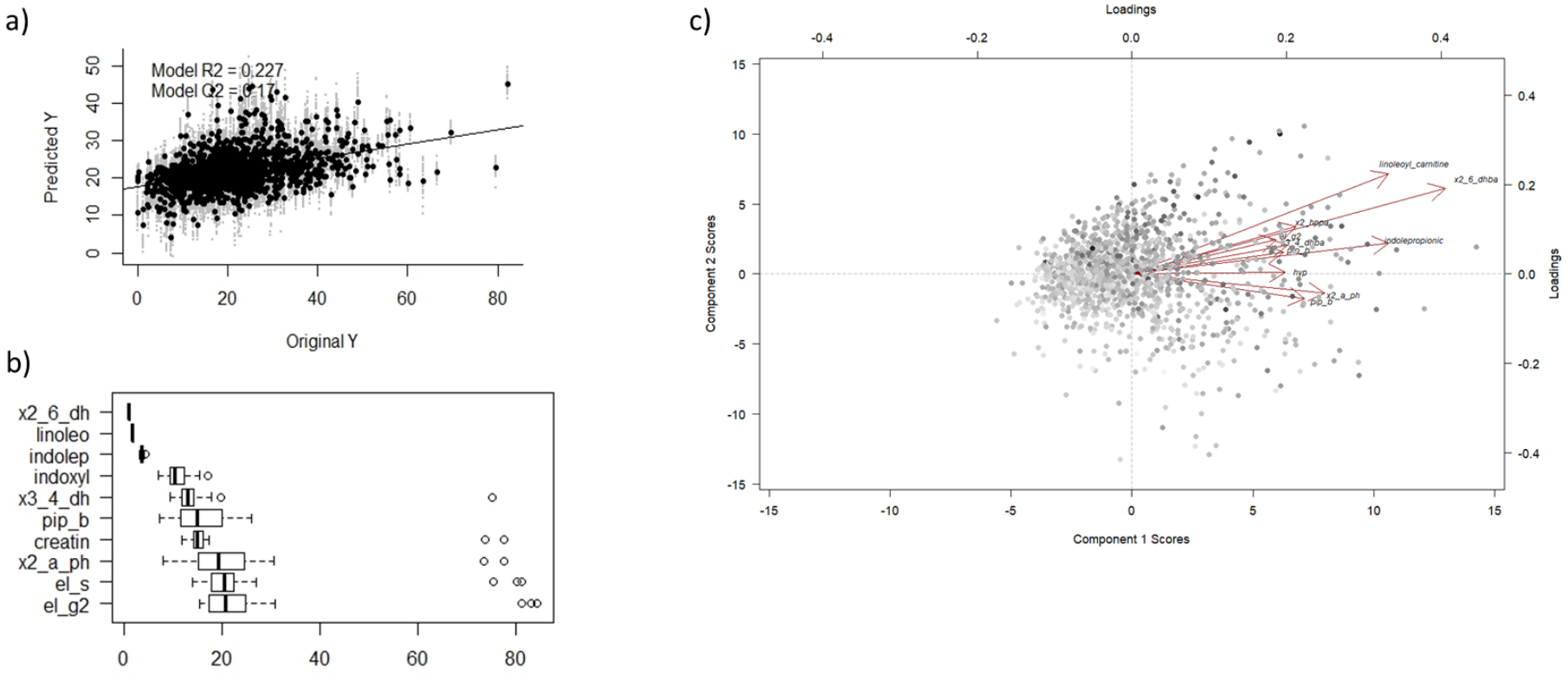
Supplementary Figure 1. Flowchart of participants at each time-point.



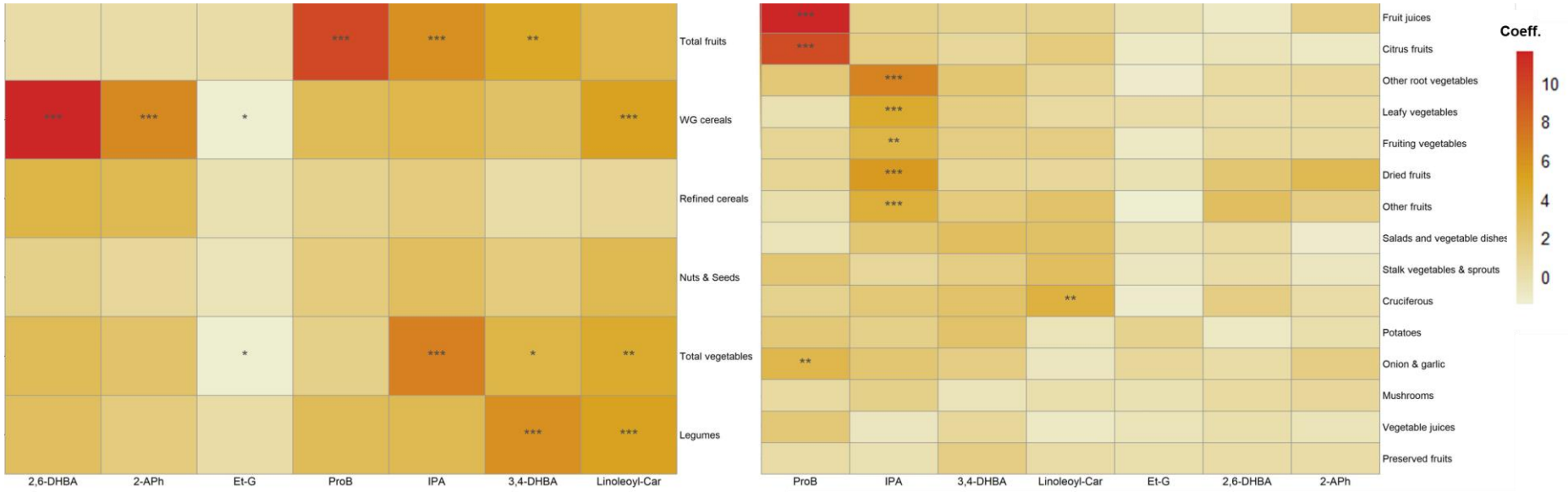
Supplementary Figure 2. Changes in dietary fibre intake and selected metabolites during the one-year study period.



Box plots and spaghetti plots including all the data from all the participants available (n=624, k=1,353). Intraclass correlation coefficients were calculated from linear mixed models adjusted for age, sex, BMI and time with random intercepts. n=participants, k=observations



Supplementary Figure 3. MUVR-PLS analysis showing metabolites associated with dietary fibre intake (n=624, k=1,353). a) Predicted fibre intake and self-reported fibre intake according to MUVR-PLS. b) Boxplot of the variable importance rank per-repetition ($p<0.0001$). Lower rank means higher importance. c) Loading plot of the PLS model. x2_6_dh, 2,6-dihydroxybenzoic acid; linoleo, linoleoyl carnitine; indolep, indolepropionic acid; indoxyl, indoxyl sulfate; x3_4_dh, 3,4-dihydroxybenzoic acid; pip_b, pipecolic acid betaine; x2_a_ph, 2-aminophenol; el_s, enterolactone-sulfate; el_g2, enterolactone-glucuronide.



Supplementary Figure 4. Heatmap showing the association between metabolites and food groups in the DCH_NG MAX study. Coefficients calculated using linear mixed models in the whole study population (n=624, k=1,353). 2,6-DHBA, 2,6-dihydroxybenzoic acid2-APh, 2-Aminophenol; 3,4-DHBA, 3,4-dihydroxybenzoic acid; IPA, indolepropionic acid; ProB, proline betaine; Car, carnitine; Et-G, ethyl-glucuronide. *****FDR-adjusted p-value<0.05, ** FDR-adjusted p-value<0.01, *** FDR-adjusted p-value <0.001 using age-, sex- and time-adjusted linear mixed models with random intercepts. n=participants, k=observations. Contributions to total fibre intake of the major food groups were: Total fruits (12%), WG cereals (41%), refined cereals (12%), Nuts & Seeds (5%), Total vegetables (22%), and Legumes (1%).