

Dietary intake of fat and fibre according to reference values relates to higher gut microbiota richness in overweight pregnant women

Henna R  yti  ¹, Kati Mekkala¹, Tero Vahlberg² and Kirsi Laitinen^{1*}

¹*Institute of Biomedicine, University of Turku and Turku University Hospital, 20014 University of Turku, Turku, Finland*

²*Department of Clinical Medicine, Biostatistics, University of Turku and Turku University Hospital, 20014 University of Turku, Turku, Finland*

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Abstract

The diet–microbiota–metabolism relationships during pregnancy are mostly unknown. We explored the effect of the habitual diet and adherence to the dietary reference values on gut microbiota composition and diversity. Further, the association of gut microbiota with serum lipidomics and low-grade inflammation was evaluated. Overweight and obese women (BMI 30.7 (SD 4.4) kg/m², *n* 100) were studied at early pregnancy (≤17 weeks). Intakes of nutrients were calculated from 3-d food diaries. Faecal microbiota composition was analysed using 16S rRNA gene sequencing. Fasting serum lipidomic profiles were determined by NMR. High-sensitivity C-reactive protein, glycoprotein acetylation (GlycA) and lipopolysaccharide activity were used as markers for low-grade inflammation. The recommended dietary intake of fibre and fat was related to higher gut microbiota richness and lower abundance of Bacteroidaceae. Correlations were observed between gut microbiota richness and GlycA and between a few microbiota genera and serum lipoprotein particles. As a conclusion, adherence to the dietary reference intake of fat and fibre was associated with beneficial gut microbiota composition, which again contributed to lipidomic profile. Higher gut microbiota richness and nutrient intakes were linked to a lower level of low-grade inflammation marker GlycA. This finding offers novel insights and opportunities for dietary modification during pregnancy with potential of improving the health of the mother and the child.

Key words: Diets: Fibres: Fats: Microbiota richness: Relative abundance: Glycoprotein acetylation: Low-grade inflammation

Human gut microbiota contributes to health by regulating host metabolism. Dysbiotic composition and decreased diversity of this microbial organ are linked to inflammatory diseases of the gut and to systemic conditions, such as obesity and type 2 diabetes^(1,2). Of particular significance are the possible health effects of maternal microbiota composition and function on the health of both the mother and child.

Pregnancy may affect the mother's gut microbiota composition and richness⁽³⁾. A recent study suggest that the colonisation of a child's gut begins *in utero*⁽⁴⁾, and as early microbiota may affect a child's health in later life, maintaining balanced microbiota in the mother during pregnancy is important. Pregnancy is a particularly critical period of life in which the health of the fetus is consolidated⁽⁵⁾, and the effects of pregnancy can be observed in the offspring throughout later life⁽⁶⁾. Furthermore, metabolic complications manifested during pregnancy also affect the mother's long-term health⁽⁷⁾. Thus, pregnancy offers a window of opportunity to improve the health of both the mother and child by potentially modifying gut microbiota composition and activity. To this end, a better understanding of the diet–microbiota–metabolism relationship during pregnancy is warranted.

Although the adult gut microbiota is generally considered consolidated, external factors, such as antibiotic treatment, diseases and diet, may modify the composition and activity of the gut ecosystem⁽⁸⁾. Diet and dietary patterns have significant effects on the composition of the microbiota and the metabolites produced. Short-term dietary interventions are shown to rapidly alter the structure and function of the microbiota: for example, the inclusion of high-fibre or high-fat intakes, as well as increased protein and animal-based foods in dietary interventions, results in rapid changes in the relative abundance of microbial phyla and genera in study subjects^(9–11). Moreover, long-term dietary patterns containing high amounts of fibre, fruit and vegetables lead to the accumulation of carbohydrate-digesting bacteria in the gut^(12,13), and the original African diet⁽¹⁴⁾ and the Mediterranean diet⁽¹⁵⁾ promote a different type of microbiota compared with that promoted by the 'typical' Western diet. Thus, diets that vary heavily in their content of plant-derived fibre and animal-derived fat and protein may significantly affect the composition and divergence of gut microbiota. The current Nordic nutritional recommendations encourage people to consume more fruit, vegetables and whole-grain products to increase their fibre

Abbreviations: E%, percentage of total energy intake; GlycA, glycoprotein acetylation; hs-CRP, high-sensitivity C-reactive protein; LC-PUFA, long-chain PUFA; LPS, lipopolysaccharide; OTU, operational taxonomic units.

* **Corresponding author:** K. Laitinen, email kirsi.laitinen@utu.fi

intake and to consume more fish, nuts, seeds and vegetable oils and to limit their intake of SFA⁽¹⁶⁾. A generally healthy and diverse diet with a high-fibre and moderate-fat intake is also considered beneficial regarding microbiota composition⁽¹⁷⁾ and richness⁽¹³⁾. Nevertheless, the effects of the recommended diet on microbiota during pregnancy have not yet been investigated to a large extent.

In addition to gut microbiota richness and composition, differing dietary components and variability of the micronutrients and macronutrients may affect the metabolites produced by the gut microbiota. Microbes convert dietary ingredients into new molecules that interact with the host and generate a repertoire of signals that have systemic effects. For example, SCFA produced mainly from dietary carbohydrates are important sources of energy for epithelial cells locally, having thus a beneficial impact on intestinal epithelial integrity. In our previous study, we observed that higher dietary intake of fibre was related to lower concentration of serum zonulin – that is lower intestinal permeability⁽¹⁸⁾. SCFA are also related to the release of satiety hormones, which play a role in food digestion, insulin actions and feelings of satiety⁽¹⁷⁾. Although not well understood, certain metabolites can have negative or positive effects on systemic health depending on the microbiota composition and its fermentation capabilities^(19,20). One means of evaluating the metabolic functionality of the microbiota is to measure the metabolic profile from serum.

An exploration of the interactions of the diet with microbiota and of microbiota with serum metabolic activity may offer novel insights and opportunities for dietary manipulation with the aim of improving the health of the mother and child. The aim of this study was to explore the effects of habitual diet and adherence to the recommended diet on gut microbiota, serum lipidomics and low-grade inflammation and further the relationship of gut microbiota composition to serum lipidomics and inflammatory markers in overweight and obese pregnant women.

Methods

In all, 100 overweight women in early pregnancy (≤ 17 weeks of gestation) were included in this study, of which complete data for microbiota, diet and lipidomics were available from eighty-eight subjects. The women were participating in an ongoing mother–infant dietary intervention trial (ClinicalTrials.gov, NCT01922791) conducted in southwest Finland. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Hospital District of Southwest Finland (permission no. 115/180/2012). Written informed consent was obtained from all subjects. The samples and data were collected at their first study visit, which served as the baseline of the intervention trial. The characteristics of the women are shown in Table 1.

Gut microbiota profiling

Faecal samples from mothers were collected in sterile plastic pots. Samples were collected the morning of the study visit or

Table 1. Characteristics of the pregnant, overweight women (*n* 100) (Mean values and standard deviations)

	Mean	SD
Age (years)	30.1	4.7
Week of gestation	13.3	2.4
Pre-pregnancy BMI (kg/m ²)	30.2	4.6
Proportion of overweight (BMI 25–30 kg/m ²)	51	
Proportion of obese (BMI ≥ 30 kg/m ²)	49	
Education		
Proportion of highly educated (college or university degree)	50	

the previous evening, delivered to the study unit and kept at +4C until DNA extraction. DNA was extracted and the DNA samples were sequenced as previously described⁽¹⁸⁾ (the Sequencing and Bioinformatics Service at the Fundaci  n para el Fomento de la Investigaci  n Sanitaria y Biom  dica de la Comunitat Valenciana, Valencia, Spain).

Raw sequences, 41 000–118 000/sample, were processed using the QIIME software package. Operational taxonomic units (OTU) were picked at 97% similarity against the GreenGenes database and matched with known bacterial genomes to identify members of the faecal community. On the basis of the sequences, a total of 731 OTU were detected, and the relative abundance was determined using these OTU. The α diversity, measured as Chao1, observed OTU, phylogenetic diversity (PD) and the Shannon index⁽²¹⁾, was calculated using α rarefaction values at sequence 36 382. The abundance of bacteria $>1\%$ of total microbiota was considered to be reliable and taken into further analyses.

Dietary intake

Food diaries, 3 d, were recorded by the women within the week before the study visit. The subjects were provided oral and written instructions regarding how to record food intake, and during the study visit the diaries were checked for completeness and accuracy with the help of a portion picture booklet. Mean daily intakes of energy, energy-yielding nutrients (primary measure) and vitamins and minerals were calculated using computerised software (Aivo diet 2.0.2.3; Aivo Finland Oy).

Serum metabolites

On the morning of the study visit, a 10-h fasting blood sample was drawn from the antecubital vein of the participants. Lipidomics and glycoprotein acetylation (GlycA) were quantified from serum samples using a commercial high-throughput proton NMR metabolomics platform (Brainshake Ltd). Details of the experimentation and applications of the NMR metabolomics platform have been previously described^(22,23).

The fourteen lipoprotein subclass sizes were defined as follows: six VLDL subclasses (particle diameters 75 nm (XXL) and upwards and average particle diameters of 64.0 (XL), 53.6 (L), 44.5 (M), 36.8 (S) and 31.3 (XS) nm); intermediate-density lipoproteins (28.6 nm); three LDL subclasses (25.5 (L), 23.0 (M) and 18 (S) nm); and four HDL subclasses (14.3 (XL), 12.1 (L), 10.9 (M)

and 8.7 (S)nm). The mean sizes of the VLDL, LDL and HDL particles were calculated by weighing the corresponding subclass diameters with their particle concentrations.

High-sensitivity C-reactive protein (hs-CRP) was determined using an automated colorimetric immunoassay on the Dade Behring Dimension RXL autoanalyzer (Siemens Healthcare), GlycA was determined by NMR, serum lipopolysaccharide (LPS) activity measured by Limulus amoebocyte lysate assay and zonulin by ELISA, as reported in Mekkala *et al.*⁽²⁴⁾. GlycA is a novel inflammatory biomarker that is composed of a complex of heterogeneous NMR signal containing *N*-acetyl sugar groups originating from multiple acute-phase circulating glycoproteins: α 1-acid glycoprotein, haptoglobin, α 1-antitrypsin, α 1-antichymotrypsin and transferrin⁽²⁵⁾.

Statistics

All of the statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.) and SPSS version 22.0 (IBM Inc.). The Kolmogorov–Smirnov test and graphical analysis of histograms was used to determine the normality of the data. Not all of the variables were normally distributed, and parametric and non-parametric tests were used accordingly. Pearson's and Spearman's correlations were used to determine the associations between parameters. *P* values (impact of dietary intake on relative abundance of gut microbiota and impact of gut microbiota on serum lipidomics) were adjusted for multiple comparison using the Benjamini–Hochberg method to control the false discovery rate at each taxonomic level. Similarly, when comparing the gut microbiota richness with serum lipidomics and the markers of low-grade inflammation with nutrient intakes Benjamini–Hochberg method was used. Adjusted $P < 0.05$ was considered significant in correlation analysis. The associations between dietary intakes and gut microbiota richness were also analysed after adjustment for pre-pregnancy BMI using linear regression analysis. In group comparisons, Tukey's honest significant difference (HSD) tests were performed following significant one-way ANOVA for comparing dietary intake and serum zonulin concentration and Mann–Whitney *U* tests using Bonferroni corrections following significant Kruskal–Wallis tests for comparing inflammatory markers, serum lipidomics and relative abundance of gut microbiota and gut microbiota richness among the three diet groups. When comparing the relative abundance of the gut microbiota and lipidomics among the three dietary groups in Kruskal–Wallis test, the bacteria with adjusted $P < 0.05$ were considered significant and were chosen for pairwise comparison by Mann–Whitney *U* test. This study was an exploratory analysis of the observational study, and thus no calculations for required sample size were performed.

Results

Dietary intake of nutrients relates to microbiota diversity and composition and low-grade inflammation

The intakes of dietary fibre (g) and fat (as percentage of total energy intake (E%)) were correlated with indexes describing the

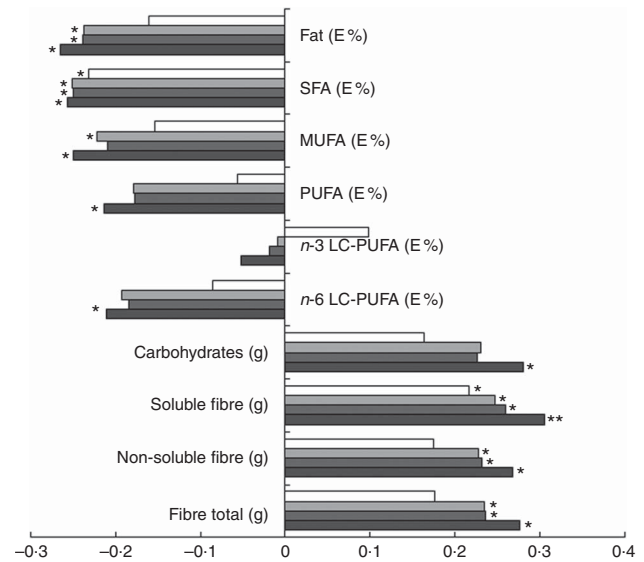


Fig. 1. Pearson's correlations between microbiota diversity and richness (Shannon index (□), observed number of operational taxonomic units (■), Chao 1 (■) and phylogenetic diversity (■)) and the intakes of energy-yielding nutrients and dietary fats (percentage of total energy intake (E%)) and carbohydrates and fibre (g/d). LC-PUFA, long-chain PUFA. Statistically significant: * $P < 0.05$, ** $P < 0.01$.

gut microbiota diversity and richness, as presented in Fig. 1. The intakes of fibre were consistently positively and the intakes of total fat and different fat types were negatively associated with gut microbiota diversity and richness. Dietary fat quality appeared to manifest diverse impact to microbiota as SFA were statistically significantly negatively associated with all indexes, whereas *n*-3 long-chain PUFA (LC-PUFA) showed no correlation. No statistically significant correlations were observed between protein or total energy intakes with indexes of gut microbiota richness. Further evaluations by linear regression analyses showed that after adjustment for BMI the observed associations remained statistically significant (online Supplementary Table S1).

The significant associations between gut microbiota and different dietary nutrients are shown in a heatmap (Fig. 2). Intriguingly, most striking correlations were found in intakes of fibre (g) and fat (E%). Fibre intake was correlated with the phylum Firmicutes in unidentified family of order Clostridiales and in the Barnsiellaceae family, belonging to the phylum Bacteroidetes. Negative correlations were found between the intake of fat (E%) and SFA (E%) and relative abundance in the family Barnsiellaceae.

Of the vitamins and minerals, fewer associations were detected compared with energy-yielding nutrients; the strongest correlations were seen for the phylum Firmicutes that correlated positively with the intake of vitamin A (vitamin A expressed as retinol activity equivalents, comprising the retinols and carotenoids) and β -carotene, although no associations remained statistically significant after adjusting for multiple testing. When evaluating the association between the intake of dietary nutrients and markers of low-grade inflammation, multiple nutrients correlated with GlycA including fibre, LC-PUFA and *n*-3 LC-PUFA and several vitamins and minerals (Table 2), but no correlations were detected between any of the nutrient and

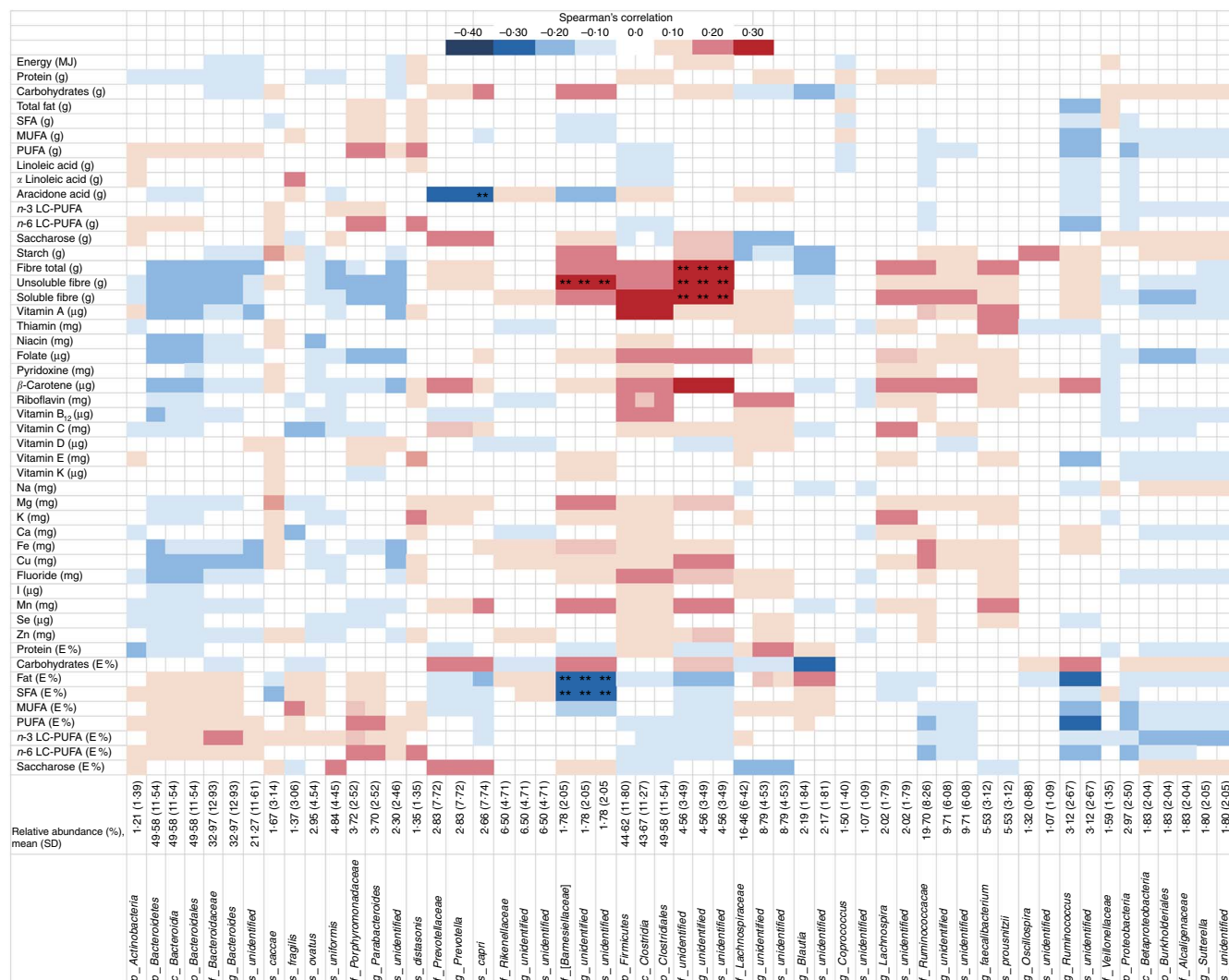


Fig. 2. Heatmap showing correlations between gut microbes and dietary nutrients (n 88). The intensity of the colours represents the degree of Spearman's correlation between the microbial groups and the nutrients as grams and percentage of total energy (E%). Red, positive correlation; Blue, negative correlation; LC-PUFA, long-chain PUFA. ** Benjamini-Hochberg-adjusted $P < 0.05$. Vitamin A: retinol activity equivalents comprising retinols and carotenoids. p, Phylum; c, class; o, order; f, family; g, genus; s, species.

hs-CRP (adjusted $P > 0.2$ for all; data not shown) and LPS (adjusted $P > 0.7$ for all; data not shown).

High-fibre and high-fat consumers differ significantly in their microbiota

Because of the opposing relations of dietary fibre and fatty acids on gut microbiota richness (Fig. 1), the microbiota data were further analysed in relation to adherence with nutritional recommendations. The subjects were grouped according to adherence to the dietary reference values⁽¹⁶⁾ (Table 3); group 1 was the low-fibre/moderate-fat group (n 57) and consisted of women whose fibre intake was below the reference value (< 25 g/d) and whose total fat intake was within the reference intakes (25–40 E%). Group 2 was the high-fibre/moderate-fat group (n 18), and these women consumed the recommended level of fibre (≥ 25 g/d), and the total fat intake was within the

reference intake. Group 3 was the low-fibre/high-fat group (n 13), and their fat intake exceeded the reference level of total fat intake – that is ≥ 40 % of total energy. Furthermore, their SFA consumption also exceeded the reference intake (< 10 E%), and the consumption of each fat type, that is, the SFA, MUFA and PUFA intakes, in addition to total fat, was statistically significantly higher than that in the other two groups. They also consumed less fibre and total carbohydrates than recommended (45–60 % of total energy), indicating that they had a high-fat and low-fibre diet. Total energy intake significantly differed among the groups: the high-fibre group had a higher energy intake than the other two groups.

The identified dietary groups were compared with respect to their gut microbiota richness and composition, and statistically significant differences were observed. Chao1 index (mean 406.2 (SD 44.4) *v.* 341.0 (SD 57.9), $P = 0.006$), PD (39.0 (SD 4.5) *v.* 31.3 (SD 6.7), $P = 0.003$) and observed number of OTU (355.8 (SD 38.7) *v.* 293.8 (SD 59.0), $P = 0.003$) were higher in the high-fibre/moderate-fat

group compared with the low-fibre/high-fat group, whereas low-fibre/moderate-fat group did not differ from the other groups (Chao 1 index: 380.0 (SD 57.3), PD: 35.8 (SD 5.9), observed number of OTU: 333.0 (SD 55.2), $P > 0.084$ for high-fibre/moderate-fat group, $P > 0.09$ for low-fibre/high-fat group). Moreover, the relative abundance of specific microbial phyla, families and genera were significantly different among the three dietary groups (online Supplementary Fig. S1). After adjustment for multiple testing, the difference among the dietary groups was evident in the Bacteroidaceae family: the high-fibre/moderate-fat group had a lower relative abundance of Bacteroidaceae (25.6 (SD 10.6)%) in comparison with the low-fibre/high-fat group (42.4 (SD 14.6)%, $P = 0.01$) and the low-fibre/moderate-fat group (33.2 (SD 11.9)%,

$P = 0.02$). Similar differences were detected in the genus *Bacteroides*, where differences were observed between high-fibre/moderate-fat and low-fibre/moderate-fat (25.6 (SD 10.6) v. 33.2 (SD 11.9)%, $P = 0.02$) and between high-fibre/moderate-fat and low-fibre/high-fat group (25.6 (SD 10.6) v. 42.4 (14.6)%, $P = 0.01$) (Table 4). No statistically significant differences were detected in markers of low-grade inflammation (hs-CRP: $P = 0.58$, GlycA: $P = 0.16$, LPS activity: $P = 0.1$, serum lipidomic variables (adjusted $P < 0.07$ after Kruskal–Wallis test) or zonulin concentration ($P = 0.40$) among the three diet groups (online Supplementary Table S2).

Gut microbiota richness correlates with low-grade inflammation

Higher Chao1 index ($r = -0.28$, $P = 0.007$, Spearman's correlation coefficients), PD ($r = -0.24$, $P = 0.022$) and observed number of OTU ($r = -0.27$, $P = 0.009$) correlated with lower concentration of GlycA, but no association was detected between any of the indexes of gut microbiota richness and hs-CRP ($P = 0.22$, $P = 0.27$ and $P = 0.26$, respectively) or serum LPS activity ($P = 0.82$, $P = 0.83$ and $P = 0.80$, respectively).

Gut microbiota correlates with serum lipidomics

Several correlations were observed (Fig. 3) between relative abundance of gut microbiota and serum lipidomics profiles, especially in genus levels of the family of Lachnospiraceae, which belongs to phylum Firmicutes. Opposite findings were found in genus level of this family: *Lachnospira* was negatively and *Blautia* positively correlated with concentrations of various sized VLDL particles and TAG in VLDL. Genus *Lachnospira* was also negatively associated with serum TAG. Genus *Blautia* was positively associated with VLDL diameter, but negatively with the diameters of LDL and HDL. No statistically significant correlations were detected between gut microbiota richness indexes and serum lipidomics variables (all adjusted $P > 0.59$, Spearman's correlation analysis).

Table 2. Statistically significant correlations between dietary intakes of nutrients and glycoprotein acetylation ($n = 95$)

	r^*	Unadjusted P	Adjusted P^\dagger
Energy	0.245	0.02	0.03
Fibre total	0.316	0.002	0.01
Non-soluble fibre	0.353	<0.001	0.009
Soluble fibre	0.323	0.001	0.01
Protein	0.301	0.003	0.01
PUFA	0.244	0.02	0.03
<i>n</i> -3 LC-PUFA	0.291	0.004	0.01
MUFA	0.230	0.02	0.04
Vitamin E	0.279	0.006	0.02
Pyridoxine	0.303	0.003	0.01
Niacin	0.302	0.003	0.01
Riboflavin	0.262	0.01	0.03
Thiamine	0.233	0.02	0.04
Cu	0.293	0.004	0.01
Ca	0.270	0.008	0.02
F	0.343	<0.001	0.009
Fe	0.289	0.004	0.01
Mg	0.351	<0.001	0.009
Mn	0.246	0.02	0.03
K	0.307	0.003	0.01
Se	0.256	0.01	0.03
Zn	0.256	0.01	0.03

LC-PUFA, long-chain PUFA.

* Spearman's correlation coefficient.

† Benjamini–Hochberg-adjusted P value.

Table 3. Daily intakes of energy, energy-yielding nutrients and fibre in the three identified diet groups‡ (Mean values and standard deviations)

	Low fibre/moderate fat ($n = 57$)		High fibre/moderate fat ($n = 18$)		Low fibre/high fat ($n = 13$)		All participants ($n = 88$)		Nutrition reference values ⁽¹⁶⁾
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Fibre (g)	17.6	4.5	29.0***	3.0	17.8†††	5.9	20.0	6.4	25–35
Fat (E%)	32.0	5.9	32.7	4.8	43.3***†††	2.3	33.8	6.6	25–40 E%
SFA (E%)	11.5	2.9	11.2	2.2	15.0***††	3.1	11.9	3.1	<10 E%
MUFA (E%)	11.0	2.4	10.9	2.3	15.4***†††	1.8	11.6	2.8	10–20 E%
PUFA (E%)	5.2	1.4	5.0	1.4	7.3***†††	1.8	5.5	1.6	5–10 E%
Carbohydrates (E%)	47.6	6.2	48.8	4.3	36.9***†††	4.2	46.3	6.8	45–60 E%
Protein (E%)	18.4	5.1	15.9	2.0	17.9	3.0	17.8	4.4	10–20 E%
Energy (MJ)	7.5	1.6	9.7***	1.4	8.3†††	1.7	8.1	1.8	

E%, percentage of total energy intake.

*** Significant difference between the low-fibre/moderate-fat group and the high-fibre/moderate-fat or low-fibre/high-fat group ($P < 0.001$; Tukey's honest significant difference test following a significant one-way ANOVA).

Significant difference between the high-fibre/moderate-fat and low-fibre/high-fat groups: †† $P < 0.01$, ††† $P < 0.001$ (Tukey's honest significant difference test following a significant one-way ANOVA).

‡ Only three subjects recorded a high daily intake of both fibre and fat: the two subjects with the lowest fibre intake (26.3 and 27.1 g/d) and the highest fat intake (43.2 and 41.9 E%) were placed in the high-fat group, and the subject with the highest fibre and the lowest fat intake (29 g/d and 41.7 E%) was placed in the high-fibre group.

Table 4. Relative abundance of gut microbiota between the three dietary groups (Mean values and standard deviations)

	Relative abundance (% of microbiota)						Kruskal–Wallis tests		Mann–Whitney <i>U</i> tests, <i>P</i> with Bonferroni corrections			
	Low fibre/moderate fat (<i>n</i> 57)		High fibre/moderate fat (<i>n</i> 18)		Low fibre/high fat (<i>n</i> 13)							
	Mean	SD	Mean	SD	Mean	SD	<i>P</i>	Benjamini–Hochberg-adjusted (<i>P</i>)	Low fibre/moderate fat v. high fibre/moderate fat	High fibre/moderate fat v. low fibre/high fat	Low fibre/moderate fat v. low fibre/high fat	
<i>o__Bacteroidales;f__Bacteroidaceae</i>	33.2	11.9	25.6	10.6	42.4	14.6	0.002	0.02*	0.02*	0.01*	0.10	
<i>g__Bacteroides</i>	33.2	11.9	25.6	10.6	42.4	14.6	0.002	0.03*	0.02*	0.01*	0.09	
<i>o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__copri</i>	2.0	5.6	6.7	13.5	0.003	0.003	0.05	0.25	1.00	0.05	0.10	
<i>o__Clostridiales;f__g__unidentified</i>	4.5	3.3	6.2	4.3	2.4	1.7	0.01	0.06	0.36	0.01	0.09	
<i>s__unidentified</i>	4.5	3.3	6.2	4.3	2.4	1.7	0.01	0.09	0.36	0.01	0.54	
<i>o__Clostridiales;f__Lachnospiraceae;g__Blautia</i>	4.5	3.3	6.2	4.3	2.4	1.7	0.01	0.20	0.36	0.01	0.06	
<i>s__unidentified</i>	2.4	2.2	1.4	0.9	2.4	0.9	0.01	0.09	0.09	0.01	0.09	
<i>o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus</i>	2.4	2.1	1.4	0.9	2.4	1.0	0.02	0.20	0.09	0.02	0.62	
<i>s__unidentified</i>	3.3	2.8	3.7	2.6	1.6	1.7	0.03	0.12	1.00	0.03	0.06	
	3.3	2.8	3.7	2.6	1.6	1.7	0.03	0.21	1.00	0.03	0.13	

* Significant *P* value that takes into account Benjamini–Hochberg adjustment.

Discussion

This study showed that diet composition, particularly fibre and fat, affects the gut microbiota diversity and relative abundance of gut microbes of overweight, pregnant women. Furthermore, specific bacterial groups were associated with serum lipoprotein concentrations. We also demonstrated that higher gut microbiota richness is negatively linked with low-grade inflammation marker GlycA, which was further related to intake of several nutrients including fibre and LC-PUFA. We did not observe similar relationship between hs-CRP and gut microbiota richness, suggesting that GlycA may have distinct inflammatory pathway compared with CRP. This study highlights the importance of diet in modulating both beneficially and detrimentally gut microbiota composition and again metabolism. This opens novel opportunities in designing approaches for diet modification to induce beneficial changes in microbiota because decreased diversity of the microbiota has been consistently linked to metabolic disturbances, such as obesity and type 2 diabetes^(26,27). Instead, high diversity is the hallmark of a well-functioning, healthy gut ecosystem⁽²¹⁾.

The dietary intake of the pregnant women was similar to that previously reported⁽²⁸⁾. In the present study, the deviations from the dietary reference intakes were most evident regarding intakes of fibre and SFA; most of the pregnant women had a fibre intake that was too low and a SFA intake that was too high. These factors also had the strongest correlations with the gut microbiota richness. The absolute daily intake of fibre was positively associated with increased gut microbiota richness, whereas the intakes of total fat, SFA, MUFA and *n*-6 LC-PUFA (E%) were inversely associated with these parameters. Similar associations were also observed when comparing the identified dietary groups: the high-fibre intake (high-fibre/moderate-fat group), and adherence to the dietary reference intake overall, was linked with a higher richness of the gut microbiota; however, a high-fat consumption together with low-fibre and low-carbohydrate consumption (low-fibre/high-fat group) was linked to significantly lower richness. Consistent evidence was found that, in addition to the total dietary fat intake, the quality of fat (particularly a high intake of SFA) may be potentially harmful. Negative associations were evident with SFA and all indexes describing the gut microbiota richness. *n*-3 LC-PUFA is of potential interest, as it appeared to differ from other fatty acid types with positive correlation with one of the richness indexes, although not statistically significantly. In previous studies, healthy eating patterns⁽¹³⁾ and dietary diversity⁽¹⁷⁾ have been associated with increased diversity of the gut microbiota. In addition, an experimental evidence indicates an impact of diet fat quality with microbiota composition⁽²⁹⁾; however, no effect on maternal gut microbiota composition at late pregnancy was detected when mothers consumed salmon, rich in PUFA, from mid-pregnancy until delivery⁽³⁰⁾.

When we evaluated the dietary nutrients and specific microbial groups, the most striking correlations were found for fibre and fat. Fibre intake was correlated with microbes of the Firmicutes and Bacteroidetes phylum; both negative and positive correlations were found in family and genus levels of Bacteroidetes phylum. After adjusting for multiple testing, the negative association between Bacteroidetes and fibre did not

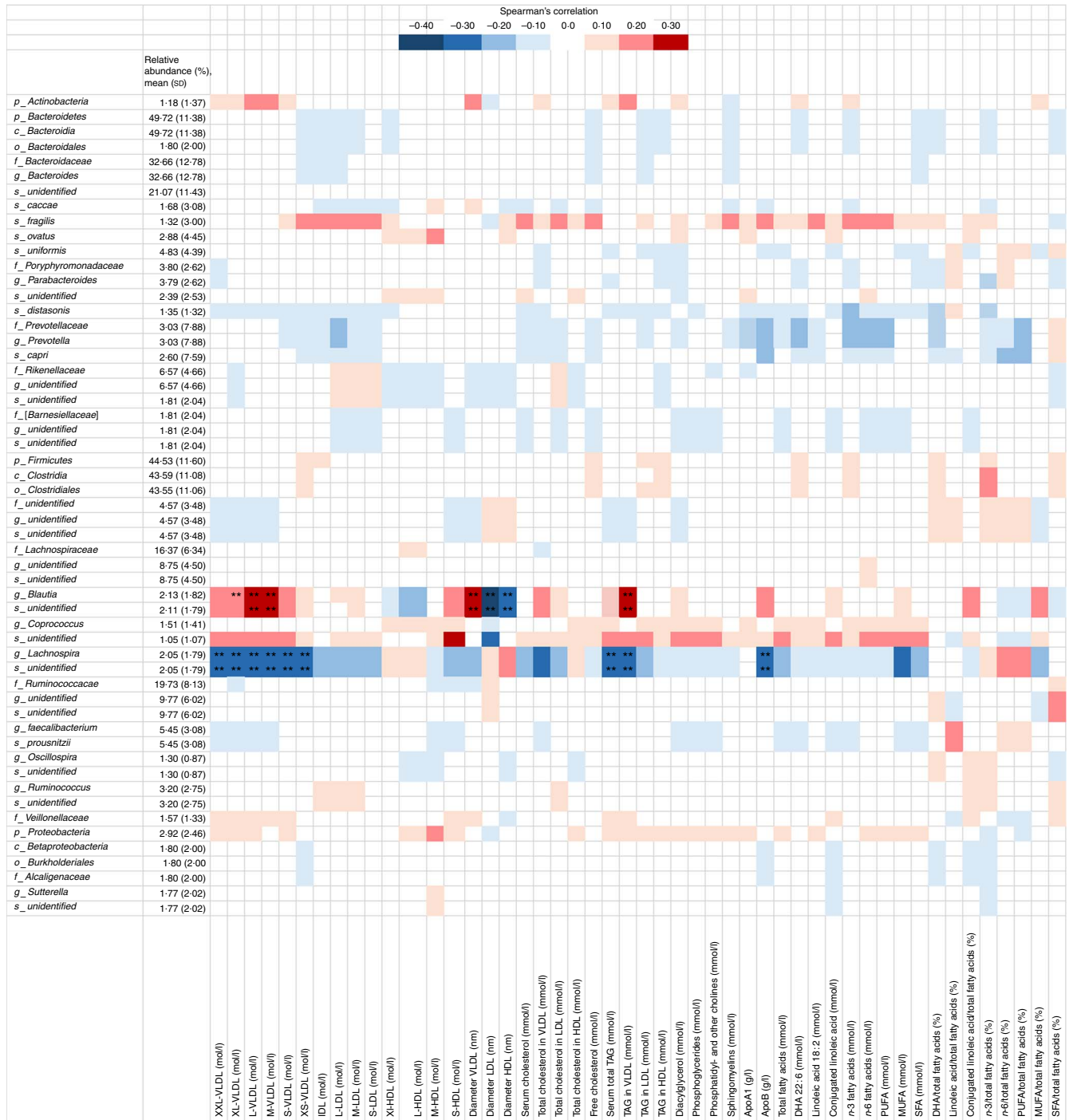


Fig. 3. Heatmap showing correlations between gut microbes and lipidomics (n 92) from the serum lipidomics analysis. The intensity of the colours represents the degree of Spearman's correlation between the genera and the nutrients. Red, positive correlation; blue, negative correlations; IDL, intermediate-density lipoproteins. ** False discovery rate <0.05. XXL, extremely large; XL, very large; L, large; S, small; L, large; M, medium; p, phylum; c, class; o, order; f, family; g, genus; s, species.

remain statistically significant; however, the lowest relative abundance of the family Bacteroidaceae, belonging to the phylum Bacteroidetes, was detected in the diet group with high-fibre/moderate-fat intake. Similar associations were detected in a previous study, in which Firmicutes were positively and Bacteroidetes negatively associated with fibre intake in non-pregnant population⁽¹²⁾. Of the association of specific microbiota on nutrient intake, Barnesiellaceae responded mostly on

nutrient intake; in addition to the negative correlation with fat intake, Barnesiellaceae showed a positive correlation with fibre. Also fibre intake was positively correlated with an unknown family of order Clostridiales. In a recent study with pregnant women, higher intake of vitamin D and retinol at the second trimester was associated with an increase in the relative abundance of *Proteobacteria* after delivery⁽³¹⁾. In our study, we did not observe any associations between relative abundances of

gut microbiota and vitamins, when conducting the analyses using robust multiple testing corrections.

When the serum lipidomics results were evaluated against the microbiota data, strongest associations were observed with Lachnospiraceae family, with genus *Lachnospira* having negative association with different lipoprotein particles and serum TAG and *Blautia* having a positive association with different lipoprotein particles. *Lachnospira* relative abundance was inversely associated with serum VLDL particles, which together with small-sized LDL particles are considered the most harmful types of lipoproteins in regard to atherosclerosis development⁽³²⁾. Recent findings have linked gut microbiota with lipid metabolism in humans and in mice, and causality has been supported by animal trials, as reviewed in Wang *et al.*⁽³³⁾. Another recent study⁽³⁴⁾ showed that the gut microbiota explains part of the variation in blood lipid concentrations in humans; the presence or absence of certain species in the gut may be the root cause for aberrant blood lipid levels.

We also detected a relation between gut microbiota and low-grade inflammation as higher microbiota richness was linked to lower level of GlycA. GlycA has been related to chronic inflammation⁽³⁵⁾ and clinical manifestations including type 2 diabetes and CVD in non-pregnant subjects^(36–38). GlycA has also been associated with metabolic risk markers in pregnant women⁽²⁴⁾. We also observed an association between lower intake of multiple nutrients, including fibre, and higher GlycA. Therefore, we would like to propose that the beneficial impact of higher dietary fibre intake may thus take place through the effect on gut microbiota richness. The other nutrients correlating with GlycA, including *n*-3 LC-PUFA and vitamins and minerals, may have immunomodulatory effects via different mechanisms; *n*-3 LC-PUFA serve as precursors for anti-inflammatory eicosanoid synthesis and vitamins act as antioxidants⁽³⁹⁾. Yet, the mechanisms may also be mediated through modulating gut microbiota composition⁽⁴⁰⁾. Intriguingly, we did not detect the relationship between microbiota or intakes of nutrients and serum LPS activity or the more traditionally used marker of low-grade inflammation, hs-CRP.

There are some limitations in the current study that should be considered when interpreting the results. First, the overweight study participants may have underreported their dietary intake⁽⁴¹⁾. In the comparison of the subject groups according to the adherence to the dietary recommendations, significant differences in total energy intake, but not in BMI or body weight, were observed among the three generated groups: total energy intake was the lowest in the low-fibre/moderate-fat group, and if this was a result of underreporting the group division may have been affected, and thus the observed differences in microbiota richness and composition. However, we used the proportional intake of energy-yielding nutrients (E%) as a primary measure, which is unlikely affected by underreporting. Indeed, the division of the subjects into three different dietary groups according to adherence to nutritional recommendations based on the food diary data resulted in significant differences in several measured parameters and was reflected in the richness and diversity, as well as composition, of the gut microbiota of these women. The benefit of the study setting is that the study population was very homogeneous; for example, none of

the women used metformin or any other medication. This fact is of importance because metformin treatment is known to cause uniform microbiome shifts in type 2 diabetic patients⁽⁴²⁾.

Pregnancy offers a window of opportunity to affect the health of both the mother and child. A disturbed balance of major microbial groups and the decreased numbers of, for example, anti-inflammatory species detected in obesity leads to an altered metabolite profile and anti- and proinflammatory factors produced by the microbiota^(2,26,43). During pregnancy, obesity and altered microbiota composition may be of high importance because these factors affect the health of the developing child⁽⁴⁴⁾ also shown in recent experimental study in obese rats⁽⁴⁵⁾. Therefore, targeting the maternal microbiota may offer opportunities to effectively and safely improve the long-term health of both the mother and child.

The findings from the current study support the association of diet and dietary patterns with the gut microbiota and further with a novel inflammatory marker, GlycA. Our results illustrate that adherence to dietary reference intake was linked to a richer gut microbiota, which further related to lower maternal inflammatory status, measured as GlycA. In addition, diet quantity, particularly absolute amount of fibre and fat consumed, and further the fat quality are related to the composition of the gut microbiota. Even subtle differences in the typical Western diet may result in significant differences in the gut microbiota of an otherwise homogeneous population; adherence to dietary reference intake was linked to a richer gut microbiota. Thus, dietary manipulation of the microbiota could be an effective and easily applicable means for improving the balanced microbiota during pregnancy, a crucial time that may have long-term effects on the future health of the next generation.

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K. L. and K. M. designed the study; H. R. contributed to the design; H. R. and K. M. analysed the data; H. R., K. M. and T. V. conducted statistical analyses; H. R. wrote the first draft of the paper; all the authors contributed to writing the paper and accepted the final version.

None of the authors has any conflicts of interest to declare.

Supplementary material

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114517002100>

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