Nutrition, Metabolism and Cardiovascular Diseases xxx (xxxx) xxx

Contents lists available at ScienceDirect

Nutrition, Metabolism and Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Links between gut microbiota with specific serum metabolite groups in pregnant women with overweight or obesity

Mrunalini Lotankar [a,b], Noora Houttu [a,b], Chouaib Benchraka [c], Leo Lahti [c], Kirsi Laitinen [a,b,d,*]

[a] Integrative Physiology and Pharmacology Unit, Institute of Biomedicine, Faculty of Medicine, University of Turku, Finland
[b] Nutrition and Food Research Center, Faculty of Medicine, University of Turku, Turku, Finland
[c] Department of Computing, Faculty of Technology, University of Turku, Turku, Finland
[d] Department of Obstetrics and Gynecology, Turku University Hospital, Wellbeing Services County of Southwest Finland, Turku, Finland

ARTICLE INFO

Handling Editor: Dr. A. Siani

Keywords:
Gut microbiota
Metagenomics
NMR metabolomics
Pregnancy
Overweight
Obesity

ABSTRACT

Background and aim: Gut microbiota may regulate metabolism but is incompletely characterized in pregnancy. Our objective was to investigate the relations using omics techniques.
Methods and results: In a cross-sectional setting, fecal and serum samples of 361 healthy pregnant women with overweight or obesity were analyzed with a combinatorial approach of metagenomics and targeted NMR-based metabolomics, with statistical and machine learning techniques to identify and analyze the extent to which the gut microbiota composition and predicted functions would be reflected in the serum metabolome. We identified five biclusters, each of which consisted of a set of gut microbial species and serum metabolites with correlated abundance profiles. Two of the biclusters included metabolites that have been linked to the cardiovascular health; one was linked with factors known to increase the risk i.e., various sizes of lipoprotein subclasses (VLDL and LDL), subclasses of relative lipoprotein lipid concentrations (VLDL, IDL, and LDL), apolipoprotein B, and an inflammation marker, glycoprotein acetylation. These metabolites were associated with abundances of species such as, *Enterocloster bolteae* and *Ruminococcus gnavus*. The second bicluster included metabolites linked with a reduced cardiovascular risk, such as different sizes of HDL (high-density lipoprotein), subclasses for relative lipoprotein lipid concentrations and mean diameter for HDL particles, and fatty acid ratios. These metabolites were associated with abundances of species, such as *Bacteroides cellulosilyticus* and *Alistipes finegoldii*. We did not observe any biclusters between predicted pathways and serum metabolites.
Conclusion: Overall, we identified five biclusters of co-abundant gut bacteria and serum metabolites , of which two were linked to pro-atherogenic and anti-atherogenic properties.
Trial registration: www.ClinicalTrials. Gov: NCT01922791.

1. Introduction

It is necessary that metabolic changes take place in pregnant women in order to ensure the flow of nutrients to the fetus and thereby to support its normal development and growth. Thus, modifications occur in glucose, lipid, and energy metabolism as well as in insulin sensitivity [1,2]. The maternal microbiota has also been shown to change in terms of diversity and abundances, these alterations have been related to the metabolic adaptations [3]. Maternal overweight and obesity status seem to contribute significantly to the metabolic aberrations as evidenced by results emerging from metabolomics studies. As compared to pregnant women with normal weight, those with obesity exhibited elevated levels of a variety of lipoprotein classes including all subclasses of very low-density lipoprotein (VLDL), small high-density (HDL) particles, monounsaturated fatty acids (MUFAs), saturated fatty acids (SFAs), branched chain as well as aromatic amino acids, and an inflammation

Abbreviations: BMI, body mass index; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; FAs, fatty acids; GlycA, glycoprotein acetylation; FDR, false discovery rate.

* Corresponding author. Kiinamyllynkatu 10, 20520, Turku, Finland.
E-mail address: kirsi.laitinen@utu.fi (K. Laitinen).

https://doi.org/10.1016/j.numecd.2025.104095
Received 27 June 2024; Received in revised form 10 April 2025; Accepted 14 April 2025
Available online 16 April 2025

0939-4753/© 2025 The Authors. Published by Elsevier B.V. on behalf of The Italian Diabetes Society, the Italian Society for the Study of Atherosclerosis, the Italian Society of Human Nutrition and the Department of Clinical Medicine and Surgery, Federico II University. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

marker, glycoprotein acetylation (GlycA) [4]. Maternal obesity may also induce changes in the gut microbiota including a decreased beta diversity and an altered bacterial composition [3,5]. These obesity-induced alterations in the metabolism and gut microbiota during pregnancy may have health consequences for both the mother and child e.g., an increased risk of gestational diabetes mellitus [6] and in the long-term, an elevated risk of type-2 diabetes [7].

Rather few studies have examined the gut microbiota-serum metabolite interaction in pregnant women with most focusing on either a specific health condition, e.g., glucose levels in hyperglycemia during pregnancy [8], serum metabolite profile in intrahepatic cholestasis in pregnant women [9], or alternatively with the association between the gut microbiota with a specific metabolite i.e., glycoalbumin levels, in healthy pregnant women [10]. One recent study investigated dynamics of gut microbiota and serum metabolomes during and after pregnancy longitudinally [11]. There is another report of an association between the gut microbiota with a comprehensive metabolite profile in pregnant women with overweight and obesity [5]. However, the findings of these previous studies were based on 16 rRNA amplicon sequencing, which can provide a robust taxonomic classification of gut microbiota at the genus level. Here, we have extended the analysis of gut microbiota-serum metabolite associations to a level i.e., to distinct microbial species and predicted functions as enabled by metagenomics profiling of gut microbiota. Furthermore, the serum metabolic profile was analyzed by nuclear magnetic resonance (NMR) spectroscopy, which provided a comprehensive analysis of more than 200 metabolites, including lipoprotein subclasses and their ratios, biomarkers for lipid and glucose metabolism, amino acids, ketone bodies and GlycA, which is a marker of low-grade inflammation. Many of these metabolites and their ratios are considered as markers of an individual's cardiovascular risk [12,13]. We speculated that a deeper understanding of the gut microbiota-serum metabolism interactions in pregnant women at risk for health complications, i.e., those with overweight or obesity, would provide valuable insights when designing new dietary intervention approaches especially aimed at those women with an elevated cardiovascular risk.

Our primary aim was to investigate the extent to which the composition of the gut microbiota would be reflected in serum metabolomic profiles with a bicluster analysis during early pregnancy.

2. Methods

2.1. Study population

The study subjects were pregnant women with overweight or obesity, participating in a mother-infant dietary single-center intervention trial (ClinicalTrials. gov, NCT01922791) being conducted in University of Turku and Turku University Hospital, Turku, Finland [14]. The following were the inclusion criteria for the current study. As the study focuses on at-risk group of pregnant women, those with overweight (self-reported pre-pregnancy body mass index (BMI) $\geq$ 25 kg/m^2) were included. Here, we have investigated the associations between gut microbiota and serum metabolites cross-sectionally at the baseline of the study (i.e., prior to the initiation of the intervention), at a mean 13.9 (SD 2.1) weeks of gestation (i.e., early pregnancy phase). Only those women, who had not consumed antibiotics within 8 weeks before fecal sampling, had provided both fecal and serum samples for metagenomics and metabolomics analyses, respectively, were included in this study. The exclusion criteria were gestational diabetes mellitus diagnosed before entering the study, multifetal pregnancy, and the presence of metabolic or inflammatory diseases. To avoid potential confounding effects of these factors, such as use of antibiotics, pre-existing diabetes status, the participants were excluded based on the aforementioned criteria. Data on the clinical characteristics were collected with questionnaires and interviews during a study visit [14].

Ultimately, 361 pregnant women with overweight or obesity out of 439 recruited were included in the current study.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki as revised in 2013, and all procedures that involved human subjects were approved by the Ethics Committee of the Hospital District of Southwest Finland (permission number 115/180/2012). Written informed consent was provided by the study participants.

2.1.1. Characteristics of the study population

The characteristics of the study participants are shown in Table 1. The majority of the pregnant women, 63 % (227/361), were with overweight, the others (134/361 = 37 %) were with obesity. Overall, 57 % of the women had completed a higher level of education (college or university degree) and 48 % were expecting their first child.

2.2. Gut microbiota profiling

Fecal samples were collected in sterile plastic pots on the morning of the study visit or the previous evening and kept at −20 °C until DNA extraction. DNA was extracted by using a GTX stool extraction kit and a fully automated GenoXTractmachine (Hain Lifescience). Before extraction, mechanical lysis was performed by bead-beating the samples in ceramic bead tubes with a MOBIO PowerLyzer 24 Bench Top Bead-Based Homogenizer (MO BIO Laboratories, Inc., Carlsbad, CA). Details regarding metagenomics sequencing and gut microbiota profiling have been described previously [15]. In brief, the genomic DNA was fragmented to a length of approximately 350bp and used for library construction using NEBNext Ultra II Library Prep Kit for Illumina (New England Biolabs). The library was sequenced using 2 × 150bp paired-end sequencing on an Illumina HiSeq platform. A total of 767 samples were sequenced for two time points during pregnancy (early and late pregnancy). The raw FASTQ files were quality controlled using KneadData (v. 0.6.1) to remove low-quality bases and reads derived from the host genome as follows- Using Trimmomatic (v. 0.36), the reads were quality trimmed by removing Nextera adapters, leading or trailing bases with a Phred score below 20, and trailing bases in which the Phred score over a window of size 4 drops below 20. Trimmed reads shorter than 100 bases were discarded. Reads that mapped to the human reference genome GRCh38 (with Bowtie 2 v. 0.2.3.2 using default settings) were discarded. Read pairs in which both reads passed filtering were retained; these were classified as high quality non-host (HQNH) reads. For each sample, the average sequencing depth was 25.3 million (M) read pairs. On an average per sample, 5.4 M (~21 %) reads were discarded for their low quality, 0.1 M (~0.4 %) reads were mapped to the host genome, and 2.2 M (8.7 %) reads were not mapped to the gene catalog. On an average for each sample, about 17.5 M (~70 %) reads were mapped to the gene catalog. The analysis of the microbial composition and pathway profiling (predicted functions) was performed using MetaPhlAn2 V.2.6.0 [16] and HUMAnN2 pipeline V.0.11.1 [17], respectively. To avoid the potential confounding effect of intervention

Table 1
Baseline characteristics of the study population.

Characteristics	All women
n	361
Age, years	30.4 [27.6, 33.8]
Weight, Kg	82.1 [74.4, 90.3]
Pre-pregnancy BMI (Kg/m^2)	28.4 [26.4, 31.6]
BMI Status	
Overweight	227 (62.9)
Obese	134 (37.1)
College or university education	207 (57.3)
Smokers before pregnancy	73 (20.2)
Expecting her first child	176 (48.8)

The values represent median with interquartile ranges, [IQR] and percentages (n = Number of all pregnant women with overweight or obesity).

on the gut microbiota-serum metabolite associations , only baseline samples were considered in the current analysis (n = 361).

2.3. Metabolite profiling

Fasting (overnight for at least 9 h) blood samples were drawn from the antecubital vein. The serum was separated, and frozen in aliquots at −80 °C. These serum samples were analyzed using a high-throughput NMR spectroscopy platform (Nightingale, Helsinki, Finland) as described previously [13]. The platform measures 228 metabolites, including lipoprotein subclasses and their ratios, biomarkers for lipid and glucose metabolism, amino acids, ketone bodies and GlycA, a marker of low-grade inflammation.

2.4. Statistical and bioinformatics analysis

When quantifying the association between alpha diversity and individual metabolites, community richness (observed) and diversity (Shannon index) were calculated with the *mia* R/Bioconductor package [18]. Relative abundances were used for species, while metabolites were log10 transformed. Spearman correlations between alpha diversity and individual metabolite abundances were calculated with multiple correction being conducted with FDR (False Discovery rate, Benjamini-Hochberg method; R function *p.adjust*). FDR <0.05 was considered statistically significant.

Spearman correlation coefficients were calculated to assess the associations between species and serum metabolite abundances, and between the predicted functions and serum metabolite abundances. Positive and negative correlations that exceed the absolute value of Spearman correlation coefficient ($|r| > 0.15$) are reported. For correlation analysis, the species and predicted function abundances were centered log-ratio (clr) transformed to remove compositional bias. However, a shortcoming in cross-associating individual species and metabolites is that it does not characterize collinear variation in many species and metabolites that tend to co-vary as larger groups of multiple species and metabolites. Hence, we rearranged the correlation coefficient matrix between species-serum metabolites abundances with the BCplaid biclustering algorithm [19,20], which has been designed to detect informative groups of co-varying features. We used the *biclust* package in R [21] and the optimal number of biclusters was determined based on the default algorithm in biclust. In order to avoid overfitting to the data [22] we used a single biclustering algorithm. All analyses were performed in an R environment.

3. Results

3.1. Relations between species richness and diversity with serum metabolites

In this study, we did not observe any statistically significant relation between microbial species richness (richness observed) and diversity (Shannon diversity) ($P > 0.05$) with serum metabolites (Supplementary table, S1).

3.2. Correlations between species abundances with serum metabolites

The correlations of individual species with serum metabolites are shown as heatmap in Fig. 1. Both positive and negative correlations ($|r| > 0.15$) were identified (Supplementary Tables S2 and S3). For example, abundances of *Streptococcus salivarius* positively correlated with triglycerides in large HDL and triglycerides in very large HDL and abundances of *Eubacterium rectale* negatively correlated with cholesterol esters in small HDL and total cholesterol in small HDL (see Fig. 1).

3.3. Correlations between predicted functions with serum metabolites

Fig. 2 represents the associations between the predicted functions and serum metabolites and the identified correlations are reported in Supplementary Tables S4 and S5. Abundances of functional pathway related to starch degradation (PWY-6737, starch degradation V) positively correlated with free cholesterol to total lipids ratio in small VLDL and negatively with GlycA.

3.4. Biclusters of gut microbial species with serum metabolites

Five sets of microbial species that were linked with a metabolite cluster were identified by biclustering (Supplementary Table S6). Of the clusters, two (bicluster number 4 and 5) manifested with properties that have been associated with cardiovascular health.

The first bicluster (bicluster number 4) of these two included species from the phyla Bacteroidota (formerly, Bacteroides) (unclassified *Paraprevotella*) and Bacillota (formerly, Firmicutes) (*Clostridium bolteae, Ruminococcus gnavus,* unclassified *Peptostreptococcaceae*) [23]; this was linked to a group of metabolites that consisted of mostly several sizes of VLDL particles and their relative concentrations, along with the relative concentrations of large LDL and IDL. Other metabolites including triglycerides in VLDL, cholesterol, apolipoproteins, 3-hydroxybutyrate and GlycA were also present in this bicluster.

The second bicluster (bicluster number 5) contained four Bacteroidota (*Bacteroides cellulosilyticus*, *B. bacterium ph8*, *Alistipes finegoldii*, *A. shahii*), four Bacillota (*Coprococcus catus, Coprococcus* sp ART55 1, *Lachnospiraceae bacterium* 8 1 57FAA, *Roseburia hominis*), and single Actinomycetota (formerly, Actinobacterium) (*Adlercreutzia equolifaciens*) and Pseudomonadota (formerly, Proteobacterium) species (*Burkholderiales bacterium* 1 1 47). The metabolites linked to this bicluster included very large and large HDL particles, the relative concentrations of small and large HDL particles, and the mean diameter of HDL particle, along with relative concentrations of very small VLDL, large LDL, IDL, fatty acid ratios and cholesterol.

The remaining three biclusters were composed as follows; the first (bicluster number 1) included seven Bacteroidota (*Bacteroides fragilis, B. salyersiae, Parabacteroides goldsteinii, Paraprevotella clara, P. xylaniphila, Alistipes senegalensis, Eubacterium ventriosum*), six Bacillota (*Eubacterium eligens*, *Dorea longicatena*, *Lachnospiraceae bacterium* 3 1 46 FAA, unclassified *Roseburia*, unclassified *Peptostreptococcaceae*, and unclassified *Veillonella*), and a Pseudomonadota species (*Haemophilus parainfluenzae*). The metabolites included in this bicluster were large and very large HDL particles, the relative concentrations for different sizes of VLDL, IDL, and large HDL particles, cholesterol, cholesteryl esters, sphingomyelins, fatty acids (FAs) such as linoleic acid, omega-6 fatty acids, and polyunsaturated fatty acids (PUFAs). The second bicluster (bicluster number 2) included one Bacteroidota (*Bacteroides vulgatus*) and three Bacillota species (*C. bolteae, R. gnavus,* unclassified *Oscillibacter*). In this bicluster, large and very large HDL particles, the relative concentrations of different sizes of VLDL, very large and large HDL, IDL, large LDL particles; lipoprotein particle sizes for LDL and HDL, along with cholesterol and fatty acid ratios were evident. The species observed in the remaining bicluster (bicluster number 3) were eight Bacillota (*E. eligens, Anaerostipes hadrus, Coprococcus catus, Coprococcus* sp. ART 55 1, *Roseburia hominis, Roseburia unclassified, Ruminococcus callidus,* and *Subdoligranulum unclassified*), four Bacteroidota (*Alistipes finegoldii, A. putredinis, A. shahii,* and *E. ventriosum*), and two Pseudomonadota (*Parasutterella excrementihominis* and *Burkholderiales bacterium* 1 1 47), with the metabolites in this bicluster consisting of various sizes of VLDL particles and mean diameter of VLDL particles, small and medium HDL particles and relative concentrations of medium and large HDL particles, apolipoproteins, a branched chain amino acid-isoleucine, as well as GlycA.

We did not observe any biclusters between predicted functional pathways and the serum metabolites.

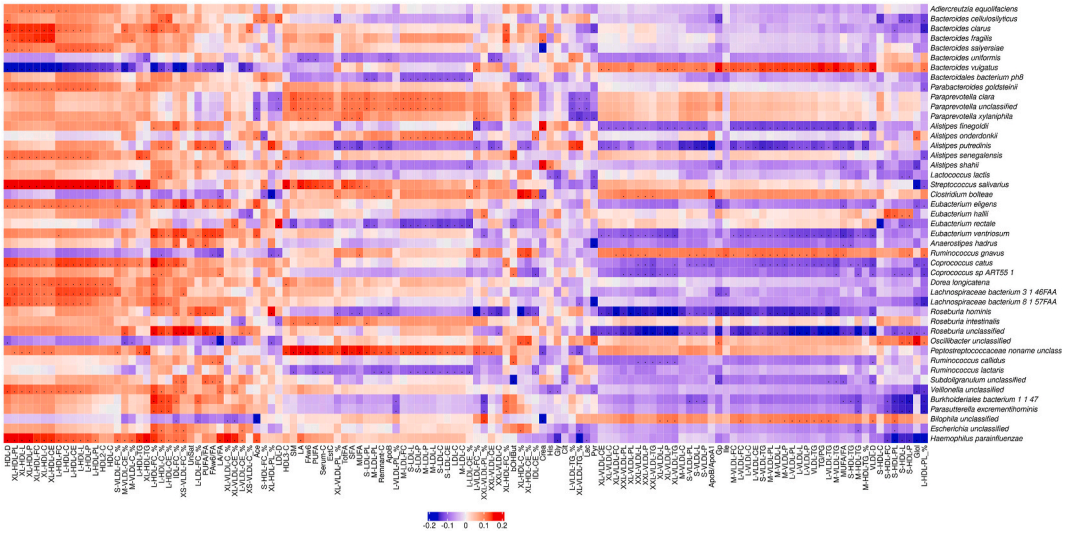

Fig. 1. Heatmap shows the associations between microbial taxa and serum metabolites for all pregnant women. The colors red and blue denote positive and negative associations respectively. The intensity of the color depends on the value of the Spearman correlation coefficient. Symbol (.) denotes significant correlations (unadjusted p-value <0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

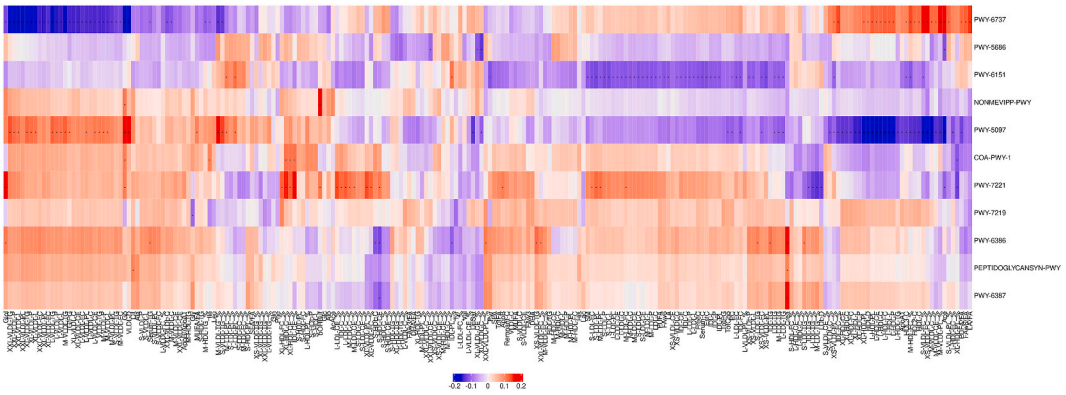

Fig. 2. Heatmap shows the associations between predicted functions and serum metabolites for all pregnant women. The colors red and blue denote positive and negative associations respectively. The intensity of the color depends on the value of the Spearman correlation coefficient. Pathways: PWY-6737: starch degradation V, PWY-5686: UMP biosynthesis, PWY-6151: *S*-adenosyl-L-methionine cycle I, NONMEVIPP-PWY: methylerythritol phosphate pathway I, PWY-5097: L-lysine biosynthesis VI, COA-PWY-1: coenzyme A biosynthesis II (mammalian), PWY-7221: guanosine ribonucleotides de novo biosynthesis, PWY-7219: adenosine ribonucleotides de novo biosynthesis, PWY-6386: UDP-*N*-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing), PEPTIDOGLYCANSYN-PWY: peptidoglycan biosynthesis I (*meso*-diaminopimelate containing), PWY-6387: UDP-*N*-acetylmuramoyl-pentapeptide biosynthesis I (*meso*-diaminopimelate containing). Symbol (.) denotes significant correlations (unadjusted p-value <0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

By using a biclustering approach, we identified five sets of microbial species that were related with groups of serum metabolites in women with overweight and obesity during the early stage of their pregnancy. Interestingly, two of these biclusters (bicluster number 4 and 5) pointed to a relation with the women's cardiovascular health.

We identified one bicluster (bicluster number 4) which included many metabolites such as subclasses of VLDL particles and their relative concentrations, along with the relative concentrations of large LDL and IDL, triglycerides in VLDL, cholesterol in LDL, ketone body- 3-hydroxybutyrate, apolipoproteins-apolipoprotein B, the ratio of apolipoprotein B to apolipoprotein A-I, total FAs, omega-6 fatty acids, PUFAs, MUFAs, and SFAs as well as an inflammation marker, GlycA. This cluster may be considered to highlight the links between the gut microbiota and serum metabolites that are adversely related to the cardiovascular health, i.e., there is previous evidence demonstrating their links with the risk of cardiovascular diseases (CVD). A prospective study of three population-based cohorts, revealed an increased CVD risk in individuals with higher concentrations of certain circulating metabolites such as lipid concentrations within the LDL, IDL, medium-VLDL and small-VLDL forms [24]. Similarly, another prospective population-based study reported a stronger association of LDL, VLDL, apolipoprotein B, and triglycerides in all lipoprotein classes with incident CVD [25]. An abundance of subclasses of VLDL, LDL and IDL has been associated with metabolic diseases, CVD, obesity and type-2 diabetes [26,27]. Bicluster number 4 contained the following microbial species; *Enterocloster bolteae* (formerly known as, *Clostridium bolteae*) [28], *Ruminococcus gnavus,* unclassified *Paraprevotella,* unclassified *Peptostreptococcaceae* which have been reported previously to associate with disease conditions such as type-2 diabetes [29], insulin resistance, dyslipidemia, and inflammation, cardio-metabolic diseases [30,31], Crohn's disease [32,33] and cancer [34,35]. When viewed through the perspective of these previous relations with the disease risk, the species in bicluster number 4 could be argued to be associated with adverse cardiovascular health effects.

On the other hand, the metabolites observed in bicluster number 5

were subclasses of very large and large HDL particles, the relative concentrations of small and large HDL, cholesterol, HDL-C, HDL-2C, and HDL3 and mean HDL particle size, the ratio of linoleic acid (C18:2) to total FAs and the ratio of PUFAs to total FAs. This cluster may be beneficial with respect to the cardiovascular health, as these metabolites have been considered to exert a protective effect on the incident CVD risk. This was demonstrated in a cohort study in which long-chain FAs, such as long-chain PUFA and those with a higher carbon atom number (18:0) were reported to exert a protective effect on the incident CVD [36]. Similarly, another study identified an inverse association between HDL cholesterol [37], lower concentrations of lipids within large HDL particles [24] and CVD risk. There are also reports of an association between very large and large HDL particles as well as mean HDL particle size with a reduced CVD risk; however, this association was inverse with small and medium HDL particles [27,38,39].

The species in this bicluster (bicluster number 5) consisted predominantly of short-chain fatty acid (SCFA) producers- *Alistipes shahii, Coprococcus catus, Coprococcus* sp ART55 1*, Roseburia hominis, Alistipes finegoldii*, an equol producer- *Adlercreutzia equolifaciens,* a cellulose degrader-*Bacteroides cellulosilyticus* and a glucose uptake regulator-*Lachnospiraceae bacterium* 8 1 57FAA. Two other species, *Bacteroidales bacterium ph8, Burkholderiales bacterium* 1 1 47 have been reported to exhibit an association with certain chronic diseases [40]. Most of the observed bacterial species are SCFA producers i.e., they synthesize butyrate, acetate as well as succinate. It has been demonstrated that the production of SCFAs confers beneficial effects on glucose and lipid metabolism, insulin sensitivity and inflammation status [41,42]. The overall beneficial status of this bicluster (bicluster number 5) metabolites might be due to the presence of these SCFAs as well as other microbial metabolites.

When considered on the basis of the literature two biclusters (bicluster number 4 and 5) seemed to exhibit properties towards pro-atherogenic and anti-atherogenic profiles, respectively. We speculate that these microbiota modifications could result in changes in metabolites and therefore induce either positive or negative health effects.

We also describe here the specific correlations between species abundances and predicted functions with serum metabolites in all the studied pregnant women based on Spearman correlation coefficient values, although the observed associations tended to be weak ($|r| > 0.15$). These correlations were with Lachnospiraceae species, *Roseburia* and *Eubacterium rectale,* with different sizes and subclasses of VLDL, LDL, IDL and HDL. Previously, associations have been identified between the family *Lachnospiraceae,* LDL [43] and subclasses of various sizes of VLDL and HDL [27]. The correlations detected here suggest that microbial species might play a role in lipid metabolism.

We also observed correlations between the relative concentrations of different sizes and subclasses of VLDL, IDL and LDL as well as acetate, glycerol and GlycA with predicted functions. The correlated pathways belong to the super-classes of biosynthesis and degradation as predicted by the MetaCyc database [44]; i.e., cell structure biosynthesis (UDP--*N*-acetylmuramoyl-pentapeptide biosynthesis I and II), purine nucleotide biosynthesis (guanosine ribonucleotides de novo biosynthesis), amino acid biosynthesis (L-lysine biosynthesis VI), secondary metabolite biosynthesis (methylerythritol phosphate pathway I) and polysaccharide degradation (starch degradation V) [44]. Considering the complexities of human as well as microbial metabolism, the exact biological relevance of the reported associations remains somewhat unclear.

However, we did not observe any significant relationship between microbial species richness and alpha diversity with the individual serum metabolites. In previous reports, richness has been positively associated with certain amino acids such as glutamine, histidine and tyrosine, glycolysis-related metabolites, choline, creatinine [45] (untargeted NMR signals for metabolites) as well as with acetate [46]. In one publication, the levels of acetate also influenced microbial diversity [46]. Inverse associations between microbial richness and metabolite signals related to lipids, esters and ketone have also been reported [45]. These differences might be attributed to the differing characteristics of the participants. In the present trial, the participants were pregnant women with overweight or obesity, whereas one of the studies included only middle aged Finnish men (average age 61 years) [46], and the other included only healthy men and women whilst obesity status was not reported [45].

There is a paucity of evidence for the relations between gut microbiota and serum metabolites at the species level. Here, the strength of our study lies in using gut microbial data based on metagenomic sequencing, which provides taxonomic resolution at the species level as well as using serum metabolite data assayed with NMR spectroscopy, which provides a well-characterized profile of many metabolites. We identified individual as well as group level associations between gut microbiota and metabolites and applied a biclustering method, which has previously been used to identify group-level associations between the gut microbiota and metabolites in human and animal studies [47,48]. Biclustering simultaneously clusters rows and columns of a data matrix. We used the BCplaid algorithm, which is considered as a refinement of the original Plaid model method and has improved capability of detecting informative biclusters [21]. Here, we first calculated correlation coefficients for species abundances and serum metabolites. Then, based on the resulting correlation matrix, the biclustering algorithm was applied which allowed us to reduce the dimensionality of the data and identify biclusters that were considered biologically relevant according to the available literature. Additionally, as the study setting was that of randomized control trial, the pregnant women in our study represented a homogenous population and provided relevant information such as none of the women used any antibiotics or medications. Our study included women at their early pregnancy before the initiation of the intervention. It is important to understand microbiota-metabolite associations at the baseline as a prerequisite that is necessary to map properly before proceeding to study the effects of intervention and diet.

Our study also has a few limitations. The pregnant women in our study belonged to those in the higher BMI groups, i.e., overweight or obese. This group was chosen as these individuals carry elevated risks to experience metabolic complications. However as no comparisons with normal or lean weight pregnancy were available, it was not possible to investigate the extent to which adiposity might influence gut microbiota-serum metabolite relations. The state of being overweight or obese puts a woman at risk of developing type-2 diabetes, cardiometabolic as well as other metabolic disorders. As the prenatal period affects the development and growth of the fetus, the changes and conditions occurring during this period can also influence future outcomes related to her child's health. There is a need for further studies to understand the gut microbiota-serum metabolite relations in pregnancy, and further whether they are specific to pregnancy or also take place in comparison to non-pregnant conditions. One important consideration is diet, due to its considerable effect on modulation of both gut microbiota as well as metabolites. In this exploratory study, however, diet was not included. Further studies that consider dietary data and effect of intervention in longitudinal settings are needed to understand the relationship between diet-gut microbiota and serum metabolites.

The taxonomic and predicted functional annotations were based on MetaPhlAn2 and HUMAnN2, respectively. Updated versions of these pipelines have recently become available [49,50] and might provide improvements in the taxonomic and functional classifications. However, these latest versions include remarkable recent changes in bacterial nomenclature, making the comparison with existing literature more challenging. Our findings were obtained with MetaPhlAn2 and HuMAnN2, which have become widely adopted and verified methods. This allowed us to more directly compare our findings with the wider array of existing literature. In particular due to the exploratory nature of our study, the ability to relate the findings to previous literature is essential for interpretation. Replicating the analysis with updated

taxonomic and functional mappings will be valuable for further verification but out of scope for our present study. It should also be noted that NMR platform, with its modern omics approach, provides comprehensive profile of 228 serum metabolites. Nevertheless, the high number of metabolites and requirement of multiple testing correction for statistically robust results may pose as a limitation.

Although we identified two biclusters with relation to cardiovascular health, there were some similarities in all five biclusters. This is reasonable considering the functional redundancy of the gut microbiota and metabolism. Another important point to consider is the sensitivity of the parameters in the biclustering algorithm; in other words, the choice of algorithm can affect the way in which the rows and columns of the given data matrix are assigned into biclusters, which may in turn lead to differences in the apparent structures of the biclusters. Here, we used a single algorithm to detect informative groups and avoid overfitting the data. Our cross-sectional study provides information regarding the gut microbiota-serum metabolites associations during early pregnancy, further studies are required to understand these relations during late pregnancy.

In conclusion, we applied a biclustering method and identified two gut microbial species-serum metabolite profiles, with potential pro-atherogenic and anti-atherogenic properties. Considering the limited availability of the evidence, our observations can provide insights regarding the relationship between gut microbiota and serum metabolites in an at-risk group of pregnant women. These gut microbial-serum metabolite relations could help to identify distinctive profiles for an at-risk group of pregnant women that may be relevant in terms of their cardiovascular disease risk. Understanding these relations better could identify approaches to modify gut microbiota, for e.g. with diet and subsequently enable metabolic and health benefits in the pregnant women with overweight and obesity. Further studies will be needed to understand these relations during late pregnancy, to confirm whether these profiles can be used as biomarkers, as well as design the clinical strategies to improve metabolic and overall health.

Author contributions

KL designed the original clinical study. The research reported here was designed by ML, LL and KL. NH participated in sample and data collection. ML, KL wrote the paper. LL designed and supervised statistical and bioinformatics analysis. ML, CB performed statistical and bioinformatics analyses. ML, LL and KL interpreted the results. All authors revised the manuscript. All authors read and approved the final manuscript.

Data availability statement

The data sets are not available due to their containing information that could compromise participant privacy and consent. The source code for the analyses is available at Zenodo (https://doi.org/10.5281/zenodo.15261689).

Funding

The clinical trial was supported by the State Research Funding for university-level health research in the Turku University Hospital Expert Responsibility Area, Academy of Finland (# 258,606), the Diabetes Research Foundation, the Päivikki and Sakari Sohlberg Foundation, and the Juho Vainio Foundation and for the reporting by the Finnish Foundation for Cardiovascular Research, Finnish Cultural Foundation and the Biocodex Foundation. Funding to the University of Turku for the gut microbiota and metabolomics analyses was provided by Janssen Research & Development, LLC. Personal support to ML by -University of Turku postgraduate education unit research grant and the Juho Vainio Foundation is acknowledged. LL was supported by Academy of Finland (# 330,887).

These funding sources had no role in the design, execution, analyses, interpretation of the data, or decision to submit these results.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

The authors thank all participants in the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2025.104095.

References

[1] Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr 2016;27:89–94. https://doi.org/10.5830/CVJA-2016-021.
[2] Mesa MD, Loureiro B, Iglesia I, Gonzalez SF, Olivé EL, Algar OG, et al. The evolving microbiome from pregnancy to early infancy: a comprehensive review. Nutrients 2020;12. https://doi.org/10.3390/nu12010133.
[3] Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Kling Bäckhed H, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012;150:470–80. https://doi.org/10.1016/j.cell.2012.07.008.
[4] Kivelä J, Sormunen-Harju H, Girchenko PV, Huvinen E, Stach-Lempinen B, Kajantie E, et al. Longitudinal metabolic profiling of maternal obesity, gestational diabetes, and hypertensive pregnancy disorders. J Clin Endocrinol Metab 2021;106:E4372–88. https://doi.org/10.1210/clinem/dgab475.
[5] Houttu N, Mokkala K, Laitinen K. Overweight and obesity status in pregnant women are related to intestinal microbiota and serum metabolic and inflammatory profiles. Clin Nutr 2018;37:1955–66. https://doi.org/10.1016/j.clnu.2017.12.013.
[6] Mokkala K, Vahlberg T, Pellonperä O, Houttu N, Koivuniemi E, Laitinen K. Distinct metabolic profile in early pregnancy of overweight and obese women developing gestational diabetes. J Nutr 2020;150:31–7. https://doi.org/10.1093/jn/nxz220.
[7] Zhou L, Xiao X. The role of gut microbiota in the effects of maternal obesity during pregnancy on offspring metabolism. Biosci Rep 2018;38. https://doi.org/10.1042/BSR20171234.
[8] Gao B, Zhong M, Shen Q, Wu Y, Cao M, Ju S, et al. Gut microbiota in early pregnancy among women with Hyperglycaemia vs. Normal blood glucose. BMC Pregnancy Childbirth 2020;20. https://doi.org/10.1186/s12884-020-02961-5.
[9] Li GH, Huang SJ, Li X, Liu XS, Du QL. Response of gut microbiota to serum metabolome changes in intrahepatic cholestasis of pregnant patients. World J Gastroenterol 2020;26:7338–51. https://doi.org/10.3748/wjg.v26.i46.7338.
[10] Sakurai K, Kato T, Tanabe H, Taguchi-Atarashi N, Sato Y, Eguchi A, et al. Association between gut microbiota composition and glycoalbumin level during pregnancy in Japanese women: pilot study from Chiba Study of Mother and Child Health. J Diabetes Investig 2020;11:699–706. https://doi.org/10.1111/jdi.13177.
[11] Pan R, Guo M, Chen Y, Lin G, Tian P, Wang L, et al. Dynamics of the gut microbiota and faecal and serum metabolomes during pregnancy—a longitudinal study. Nutrients 2024;16. https://doi.org/10.3390/nu16040483.
[12] Smirnov KS, Maier TV, Walker A, Heinzmann SS, Forcisi S, Martinez I, et al. Challenges of metabolomics in human gut microbiota research. Int J Med Microbiol 2016;306:266–79. https://doi.org/10.1016/j.ijmm.2016.03.006.
[13] Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet 2015;8:192–206. https://doi.org/10.1161/CIRCGENETICS.114.000216.
[14] Pellonperä O, Mokkala K, Houttu N, Vahlberg T, Koivuniemi E, Tertti K, et al. Efficacy of fish oil and/or probiotic intervention on the incidence of gestational diabetes mellitus in an at-risk group of overweight and obese women: a randomized, placebo-controlled, double-blind clinical trial. Diabetes Care 2019;42:1009–17. https://doi.org/10.2337/dc18-2591.
[15] Mokkala K, Paulin N, Houttu N, Koivuniemi E, Pellonperä O, Khan S, et al. Metagenomics analysis of gut microbiota in response to diet intervention and gestational diabetes in overweight and obese women: a randomised, double-blind, placebo-controlled clinical trial. Gut 2021;70:309–18. https://doi.org/10.1136/gutjnl-2020-321643.
[16] Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E, et al. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods 2015;12:902–3. https://doi.org/10.1038/nmeth.3589.
[17] Franzosa EA, McIver LJ, Rahnavard G, Thompson LR, Schirmer M, Weingart G, et al. Species-level functional profiling of metagenomes and metatranscriptomes. Nat Methods 2018;15:962–8. https://doi.org/10.1038/s41592-018-0176-y.
[18] Ernst FGM, Shetty SA, Borman T, Lahti L. mia: microbiome analysis.

[19] Turner H, Bailey T, Krzanowski W. Improved biclustering of microarray data demonstrated through systematic performance tests. Comput Stat Data Anal 2005; 48:235–54. https://doi.org/10.1016/j.csda.2004.02.003.
[20] Lazzeroni L, Owen A. Plaid models for gene expression data, vol. 12; 2002.
[21] Kaiser S, Santamaria R, Khamiakova T, Sill M, Theron R, Quintales L, et al. Biclust: BiCluster algorithms.
[22] Head ML, Holman L, Lanfear R, Kahn AT, Jennions MD. The extent and consequences of p-hacking in science. PLoS Biol 2015;13:e1002106. https://doi.org/10.1371/journal.pbio.1002106.
[23] Oren A, Garrity GM. Valid publication of the names of forty-two phyla of prokaryotes. Int J Syst Evol Microbiol 2021;71. https://doi.org/10.1099/ijsem.0.005056.
[24] 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. https://doi.org/10.1161/CIRCULATIONAHA.114.013116.
[25] Tzoulaki I, Castagné R, Boulangé CL, Karaman I, Chekmeneva E, Evangelou E, et al. Serum metabolic signatures of coronary and carotid atherosclerosis and subsequent cardiovascular disease. Eur Heart J 2019;40:2883–96. https://doi.org/10.1093/eurheartj/ehz235.
[26] Saba A, Oridupa O. Lipoproteins and cardiovascular diseases. In: Frank S, Kostner G, editors. Lipoproteins. Rijeka: IntechOpen; 2012. https://doi.org/10.5772/48132. Ch. 8.
[27] Vojinovic D, Radjabzadeh D, Kurilshikov A, Amin N, Wijmenga C, Franke L, et al. Relationship between gut microbiota and circulating metabolites in population-based cohorts. Nat Commun 2019;10. https://doi.org/10.1038/s41467-019-13721-1.
[28] Haas KN, Blanchard JL. Reclassification of the Clostridium clostridioforme and Clostridium sphenoides clades as Enterocloster gen. nov. and Lacrimispora gen. nov., including reclassification of 15 taxa. Int J Syst Evol Microbiol 2020;70:23–34. https://doi.org/10.1099/ijsem.0.003698.
[29] Wang L, Yu X, Xu X, Ming J, Wang Z, Gao B, et al. The fecal microbiota is already altered in normoglycemic individuals who go on to have type 2 diabetes. Front Cell Infect Microbiol 2021;11. https://doi.org/10.3389/fcimb.2021.598672.
[30] Campisciano G, de Manzini N, Delbue S, Cason C, Cosola D, Basile G, et al. The obesity-related gut bacterial and viral dysbiosis can impact the risk of colon cancer development. Microorganisms 2020;8. https://doi.org/10.3390/microorganisms8030431.
[31] Jie Z, Liang S, Ding Q, Li F, Sun X, Lin Y, et al. Dairy consumption and physical fitness tests associated with fecal microbiome in a Chinese cohort. Medicine Microecology 2021;9:100038. https://doi.org/10.1016/j.medmic.2021.100038.
[32] Henke MT, Kenny DJ, Cassilly CD, Vlamakis H, Xavier RJ, Clardy J. Ruminococcus gnavus, a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. Proc Natl Acad Sci U S A 2019;116:12672–7. https://doi.org/10.1073/pnas.1904099116.
[33] Zafar H, Saier MH. Gut Bacteroides species in health and disease. Gut Microbes 2021;13:1–20. https://doi.org/10.1080/19490976.2020.1848158.
[34] Liu B, Pan X, Yao L, Chen S, Liu Z, Han M, et al. Fecal microbiota as a non-invasive biomarker to predict the tissue iron accumulation in intestine epithelial cells and liver. https://doi.org/10.1101/612168; 2019.
[35] Mizutani S, Yamada T, Yachida S. Significance of the gut microbiome in multistep colorectal carcinogenesis. Cancer Sci 2020;111:766–73. https://doi.org/10.1111/cas.14298.
[36] Toledo E, Wang DD, Ruiz-Canela M, Clish CB, Razquin C, Zheng Y, et al. Plasma lipidomic profiles and cardiovascular events in a randomized intervention trial with the Mediterranean diet. Am J Clin Nutr 2017;106:973–83. https://doi.org/10.3945/ajcn.116.151159.
[37] Kazemian N, Mahmoudi M, Halperin F, Wu JC, Pakpour S. Gut microbiota and cardiovascular disease: opportunities and challenges. Microbiome 2020;8. https://doi.org/10.1186/s40168-020-00821-0.
[38] Kontush A. HDL particle number and size as predictors of cardiovascular disease. Front Pharmacol 2015;6. https://doi.org/10.3389/fphar.2015.00218.
[39] Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. Trends Mol Med 2011;17:594–603. https://doi.org/10.1016/j.molmed.2011.05.013.
[40] Chen L, Wang D, Garmaeva S, Kurilshikov A, Vich Vila A, Gacesa R, et al. The long-term genetic stability and individual specificity of the human gut microbiome. Cell 2021;184:2302–2315.e12. https://doi.org/10.1016/j.cell.2021.03.024.
[41] Kim MH, Yun KE, Kim J, Park E, Chang Y, Ryu S, et al. Gut microbiota and metabolic health among overweight and obese individuals. Sci Rep 2020;10. https://doi.org/10.1038/s41598-020-76474-8.
[42] Canfora EE, Meex RCR, Venema K, Blaak EE. Gut microbial metabolites in obesity, NAFLD and T2DM. Nat Rev Endocrinol 2019;15:261–73. https://doi.org/10.1038/s41574-019-0156-z.
[43] Fu J, Bonder MJ, Cenit MC, Tigchelaar EF, Maatman A, Dekens JAM, et al. The gut microbiome contributes to a substantial proportion of the variation in blood lipids. Circ Res 2015;117:817–24. https://doi.org/10.1161/CIRCRESAHA.115.306807.
[44] Caspi R, Billington R, Keseler IM, Kothari A, Krummenacker M, Midford PE, et al. The MetaCyc database of metabolic pathways and enzymes-a 2019 update. Nucleic Acids Res 2020;48. https://doi.org/10.1093/nar/gkz862. D455–D453.
[45] Partula V, Deschasaux-Tanguy M, Mondot S, Victor-Bala A, Bouchemal N, Lécuyer L, et al. Associations between untargeted plasma metabolomic signatures and gut microbiota composition in the Milieu Intérieur population of healthy adults. Br J Nutr 2021;126:982–92. https://doi.org/10.1017/S0007114520004870.
[46] Org E, Blum Y, Kasela S, Mehrabian M, Kuusisto J, Kangas AJ, et al. Relationships between gut microbiota, plasma metabolites, and metabolic syndrome traits in the METSIM cohort. Genome Biol 2017;18. https://doi.org/10.1186/s13059-017-1194-2.
[47] Lahti L, Salonen A, Kekkonen RA, Salojärvi J, Jalanka-Tuovinen J, Palva A, et al. Associations between the human intestinal microbiota, Lactobacillus rhamnosus GG and serum lipids indicated by integrated analysis of high-throughput profiling data. PeerJ 2013;2013. https://doi.org/10.7717/peerj.32.
[48] Hintikka J, Lensu S, Mäkinen E, Karvinen S, Honkanen M, Lindén J, et al. Xylo-oligosaccharides in prevention of hepatic steatosis and adipose tissue inflammation: associating taxonomic and metabolomic patterns in fecal microbiomes with biclustering. Int J Environ Res Publ Health 2021;18. https://doi.org/10.3390/ijerph18084049.
[49] Blanco-Míguez A, Beghini F, Cumbo F, McIver LJ, Thompson KN, Zolfo M, et al. Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. Nat Biotechnol 2023;41:1633–44. https://doi.org/10.1038/s41587-023-01688-w.
[50] Beghini F, McIver LJ, Blanco-Míguez A, Dubois L, Asnicar F, Maharjan S, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. Elife 2021;10. https://doi.org/10.7554/eLife.65088.