J Physiol Biochem (2018) 74:159–169
DOI 10.1007/s13105-017-0577-x

ORIGINAL ARTICLE

Maternal obesity is associated with gut microbial metabolic potential in offspring during infancy

Tomás Cerdó [1,2] · Alicia Ruiz [2,3] · Ruy Jáuregui [4] · Hatim Azaryah [1,2] · Francisco José Torres-Espínola [1,2] · Luz García-Valdés [1,2] · M. Teresa Segura [1,2] · Antonio Suárez [3] · Cristina Campoy [1,2,5,6]

Received: 2 March 2017 / Accepted: 29 June 2017 / Published online: 17 August 2017

Abstract Children born to obese mothers are at increased risk for obesity, but the mechanisms behind this association are not fully understood. Our study aimed to investigate differences in the functions encoded by the microbiome of infants at 18 months of age when the transition from early infant-feeding to solid family foods is established. To investigate the impact of maternal prepregnancy body mass index on infants' gut microbiome, faecal samples from infants born to normoweight (n = 21) and obese mothers (n = 18) were analysed by 16S rRNA gene sequencing and a functional-inference-based microbiome analysis. Our results indicated that *Firmicutes* was significantly enriched in infants born to normoweight mothers whereas *Bacteroidetes* was significantly enriched in infants born to obese women. In both microbiomes, the greatest number of genes (>50%) that were assigned a function encoded for proteins involved in "metabolism" among tier 1 KEGG Orthology (KO) categories. At lower KO functional categories, the microbiome of infants born to normoweight mothers was characterized by a significant enrichment in the abundances of "pentose phosphate pathway" (p = 0.037), "lysine biosynthesis" (p = 0.043), "glycerolipid metabolism" (p = 0.042), and "C5-branched dibasic acid metabolism" (p = 0.045). Notably, the microbiome of infants born to obese mothers was significantly enriched in "streptomycin biosynthesis" (p = 0.047), "sulphur metabolism" (p = 0.041), "taurine and hypotaurine metabolism" (p = 0.036), and "lipopolysaccharide biosynthesis" (p = 0.043). In summary, our study showed that maternal prepregnancy obesity may imprint a selective gut microbial composition during late infancy with distinct functional performances.

Keywords Gut microbiota · Metabolism · Maternal obesity · Infant · Microbiome

T.C and A.R contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s13105-017-0577-x) contains supplementary material, which is available to authorized users.

✉ Cristina Campoy
ccampoy@ugr.es

[1] Department of Pediatrics, School of Medicine, University of Granada, Granada, Spain

[2] EURISTIKOS Excellence Centre for Paediatric Research, Biomedical Research Centre, University of Granada, Granada, Spain

[3] Department of Biochemistry and Molecular Biology 2, Biomedical Research Centre, University of Granada, Granada, Spain

[4] AgResearch Grasslands, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand

[5] Biomedical Research Networking Center for Epidemiology and Public Health (CIBERESP), Carlos III Health Institute, Granada, Spain

[6] Department of Pediatrics, Faculty of Medicine, University of Granada, Avd. de la Investigación, 11, 18016 Granada, Spain

Introduction

The human gut is a bioreactor with evolutionarily conserved roles in the biotransformation of dietary ingredients into products that affect our physiology, metabolism, immune function, and development [7, 24, 53]. This ensemble of organisms consists mainly of bacterial taxa of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* phyla that have coevolved with the human host complementing the coding potential of our own genome [20, 27]. Although research interest has traditionally aimed at cataloguing the microbial species of the adult human gut microbiota and its relationship to complex diseases

[2, 30, 38, 49, 57], few studies have focused on the infant gut microbiota that have historically relied on culture-based enumeration and, more recently, on taxonomic profiling by16S rRNA gene sequence analyses [1, 4, 18]. Infancy is a critical period for gut microbial de novo assembly during which a constant dialogue with the immune system and a metabolic crosstalk with the host influences healthy growth and development [3, 11, 19]. These studies have shown that there is a rapid rate of colonization and expansion of gut bacteria from birth, a shift from a gut dominated by *Proteobacteria* to an adult-like one dominated by *Firmicutes* and *Bacteroidetes*, that achieves compositional diversity and stability by the third year of life [14]. Differential modes of birth (vaginal vs. C-section) and of type of feeding (breast milk vs. formula) are important aspects influencing the composition of the gut microbial ecosystem [48, 62]. Medical factors like prenatal and postnatal exposures to antibiotics and maternal factors like mother's prepregnancy weight affect the infant microbiota and increase the predisposition to obesity, with long-lasting effects [12, 58]. Thus, it is important to understand how these environmental factors contribute to the overall composition and function of the gut microbiota, what is the metabolic crosstalk of the gut microbiota and the infant body with potential long-term consequences.

Taking into account all of the above, the present study explores the gut microbial composition and function of healthy infants at 18 months of age born to mothers with prepregnancy normoweight or obesity. We have collected faecal samples to obtain the phylogenetic composition. As a novel approach, we have used a functional-inference-based (PICRUSt) analysis to identify the KEGG metabolic pathways of the microbial communities [31]. This strategy has allowed us to depict the potential metabolic pathways encoded by the microbiota of the infants and associate differential rates with mother's prepregnancy weight.

Materials and methods

Subjects, experimental design, and ethical guidelines

The infants included in this study were part of the PREOBE study cohort that recruited 331 mothers with normoweight, overweight, obesity, and gestational diabetes [5]. The pregnant women were recruited between 2008 and 2012 through collaboration with the two university tertiary hospitals in Granada, the San Cecilio and Mother-Infant Hospitals (Spain). Pregnant women with singleton pregnancies and aged between 18 and 45 years were included during their 12th to 34th weeks of pregnancy. The study exclusion criteria were simultaneous participation in any other research study, maternal drug use during pregnancy, diagnosed diseases other than obesity (e.g. pregestational diabetes, hypertension or preeclampsia, foetal intrauterine growth retardation, maternal infection during pregnancy, hypo/hyperthyroidism, hepatic diseases, and renal disease), or having a vegan diet. At the recruitment visit, information was collected regarding maternal prepregnancy weight and used for calculation of the prepregnancy BMI [6]. Due to drop out and availability of stool, 21 faecal samples from infants born to normoweight mothers ($18.5 \leq BMI \leq 25$ kg/m^2) and 18 faecal samples from infants born to obese mothers (≥ 30 kg/m^2) were further used in this study.

The project was approved by the Bioethical Committees for Clinical Research of the Clinical University Hospital San Cecilio and of the Mother-Infant University Hospital of Granada, Granada, Spain. An ethical approval was also obtained by the Research Bioethical Committee of the University of Granada. Written informed consent was obtained from all participants at study entry, and after, they had received full information from a research group member.

Sample collection and DNA extraction

Parents were provided with instructions and sterile bottles for sample collection. Fresh faecal samples were collected at home by the parents when children were 18 months old and stored at −20 °C for a maximum of 24 h until delivery to the laboratory, where they were frozen to −80 °C and stored until processing. Genomic DNA was extracted from faecal bacteria as previously described [22]. Briefly, faecal samples were resuspended in 1 ml of TN150 buffer (10-mM Tris-HCl pH 8.0 and 150-mM NaCl) and incubated at 80 °C for 30 min. Lysozyme (1 mg) was added to the suspension and incubated at 37 °C for 15 min. Zirconium glass beads (0.3 g) and 150 μl of buffered phenol were added and bacteria were disrupted with a mini bead beater set to 5000 rpm at 4 °C for 15 s (Biospec Products, Bartlesville, OK, USA). After centrifugation, genomic DNA was purified from the supernatant using phenol-chloroform extraction. Phenol-chloroform extractions were performed with 150 μl of phenol buffered and 150 μl of chloroform-isoamylalcohol (24:1) (*v*/*v*) solution. After an additional chloroform extraction, DNA was precipitated with two volumes of ethanol at −20 °C for 30 min. After centrifugation and washing with 70% ethanol, the pellet was resuspended in 50 μl of milliQ water. Five units of DNase-free RNase (Promega, USA) were added, and the sample was incubated at 37 °C for 15 min. Quality was checked by agarose gel electrophoresis and quantified with the Quant-iT PicoGreen dsDNA reagent and kit (Invitrogen, Darmstadt, Germany).

16S rRNA gene sequencing and data processing

Genomic DNA from faecal bacteria was used as template for 16S rRNA gene amplification using 27F and 338R universal primers for V1-V2 region as previously described [8]. Two consecutive PCR reactions integrated the sequence of the

specific Illumina multiplexing sequencing and index primers. The library was prepared by pooling equimolar ratios of amplicons and was sequenced using an Illumina MiSeq platform (University of Granada, Spain). Reads were demultiplexed and sorted, and paired ends were matched to give 240-nt reads. The data set was filtered and operational taxonomic units (OTUs) were defined at 99% similarity with MOTHUR programs unique.seqs and pre.cluster [55]. Taxonomic classifications of OTUs were assigned using the naïve Bayesian algorithm CLASSIFIER of Ribosomal Database Project [15]. OTUs were considered unassigned when confidence value score was lower than 0.8 and were annotated using upper taxonomic ranks.

Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis

Functional capacity of the microbiota was predicted based on the microbiota composition using PICRUSt [31]. Independent of the taxonomic analysis, 97% of the OTUs were picked using a closed-reference OTU picking protocol [10] and the Greengenes database (database/13_8) [40] preclustered at 97% identity. The obtained OTU table was normalized by 16S rRNA copy number, and functional genes were predicted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) catalogue [29].

Statistical and data analysis

Statistical analyses were carried out using SPSS version 19.0 (IBM SPSS, Inc., Chicago, IL) R statistical package (R Development Core Team, 2011) and STAMP. To examine the samples' taxonomic and functional profiles more generally, Statistical Analysis of Metagenomic Profiles [46] was used to compare the abundances of taxa, KEGG categories, and subcategories between infants born to normoweight and obese mothers. Data were not normally distributed and significant differences were identified with the White's non-parametric *t* test. The Benjamini-Hochberg procedure was used to control the false-discovery rate due to multiple testing. Results with a *q* value (corrected *p* value) <0.05 were retained.

α-Diversity indexes were calculated with PAST software (PAleontological STatistics free software). β-Diversity was calculated as Unifracs distance with GUnifrac package and permanova analysis of the distance between different study groups was calculated with *Adonis* function from vegan package in R software [45]. Bray-Curtis dissimilarity measures were calculated with vegan package and anosim test was used to establish the significant difference between infants born to obese and normoweight pregnant women in R software.

Results

Characteristics of participants

Two groups of infants were defined based on prepregnancy BMI calculated from self-reported prepregnancy body weight: normoweight 18.5 ≤ BMI ≤ 25 kg/m^2 and obese group ≥30 kg/m^2 (Table 1). All mothers and infants were in good health during pregnancy and infants were born at term. General characteristics were similar between the two study groups. The groups did not differ in maternal age, cultural level, smoking or alcohol consumption during pregnancy, sex, breast-feeding, and mode of delivery. Birth weight and Röhrer Ponderal index were 3265.71 ± 431.61 g and 26 ± 3.16 kg/cm^3 in infants born to normoweight mothers and 3428.33 ± 460.44 g and 27 ± 5.25 kg/cm^3 in infants born to obese mothers. We found lower weight gain during pregnancy in obese mothers compared to normoweight mothers, as it has been previously described [65]. At 18 months of age, weight and BMI of infants did not differ between study groups. As previously described [44], we observed a strong correlation between prepregnancy BMI and BMI at first prenatal study contact, which took place at 12 weeks of gestation (Pearson's correlation coefficient = 0.931, $p < 0.001$).

Gut microbial community structure is different in infants' gut microbiota depending on maternal prepregnancy BMI

A total of 1.848.304 high-quality 16S rRNA sequences were obtained from the 39 samples. Sequences were clustered into 662 OTUs and classified into 76 bacterial groups at genus level. We first examined the α-diversity (within sample ecological diversity) of the gut microbial communities. We used the Shannon diversity (SDI) ($p = 0.815$), Chao-1 indexes (estimates richness) ($p = 0.740$), and the taxa number (calculates unique OTUs) ($p = 0.743$). These diversity scores were not significantly different between the gut microbiota of infants born to normoweight and obese mothers (data not shown).

We calculated Unifrac distances (β-diversity) to determine the between-sample diversity in the gut microbial communities of infant groups. Permutational multivariate ANOVA using *Adonis* function showed that infants born to obese mothers had a significantly different microbial community structure ($p = 0.006$ for weighted and $p = 0.001$ for unweighted Unifrac distances) compared to those born to normoweight mothers. Two distinct populations representing normoweight and obese samples clustered in the principal coordinate analysis (PCoA) plot (Fig. 1a–b). Clustering analysis of individual samples using Bray-Curtis distances also showed that the gut microbial community structures were significantly different between infant groups (Fig. 1c). To exclude confounding variables

Table 1 General characteristics of the studied population

		Normal weight (n = 21)	Obese (n = 18)
Mother			
Maternal age (year)		32.52 ± 4.16	33 ± 5.62
Preconceptional maternal height (m)		1.63 ± 0.07	1.61 ± 0.07
Preconceptional maternal weight (Kg)		58.18 ± 6.29[a]	91.67 ± 13.61[b]
Preconceptional maternal BMI (kg/cm^3)		21.94 ± 1.67[a]	35.05 ± 4.27[b]
Weight gain during pregnancy (kg)		10.12 ± 5.68[a]	4.83 ± 8.66[b]
Maternal education level	Primary/secondary	10(47.62%)	11(61.11%)
	University/doctor	11(52.38%)	7(38.89%)
Smoking during pregnancy	Yes	3(14.29%)	2(11.11%)
	No	18(85.71%)	16(88.89%)
Alcohol consumption during pregnancy	Yes	2(9.52%)	0(0%)
	No	19(90.48%)	18(100%)
Mode delivery	Vaginal	17(80.95%)	14(77.78%)
	Cesarean	4(19.04%)	4(22.22%)
Newborn infant			
Gestational age (weeks)		39.85 ± 1.53	39.72 ± 1.40
Birth weight (g)		3265.71 ± 431.61	3428.33 ± 460.44
Length at birth (cm)		50.38 ± 1.71	50.44 ± 2.30
Röhrer Ponderal index (kg/cm^3)		26.00 ± 3.16	27.00 ± 5.25
Breast-feeding	Yes	15(71.43%)	11(61.11%)
	No	6(28.57%)	7 (38.89%)
Infant at 18 months			
Height (cm)		81.14 ± 3.14	80.32 ± 3.68
Weight (kg)		10.96 ± 1.06	10.73 ± 1.08
BMI (kg/m^2)		16.64 ± 1.41	16.62 ± 1.13
Gender	Male	16(76.19%)	9(50%)
	Female	5(23.80%)	9(50%)

Values listed are total for the variable (percent of total value n) or means ± SD

Across rows (a, b) indicate means that are significantly different ($p < 0.05$) using analysis of variance (White's non-parametric t test)

Lack of *superscript letters* indicate means that are not significantly different. $p > 0.05$

that may affect the relationship between maternal prepregnancy BMI and the composition of infants' gut microbiota, we examined the effect of gender, mode of delivery, and type of feeding using Unifrac β-diversity distances and redundancy analysis (RDA). The significance of separation in RDA analysis was estimated with a permutation test using 5000 permutations. Type of feeding was categorized into three groups, exclusively breast-fed, mixed fed (formula plus breast-feeding), and exclusively formula-fed at 3 and 6 months of age. To assess the influence of duration of breast-feeding in infants' gut microbiota, infants were categorized into two groups: breast-feeding with solid foods and exclusive solid food feeding at 12 and 18 months of age. No significant effect on the total microbiota profile was found except for prepregnancy mothers' BMI (Supplementary Table S1). Although the mode of delivery was not significant, we replicated a sensitivity analysis only on data from vaginally delivered infants. We found no significant association between any of these variables except for mothers' BMI (Supplementary Table S2). Thus, our findings concluded that mother's BMI is a strong factor influencing gut microbial community structure and composition of infants.

Maternal prepregnancy BMI and phylogenetic shifts in infants' gut microbiota

To address significant differences in the mean abundances of taxa, we used the White's non-parametric t test due to skewed distributions and Benjamini-Hochberg tests for multiple comparisons with a q value set at 0.05. On a compositional level, the gut microbiota of the infant was significantly different between our study groups (Fig. 2, Supplementary Tables S3, S4, and S5). In infants born to normoweight, *Firmicutes* was the dominant phylum (81.50%), followed by *Bacteroidetes* (17.20%), *Proteobacteria* (0.72%), *Unclass_Bacteria* (0.29%), and *Actinobacteria* (0.28%) (Fig. 2a–b). In infants born to obese mothers, *Firmicutes* was also the main phyla (56.95%), followed by *Bacteroidetes* (41.83%), *Proteobacteria* (0.81%), *Unclass_Bacteria* (0.24%), and *Actinobacteria* (0.17%) (Fig. 2c–d). We observed a significant overabundance of *Bacteroidetes* ($p = 0.004$) in infants born to obese mothers whereas infants born to normoweight mothers had a significant overabundance of *Firmicutes* ($p = 0.004$) in the gut microbiota (Supplementary Table S3). At family level, *Clostridiaceae* 1 ($p = 0.007$) and *Lachnospiraceae* ($p = 0.036$) were significantly enriched in infants born to normoweight

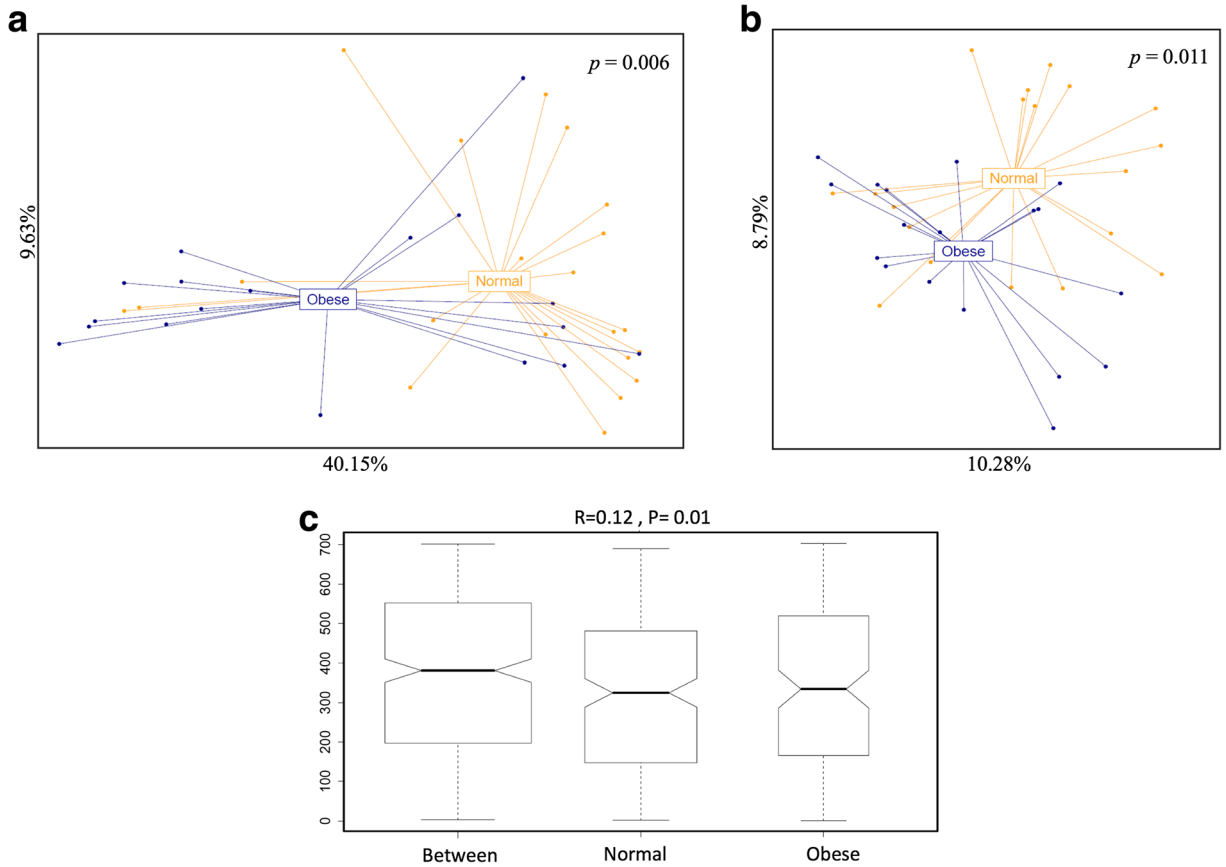

Fig. 1 Bacterial microbial community structure in infants born to normoweight and obese mothers at 18 months of age. PCoA plots of bacterial α-diversity based on weighted (**a**) and unweighted (**b**) UniFrac distances, and the Bray-Curtis dissimilarity values (**c**). Infants born to normoweight (normal) and obese mothers (obese) are coloured in *orange* and *blue*, respectively

mothers. Conversely, we observed a significant overabundance of *Porphyromonadaceae* ($p = 0.013$), *Bacteroidaceae* ($p = 0.014$), and *Prevotellaceae* ($p = 0.037$) in the gut microbiota of infants born to obese mothers (Supplementary Table S4). At genus level, the relative abundances of *Clostridium* XVIII ($p = 0.031$) and *Unclass_Clostridiaceae* 1 ($p = 0.031$) were significantly higher in infants born to normoweight mothers. In infants born to obese mothers, we observed a significant overabundance of *Parabacteroides* ($p = 0.020$), *Bacteroides* ($p = 0.014$), and *Oscillibacter* ($p = 0.019$) (Supplementary Table S5).

Profiling the metabolic capacity of bacterial communities

To identify microbial functions enriched or decreased in the infants gut microbiome depending on maternal prepregnancy BMI, an assessment of the microbial community functional potential was performed using PICRUSt metagenome predictions. Our analysis revealed similarities in the predicted functions of tier 1 KO functions between infant groups (Fig. 3). The greatest number of genes (>50%) that were assigned a function encoded for proteins involved in "metabolism" among tier 1 KO categories in the gut microbiome of infants born to normoweight and obese mothers. At tier 3 KO categories, we identified 226 predicted functions encoded in the microbiomes that were collapsed into higher categories using KEGG Pathways database (Supplementary Tables S6 and S7). To simplify analysis, a comparison of predicted functions involved in metabolism among tier 1 KO categories between both study groups was performed.

Between infant groups, a high number of significant differences in the abundances of second- and third-tier KO functional annotations were observed (Fig. 4a–b). At tier 2 KO functional annotations, the microbiome of infants born to normoweight mothers was characterized by a significant enrichment in the functional abundance of "nucleotide metabolism" ($p = 0.039$); whereas in infants born to obese mothers, the abundances of "glycan biosynthesis and metabolism" ($p = 0.042$), "metabolism of terpenoids and polyketides" ($p = 0.036$), "metabolism of other amino acids" ($p = 0.042$), and "biosynthesis of other secondary metabolites" ($p = 0.048$) were significantly increased (Fig. 4a). At tier 3 KO categories, the highest abundances of significant functional annotations in the microbiome of infants born to normoweight mothers were observed in "purine metabolism" ($p = 0.04$), "pentose phosphate pathway" ($p = 0.037$), "porphyrin and chlorophyll

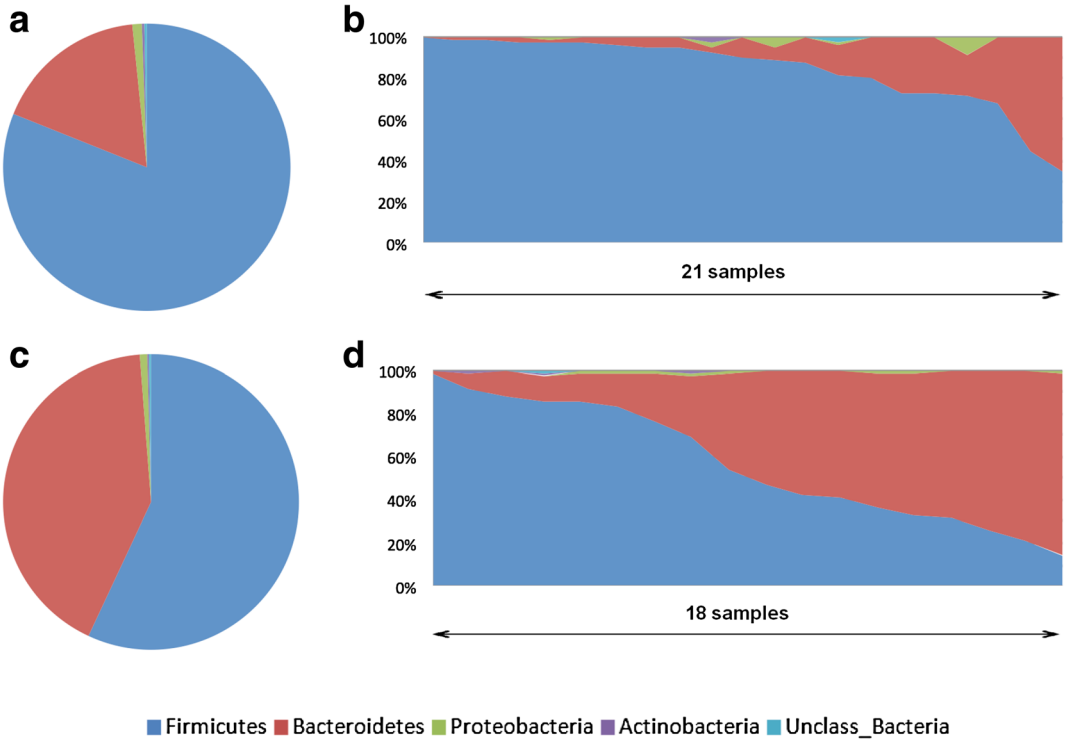

Fig. 2 Phylogenetic compositions of infants born to normoweight and obese mothers at 18 months of age. **a** and **c** Pie charts showing the distribution of phyla in infants born to normoweight mothers and obese mothers, respectively. **b** and **d** Inter-individual variation in the proportion of major phyla in infants born to normoweight mothers and obese mothers, respectively. Phyla are identified by *colour shades*: *Firmicutes* (*blue*), *Bacteroidetes* (*red*), *Proteobacteria* (*green*), *Actinobacteria* (*purple*), and *Unclass_Bacteria* (*light-blue*)

metabolism" (p = 0.038), "lysine biosynthesis" (p = 0.043), "glycerolipid metabolism" (p = 0.042), and "c5-branched dibasic acid metabolism' (p = 0.045). Notably, the microbiome of infants born to obese mothers was significantly enriched in low-abundant annotations of tier 3 KO categories. These categories were "streptomycin biosynthesis" (p = 0.047), "sulphur metabolism" (p = 0.041), "prenyltransferases" (p = 0.044), and "polyketide sugar unit biosynthesis" (p = 0.044). All categories within glycan biosynthesis and metabolism were significantly enriched in the microbiome of infants born to obese mothers: "glycosphingolipid biosynthesis globo and ganglio series" (p = 0.044, p = 0.041), "lipopolysaccharide biosynthesis" (p = 0.043), and "glycosaminoglycan degradation" (p = 0.042) (Fig. 4b).

Discussion

Maternal obesity and weight gain modify the composition and metabolism of the microbiota in the gut and breast milk during pregnancy and lactation [16, 17, 47]. Such microbial changes may be transferred to the offspring during delivery and lactation, altering microbial colonization of infant's gut [26, 58]. It is becoming increasingly clear that the gut microbiota in newborns and infants plays a key role in gut health and child development [17]. Dysbiosis of the early infant gut microbiota has been correlated with the development of childhood obesity and type 1 diabetes [11, 58]. Despite this link, few studies have addressed how maternal obesity influences the gut microbial metabolic potential during early life. Our study aimed to identify differences in the functions encoded by the microbiome of infants at 18 months of age born to prepregnancy normoweight and obese mothers. We analysed the gut microbial community composition by 16S rRNA amplicon high-throughput sequencing. Additionally, we used PICRUSt functional prediction to construct a community-level metabolic network of the microbiome and compare the abundance of pathways across infant groups [31].

Several studies have shown that the gut microbial ecosystem had higher α-diversity and lower β-diversity with no distinct clustering in children born to obese mothers compared to children born to normoweight mothers [16, 25]. These results disagree with those recently published by Laursen et al. [34]. The authors found no association between infant's gut microbiota at 9 and 18 months of age with maternal obesity. In our study, we provide evidence that maternal obesity is related to significant differences in gut microbial community structure because β-diversity metrics showed two distinct clustered groups according to maternal prepregnancy BMI. Mueller et al. showed that maternal obesity was associated with altered gut microbiota composition in neonates delivered vaginally, though not by C-section [42]. We found no effect of mode of

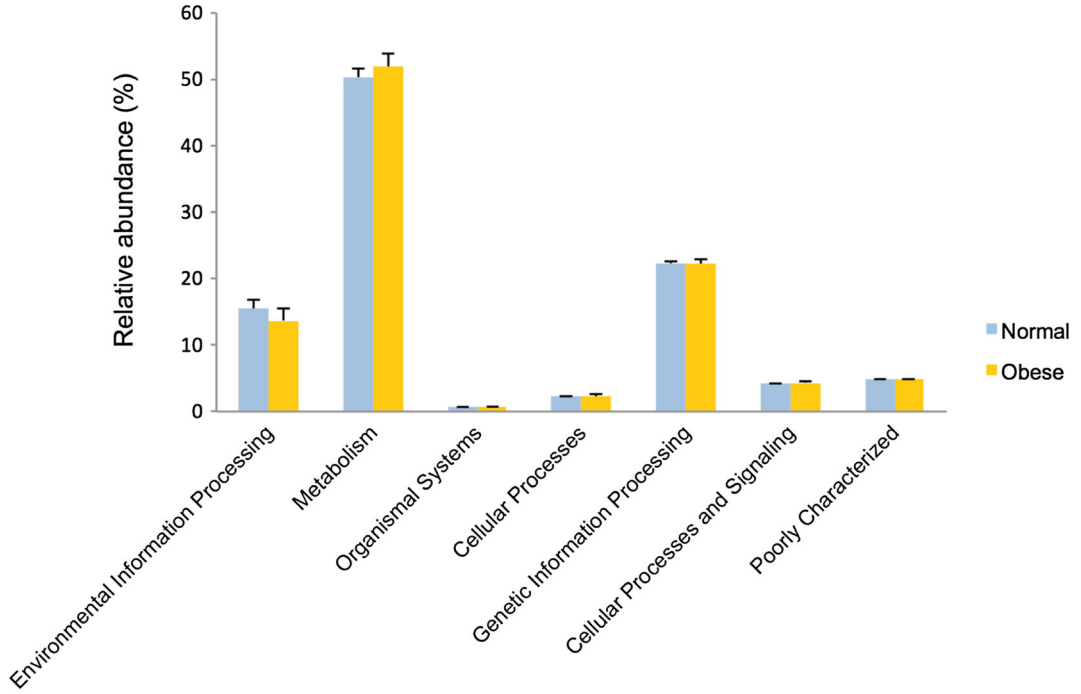

Fig. 3 Distribution of KEGG functional functional in infants born to normoweight and obese mothers. Tier 1 KEGG Orthology (*KO*) categories from infants born to normoweight mothers (normal) and infants born to obese mothers (obese) are identified by *colour shades blue* and *orange*, respectively

delivery or breast-feeding on gut microbiota, probably explained by the time of our sampling (18 months of age), when such variable is diluted by others like transition to complementary feeding. On a compositional level, we observed that *Firmicutes* was significantly enriched in children born to normoweight mothers whereas *Bacteroidetes* was significantly enriched in children born to obese women, due mainly to a significant increase in *Lachnospiraceae* and *Clostridicaeae* 1 and *Bacteroidaceae* families. At genus level, Collado et al. showed that levels of *Staphylococcus* and *Bacteroides* were significantly higher in infants born to overweight mothers during the first 6 months of life than in infants born to normoweight mothers, whereas levels of *Bifidobacterium* spp. were lower [16]. In our study, a significant overabundance of *Bacteroides*, *Parabacteroides*, and *Oscillibacter* was observed in infants born to obese mothers. Our results agree with those of Galley et al. [25]. They showed that these genera were significantly enriched in infants (aged 18 to 27 months) born to obese mothers compared to infants born to normoweight mothers. Although the genus *Bacteroides* is often associated with leanness and other desirable health traits [35, 51, 61, 63], some studies have linked these genera to obesity [28, 32, 56, 59]. Of note, differences in *Oscillibacter* have been found in prior studies of diet and obesity [13, 33, 39] that may be related to a higher consumption of fat [43]. It is plausible that differences in the sampling point (1, 6, 9, and 18 months of age) affecting feeding pattern and microbial ecosystem maturation, lifestyle, and maternal socioeconomic status explain the lack of reproducible findings. Further studies controlling for those variables in bigger cohorts are required to confirm the link between maternal obesity and offspring gut microbiota.

Our metagenomic predictions with PICRUSt showed an overall enrichment in genes involved in metabolic pathways in the gut microbiota of infants born to obese mothers. Of note, abundances of all KO modules involved in lipopolysaccharide (LPS) biosynthesis were increased. LPS derived from the outer membranes of Gram-negative bacteria has been intensively studied and is known to induce metabolic endotoxemia by promoting secretion of pro-inflammatory cytokines. The LPS from gut microbiota can induce a chronic subclinical inflammatory process and obesity, leading to insulin resistance through activation of TLR4 [9, 54]. Studies in both animal models and humans have shown that a high-fat diet can modulate the gut microbiota and increase circulating levels of LPS, probably by uptake of LPS in chylomicrons secreted from intestinal epithelial cells or through increased intestinal permeability. Therefore, the enrichment in LPS biosynthesis may predispose infants born to obese mothers to insulin resistance [58].

Our functional annotations highlight the importance of sulphur metabolism in infants born to obese mothers. This pathway is involved in the degradation of sulphur-containing compounds, such as products from the fermentation of certain amino acids (cysteine and methionine) and taurine bile acid derivatives. According to this, we also found a significantly

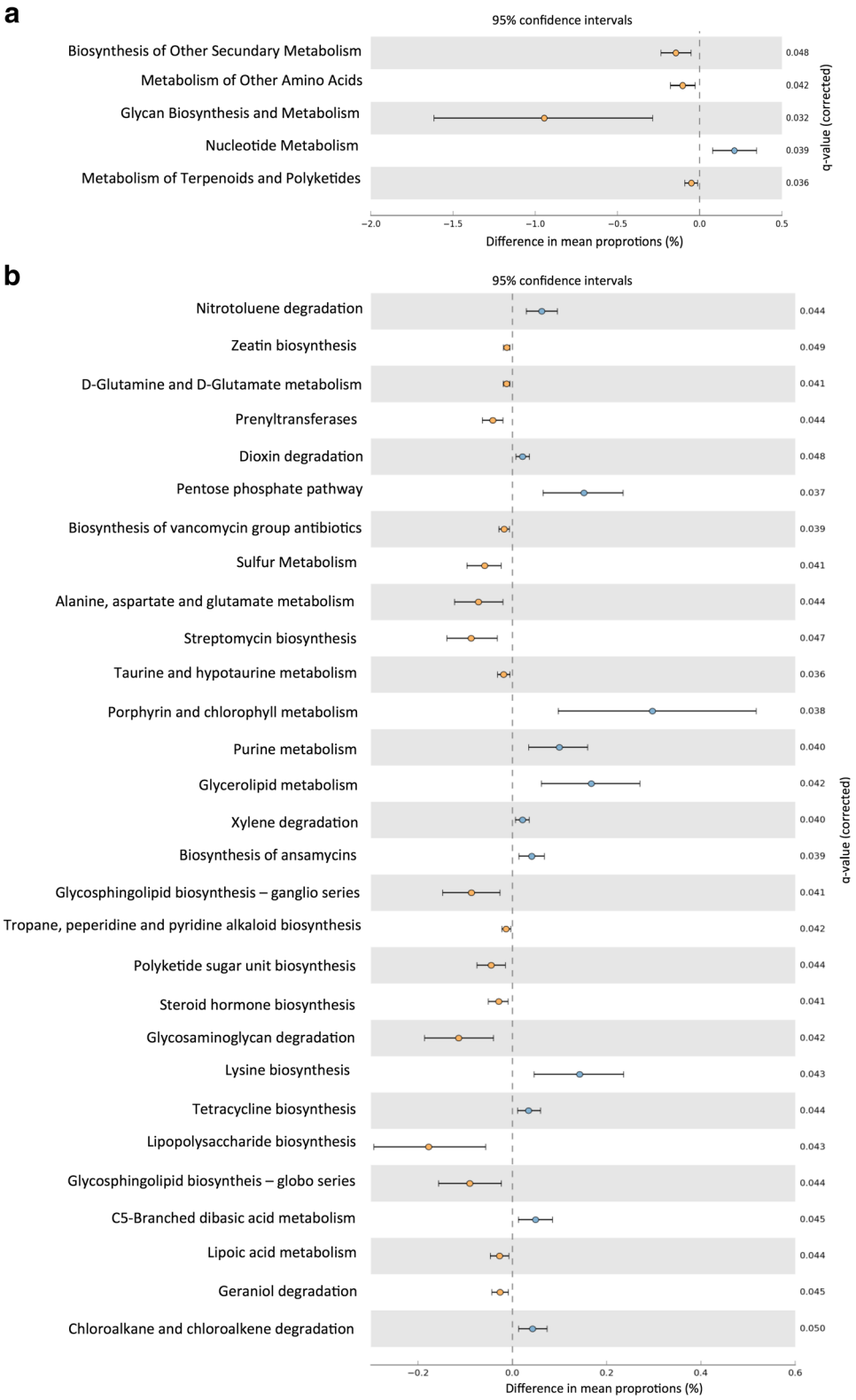

Fig. 4 Significant differences in potential metabolic capacities of the gut microbiomes between infants born to normoweight mothers (*blue*) and obese mothers (*orange*). Significance was determined by White non-parametric t test with Benjamini and Hockberg FDR correction for multiple comparisons. Only functional capacities with corrected $p < 0.05$ are shown

overrepresentation of genes involved in taurine and hypotaurine metabolism in children born to obese mothers. These pathways lead to a wide variety of compounds including hydrogen sulphide, the principal by-product of sulphur-reducing bacteria in the mammalian colon [60]. Hydrogen sulphide has well-established detrimental effects on the colonic microenvironment and epithelial health, since its excess may be detrimental for colonic epithelium energy metabolism

and DNA integrity. This result may have important long-term implications for bowel health in feeding patterns where excessive protein diets are consumed [36, 37, 64].

Despite infants born to obese mothers had higher abundances of *Bacteroides*, specialized in the degradation of dietary non-digestible carbohydrates as well as host carbohydrates including mucus, we found no enrichment in KO modules of polysaccharide degradation. The microbiota hydrolyses non-digestible carbohydrates into oligosaccharides and monosaccharides that fuel central carbon metabolism [21, 23]. Major bacterial metabolic routes are the Embden-Meyerhof-Parnas pathway (glycolysis, for six-carbon sugars) and the pentose-phosphate pathway (for five-carbon sugars) leading to phosphoenolpyruvate that is converted into fermentation products such as butyrate and propionate [41]. In our study, the microbiome of infants born to normoweight mothers has a significantly higher proportion of genes involved in lipoic acid metabolism, an essential cofactor of pyruvate dehydrogenase, and pentose-phosphate pathway. Considering that the fermentation routes were present in the gut microbiota, our results suggest an increased ability to process the larger variety of monosaccharides in the diet by the gut microbiota of infants born to normoweight mothers. Indeed, previous reports showed that normoweight children had higher levels of faecal SCFA, the end products of monosaccharide fermentation, compared to obese ones [47]. However, controversy still exists regarding the role of SCFA in obesity [50, 52]

In summary, our study showed that maternal prepregnancy obesity may imprint a selective gut microbial composition during late infancy with higher metabolic capacity. We are aware that the small sample size may preclude detecting other important differences. Still, our results showed differences in taxonomic composition and functionality of the gut microbiota across infant groups that withstood stringent correction for multiple testing. Nevertheless, future analyses enrolling a greater number of participants will help to understand the molecular factors associated to maternal prepregnancy weight that alters the microbial ecosystem during early life.

Acknowledgments This work was supported by the European Union's 7th Framework Programme under grant agreement no. 613979 (MyNewGut Project 2013/KB/613979) and no. 329812 (MC IEF, NutriOmics) and by the Spanish Ministry of Economy and Competitiveness (MINECO) BFU2012-40254-C03-01. Tomás Cerdó participated in the PhD Program in Biomedicine of the University of Granada and is a fellow of the FPI (BES-2013-065133) Program at the Spanish Ministry of Economy and Competitiveness. This article will be part of the Doctoral PhD of Tomás Cerdó.

References

1. Adlerberth I, Wold AE (2009) Establishment of the gut microbiota in Western infants. Acta paediatrica (Oslo, Norway: 1992);98:229–38
2. Allin KH, Nielsen T, Pedersen O (2015) Mechanisms in endocrinology: gut microbiota in patients with type 2 diabetes mellitus. Eur J Endocrinol 172:R167–R177
3. Arrieta M-C, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, et al. (2015) Early infancy microbial and metabolic alterations affect risk of childhood asthma Sci Transl Med, 7(307), 307ra152-307ra152
4. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P et al (2015) Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 17(5):690–703
5. Berglund SK, García-Valdés L, Torres-Espinola FJ, Segura MT, Martínez-Zaldívar C, Aguilar MJ et al (2016) Maternal, fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: an observational cohort study (PREOBE). BMC Public Health 16:207
6. Brook I, Finegold SM (1979) Bacteriology and therapy of lung abscess in children. J Pediatr 94:10–12
7. Cabreiro F, Gems D (2013) Worms need microbes too: microbiota, health and aging in *Caenorhabditis elegans*. EMBO molecular medicine 5:1300–1310
8. Camarinha-Silva A, Jauregui R, Chaves-Moreno D, Oxley AP, Schaumburg F, Becker K et al (2014) Comparing the anterior nare bacterial community of two discrete human populations using Illumina amplicon sequencing. Environ Microbiol 16:2939–2952
9. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O et al (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58:1091–1103
10. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336
11. Cerdó T, García-Valdés L, Altmäe S, Ruíz A, Suárez A, Campoy C (2016) Role of microbiota function during early life on child's neurodevelopment. Trends Food Sci Technol 57:273–288
12. Cerdó T, Ruiz A, Campoy C. (2017) Human gut microbiota and obesity during development. Adiposity-Omics and Molecular Understanding: InTech; 2017
13. Clarke SF, Murphy EF, O'Sullivan O, Ross RP, O'Toole PW, Shanahan F et al (2013) Targeting the microbiota to address diet-induced obesity: a time dependent challenge. PLoS One 8:e65790
14. Clemente JC, Ursell LK, Parfrey LW, Knight R (2012) The impact of the gut microbiota on human health: an integrative view. Cell 148:1258–1270
15. Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM et al (2007) The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. Nucleic Acids Res 35:D169–DD72
16. Collado MC, Isolauri E, Laitinen K, Salminen S (2010) Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. Am J Clin Nutr 92:1023–1030
17. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I et al (2014) Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell 158:705–721
18. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences;107:11971-5
19. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, et al (2015) High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut: gutjnl-2015-309957

20. Erkosar B, Storelli G, Defaye A, Leulier F (2013) Host-intestinal microbiota mutualism:"learning on the fly". Cell Host Microbe 13:8–14
21. Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TMS, Comelli EM (2014) Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. Nutr Diabetes 4(6):e121
22. Ferrer M, Ruiz A, Lanza F, Haange SB, Oberbach A, Till H et al (2013) Microbiota from the distal guts of lean and obese adolescents exhibit partial functional redundancy besides clear differences in community structure. Environ Microbiol 15:211–226
23. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E (2012) Microbial degradation of complex carbohydrates in the gut. Gut Microbes 3: 289–306
24. Flint HJ, Scott KP, Louis P, Duncan SH (2012) The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 9:577–589
25. Galley JD, Bailey M, Dush CK, Schoppe-Sullivan S, Christian LM (2014) Maternal obesity is associated with alterations in the gut microbiome in toddlers. PLoS One 9:e113026
26. Garcia-Mantrana I, Collado MC (2016) Obesity and overweight: impact on maternal and milk microbiome and their role for infant health and nutrition. Mol Nutr Food Res 60:1865–1875
27. Human Microbiome Project C (2012) A framework for human microbiome research. Nature 486:215–221
28. Ignacio A, Fernandes MR, Rodrigues VAA, Groppo FC, Cardoso AL, Avila-Campos MJ et al (2016) Correlation between body mass index and faecal microbiota from children. Clin Microbiol Infect 22:258. e1–258. e8
29. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M (2011) KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids research: gkr988
30. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B et al (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 498:99–103
31. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA et al (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814–821
32. La Rosa PS, Warner BB, Zhou Y, Weinstock GM, Sodergren E, Hall-Moore CM, et al (2014) Patterned progression of bacterial populations in the premature infant gut. Proceedings of the National Academy of Sciences;111:12522-7
33. Lam YY, Ha CWY, Campbell CR, Mitchell AJ, Dinudom A, Oscarsson J et al (2012) Increased gut permeability and microbiota change associate with mesenteric fat inflammation and metabolic dysfunction in diet-induced obese mice. PLoS One 7:e34233
34. Laursen MF, Andersen LBB, Michaelsen KF, Mølgaard C, Trolle E, Bahl MI et al (2016) Infant gut microbiota development is driven by transition to family foods independent of maternal obesity. Msphere 1:e00069–e00015
35. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444:1022–1023
36. Linden, D. R., Levitt, M. D., Farrugia, G., & Szurszewski, J. H (2010) Endogenous production of H2S in the gastrointestinal tract: still in search of a physiologic function. Antioxid Redox Signal, 12(9), 1135–1146
37. Linden DR. (2014) Hydrogen sulfide signaling in the gastrointestinal tract. Antioxid Redox Signal. 10;20(5):818-30. doi: 10.1089/ars.2013.5312
38. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. (2013) A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut: gutjnl-2013
39. Martínez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG et al (2013) Gut microbiome composition is linked to whole grain-induced immunological improvements. ISME J 7:269–280
40. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A et al (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME Journal 6:610–618
41. Miller TL, Wolin MJ (1996) Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. Appl Environ Microbiol 62:1589–1592
42. Mueller NT, Shin H, Pizoni A, Werlang IC, Matte U, Goldani MZ, Goldani HAS, Dominguez-Bello MG (2016) Birth mode-dependent association between pre-pregnancy maternal weight status and the neonatal intestinal microbiome. Sci Rep 6
43. Nakayama J, Yamamoto A, Palermo-Conde LA, Higashi K, Sonomoto K, Tan J, et al. (2017) Impact of westernized diet on gut microbiota in children on Leyte Island. Front Microbiol. 2017 Feb 14;8:197.
44. Natamba BK, Sanchez SE, Gelaye B, Williams MA (2016) Concordance between self-reported pre-pregnancy body mass index (BMI) and BMI measured at the first prenatal study contact. BMC Pregnancy and Childbirth 16(1):187
45. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB et al (2013) Package 'vegan'. Community Ecology package, version 2(9)
46. Parks DH, Beiko RG (2010) Identifying biologically relevant differences between metagenomic communities. Bioinformatics (Oxford, England);26:715-21
47. Payne AN, Chassard C, Zimmermann M, Müller P, Stinca S, Lacroix C (2011) The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. Nutr Diabetes 1(7):e12
48. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F et al (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490:55–60
49. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C et al (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65
50. Rahat-Rozenbloom, S., Fernandes, J., Gloor, G. B., & Wolever, T. M (2014) Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. Int J Obes, 38(12), 1525–1531
51. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science (New York, NY);341:1241214
52. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de los Reyes-Gavilán CG, Salazar N (2016) Intestinal short chain fatty acids and their link with diet and human health. Front Microbiol 7
53. Roca-Saavedra P, Mendez-Vilabrille V, Miranda JM, Nebot C, Cardelle-Cobas A, Franco CM, Cepeda A (2017) Food additives, contaminants and other minor components: effects on human gut microbiota—a review. J Physiol Biochem:1–15
54. Saad MJ, Santos A, Prada PO. (2016) Linking gut microbiota and inflammation to obesity and insulin resistance. Physiology (Bethesda). 2016 Jul;31(4):283-93. doi: 10.1152/physiol.00041.2015
55. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541
56. Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, et al (2010) Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring, Md);18:190-5

57. Shreiner AB, Kao JY, Young VB (2015) The gut microbiome in health and in disease. Curr Opin Gastroenterol 31(1):69
58. Soderborg TK, Borengasser SJ, Barbour LA, Friedman JE (2016) Microbial transmission from mothers with obesity or diabetes to infants: an innovative opportunity to interrupt a vicious cycle. Diabetologia 59:895–906
59. Tagliabue A, Elli M (2013) The role of gut microbiota in human obesity: recent findings and future perspectives. Nutrition, Metabolism, and Cardiovascular Diseases: NMCD 23:160–168
60. Tomasova L, Konopelski P, Ufnal M. (2016) Gut bacteria and hydrogen sulfide: the new old players in circulatory system homeostasis. Molecules. 2016 Nov 17;21(11). pii: E1558
61. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444: 1027–1031
62. Villanueva-Millán MJ, Pérez-Matute P, Oteo JA (2015) Gut microbiota: a key player in health and disease. A review focused on obesity. J Physiol Biochem 71(3):509–525
63. Wexler HM (2007) Bacteroides: the good, the bad, and the nitty-gritty. Clin Microbiol Rev 20:593–621
64. Yao CK, Muir JG, Gibson PR (2016) Review article: insights into colonic protein fermentation, its modulation and potential health implications. Aliment Pharmacol Ther 43:181–196
65. Yu Z, Han S, Zhu J, Sun X, Ji C, Guo X (2013) Pre-pregnancy body mass index in relation to infant birth weight and offspring overweight/obesity: a systematic review and meta-analysis. PLoS One 8(4):e61627