Luisa F. Gomez-Arango,[1,2] Helen L. Barrett,[1,2,3] H. David McIntyre,[1,4] Leonie K. Callaway,[1,2,3] Mark Morrison,[5] and Marloes Dekker Nitert,[1,2] for the SPRING Trial Group

Connections Between the Gut Microbiome and Metabolic Hormones in Early Pregnancy in Overweight and Obese Women

Diabetes 2016;65:2214–2223 | DOI: 10.2337/db16-0278

METABOLISM

Overweight and obese women are at a higher risk for gestational diabetes mellitus. The gut microbiome could modulate metabolic health and may affect insulin resistance and lipid metabolism. The aim of this study was to reveal relationships between gut microbiome composition and circulating metabolic hormones in overweight and obese pregnant women at 16 weeks' gestation. Fecal microbiota profiles from overweight (*n* = 29) and obese (*n* = 41) pregnant women were assessed by 16S rRNA sequencing. Fasting metabolic hormone (insulin, C-peptide, glucagon, incretin, and adipokine) concentrations were measured using multiplex ELISA. Metabolic hormone levels as well as microbiome profiles differed between overweight and obese women. Furthermore, changes in some metabolic hormone levels were correlated with alterations in the relative abundance of specific microbes. Adipokine levels were strongly correlated with *Ruminococcaceae* and *Lachnospiraceae,* which are dominant families in energy metabolism. Insulin was positively correlated with the genus *Collinsella.* Gastrointestinal polypeptide was positively correlated with the genus *Coprococcus* but negatively with family *Ruminococcaceae*. This study shows novel relationships between gut microbiome composition and the metabolic hormonal environment in overweight and obese pregnant women at 16 weeks' gestation. These results suggest that manipulation of the gut microbiome composition may influence pregnancy metabolism.

The increasing prevalence of maternal obesity and its subsequent health outcomes are a significant public health concern and a major challenge for obstetrics practice. In early pregnancy, overweight and obese women are at an increased risk of metabolic complications that affect placental and embryonic development (1). Metabolic adjustments, such as a decline in insulin sensitivity and an increase in nutrient absorption, are necessary to support a healthy pregnancy (2,3); however, these metabolic changes occur on top of preexisting higher levels of insulin resistance in overweight and obese pregnant women, increasing the risk of overt hyperglycemia because of a lack of sufficient insulin secretion to compensate for the increased insulin resistance (3).

The potential role of the gut microbiome (the composite of the bacteria present in the gastrointestinal tract) in pregnancy has become the subject of considerable interest. In normal pregnancy, the maternal gut microbiota changes from first to third trimester with a decline in butyrate-producing bacteria and an increase in *Bifidobacteria*, *Proteobacteria*, and lactic acid–producing bacteria. Inflammation and weight gain that occurs during pregnancy might be the result of microbe-driven processes to increase energy supply for the fetus (4). These alterations might also be linked with the maternal metabolic profile and thereby contribute to the development of pregnancy complications (5,6) as well as affect the metabolic and immunological health of the offspring (7). In summation, modifications in the metabolic hormone milieu during gestation are proposed to be linked with changes in the maternal microbiota; however, no studies

[1]School of Medicine, The University of Queensland, Brisbane, Queensland, Australia
[2]The University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia
[3]Obstetric Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia
[4]Mater Research, The University of Queensland, Brisbane, Queensland, Australia
[5]Faculty of Medicine and Biomedical Sciences, The University of Queensland Diamantina Institute, Brisbane, Queensland, Australia

Corresponding author: Marloes Dekker Nitert, m.dekker@uq.edu.au.

Received 28 February 2016 and accepted 11 May 2016.

Clinical trial reg. no. ANZCTR12611001208998, www.anzctr.org.au.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db16-0278/-/DC1.

have reported a possible maternal hormonal-microbial interaction to date.

The aim of this study was to evaluate the relationships between gut microbiome composition and the metabolic hormonal milieu in overweight and obese pregnant women in early gestation (<16 weeks). To address this relationship, fecal microbiota profiles as assessed by 16S rRNA gene amplicon sequencing were correlated with the serum concentrations of nine metabolic hormones involved in glucose and energy metabolism.

RESEARCH DESIGN AND METHODS

Study Population and Sample Collection

This study included a subset of 29 overweight and 41 obese pregnant women from the SPRING (Study of Probiotics in the Prevention of Gestational Diabetes Mellitus) cohort (8). Women with known preexisting diabetes, impaired fasting glucose, or impaired glucose tolerance and/or early-onset gestational diabetes mellitus (GDM) were excluded. The women in this subset were selected based on completion of the pregnancy with two stool samples collected. Of the women meeting these criteria, all with GDM at 28 weeks' gestation were included and those with normoglycemia were matched for BMI, maternal age, and ethnicity to encompass a broad spectrum of metabolic health. Clinical characteristics and biological samples at <16 weeks' gestation are summarized in Table 1. Fasting blood glucose, HbA_{1c}, triglyceride, total cholesterol, HDL, LDL, and VLDL levels were analyzed immediately after collection, with further serum aliquots stored at −80°C. HOMA of insulin resistance (HOMA-IR) was calculated for each participant. Refrigerated fecal samples were self-collected by each participant at home and stored within 1 day after collection at −80°C until DNA extraction.

Hormone Measurements

Stored serum samples were used to measure insulin, C-peptide, glucagon, gastrointestinal polypeptide (GIP), GLP-1, ghrelin, leptin, resistin, and visfatin by using the Bio-Plex Pro human diabetes immunoassay (Bio-Rad Laboratories, Hercules, CA) at room temperature according to the manufacturer's instructions. Serum samples were allowed to thaw overnight at 4°C. Fifty microliters of undiluted serum were loaded in each well in duplicate. Standard curves were generated for all hormones. Hormone concentrations were collected and analyzed by using a Bio-Rad Bio-Plex 200 instrument equipped with Bio-Plex Manager 6.1 software (Bio-Rad Laboratories).

Fecal DNA Extraction

The repeated bead beating and column method (9) followed by QIAGEN AllPrep DNA extraction kit were used for microbial DNA extraction from 0.25 g of thawed stool samples. Mechanical disruption was achieved using sterile zirconia beads (0.1 and 0.5 mm diameter) with homogenization for 3 min at maximum speed (30 Hz) in the TissueLyser II (QIAGEN). DNA concentration was determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies).

Microbiome Profiling

Fecal microbiome profiles were assessed by 16S rRNA gene amplicon sequencing by using the Illumina MiSeq system at The University of Queensland Australian Centre for Ecogenomics. Bacterial 16S rRNA gene sequences covering variable regions (V6–V8) were PCR amplified from purified genomic DNA by using the primers 926F (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG AAA CTY AAA KGA ATT GRC GG-3′) and 1392R (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GAC GGG CGG TGW GTR C-3′) with overhang adapters, generating a 500-base pair product. In each PCR run, a positive control (*Escherichia coli* JM109) and negative control (sterile deionized water) were added. The V6–V8 PCR amplicons were cleaned using AMPure XP beads to remove unbound primers, primer dimer species, and nucleotides. Thirty-two self-correction bar codes were added to the primers using Nextera XT Index Kit followed by a second PCR cleanup step. Library quantification, normalization, and pooling were performed according to the manufacturer's instructions.

Data files were processed using QIIME (Quantitative Insights Into Microbial Ecology) version 1.9.1 software (www.qiime.org). Forward and reverse sequence reads for each individual sample were demultiplexed, quality filtered, and joined (10). Sequences were assigned to operational taxonomic units (OTUs) with a pairwise identity threshold of 97% by using the GG (Greengenes) reference database (11). Sequences were mapped, yielding an OTU table, and a phylogenetic tree was constructed using FastTree (12), which was then used to generate unweighted and weighted UniFrac distance metrics. Any OTUs with overall relative abundance <0.0001 were excluded from further analysis. A total of 2,086,048 reads representing 787 OTUs were retained (median 26,049 reads/sample, range 3,180–88,127). To standardize sequence reads across samples, data were randomly rarefied to 3,000 sequences per sample. The relative abundances of OTUs were summarized across various taxonomic levels using default settings in QIIME.

Statistical Analysis

Pregnant women were classified according to BMI cutoff values of 25.0–30.0 kg/m^2 (overweight) and >30.0 kg/m^2 (obese). Normally distributed variables are reported as mean ± SEM; otherwise, median with interquartile range is reported (Table 1). Comparisons between the two BMI categories were performed with the Mann-Whitney *U* test. Correlations between hormonal and anthropometric variables were performed with the Spearman correlation coefficient test. Statistical analysis of the demographic and clinical data were performed using GraphPad Prism 6 software. $P < 0.05$ was considered statistically significant.

Gut microbiota diversity within samples from each individual (α-diversity) was assessed by Chao1 index,

Table 1—Maternal characteristics and biochemical data

	Overweight (n = 29)	Obese (n = 41)	P value
Age (years)	32.7 ± 1.05	34.9 ± 0.64	0.067
BMI (kg/m^2)	26.10 (25.70–27.85)	35.60 (33.15–37.39)	<0.0001
Ethnicity, %			
Caucasian	97	94	
Other	3	6	
Gestational age (days)[a]	105.2 ± 1.35	109.5 ± 1.16	0.020
Total cholesterol (mmol/L)	5.50 (4.70–6.15)	5.60 (5.00–6.08)	0.931
HDL (mmol/L)	1.60 (1.40–2.05)	1.60 (1.43–1.80)	0.591
LDL (mmol/L)	3.00 (2.65–3.60)	3.10 (2.70–3.50)	0.793
VLDL (mmol/L)	0.60 (0.45–0.85)	0.75 (0.53–0.90)	0.077
Triglycerides (mmol/L)	1.30 (0.95–1.85)	1.65 (1.23–1.90)	0.015
Random blood glucose	4.77 ± 0.18	4.97 ± 0.11	0.319
Fasting glucose (mmol/L)	4.28 ± 0.06	4.49 ± 0.05	0.015
HbA_{1c} (mmol/L)	5.00 (4.75–5.20)	5.00 (4.90–5.20)	0.465
HOMA-IR	0.77 (0.50–0.95)	1.75 (0.80–2.4)	<0.0001
Insulin (μU/L)	4.34 (2.31–5.47)	8.20 (4.51–11.43)	<0.0001
C-peptide (pg/mL)	132.0 (76.6–219.0)	186.1 (113.2–353.4)	0.032
Glucagon (pg/mL)[b]	23.1 (13.5–33.8)	31.1 (19.9–40.9)	0.170
GIP (pg/mL)	22.8 (16.8–36.5)	16.4 (12.6–22.9)	0.007
GLP-1 (pg/mL)	41.2 (26.1–55.6)	36.7 (22.6–55.1)	0.603
Ghrelin (pg/mL)	35.3 (20.8–87.2)	24.9 (14.1–65.9)	0.227
Leptin (pg/mL)	1,830 (846.9–3,531)	3,312 (1,650–7,133)	0.015
Resistin (pg/mL)	68.7 (53.7–104.5)	52.4 (33.5–76.7)	0.030
Visfatin (pg/mL)[c]	224.1 (46.7–604.0)	71.3 (27.6–356.5)	0.373
GDM diagnosis, n (%)	8 (27.6)	18 (43.9)	0.212
Asthma, n (%)*	0 (0)	2 (4.8)	ND
Vegetarian, n (%)	1 (3.4)	0 (0)	ND

Data are mean ± SEM or median (interquartile range), unless otherwise stated. Clinical characteristics and biochemical and hormonal variables of overweight and obese pregnant women at <16 weeks' gestation. The statistically significant difference between the overweight and obese groups are $P < 0.05$. ND, not determined. [a]Gestational age data from 26 overweight and 35 obese pregnant women were registered. [b]Glucagon was only detectable in 50% of pregnant women (overweight n = 17, obese n = 18). [c]Visfatin was only detectable in 27% of pregnant women and removed from further analyses (overweight n = 12, obese n = 17). *Women on active treatment for asthma.

which estimates the number of different taxa, and by Shannon diversity index, which evaluates the richness and evenness of taxa. High Shannon diversity values range from 2.5 to 3.0. Significant differences in microbial community composition between overweight and obese women or future GDM status (β-diversity) were calculated by permutational MANOVA (pMANOVA) of weighted UniFrac distances (Bray-Curtis distance method) with 999 permutations and illustrated by principal component analysis. The impact of maternal age and gestational age on microbiome composition was evaluated by redundancy analysis. For the hypothesis-driven analysis, canonical correspondence analysis (CCA) with permutation tests was computed and visualized by Calypso software (http://bioinfo.qimr.edu.au/calypso). Associations between the metadata (BMI category, hormonal measurements, glucose levels, HbA_{1c}, and clinical data) and taxa abundance were evaluated by bootstrapped Spearman rank correlation coefficient using 1,000 permutations. For all analyses, correction for multiple testing by false discovery rate (FDR) with the Benjamini-Hochberg procedure was done in QIIME, with values of <0.05 considered statistically significant.

RESULTS

Clinical Characteristics and Biochemical Variables

The clinical characteristics of the pregnant women included in this substudy at <16 weeks' gestation are presented in Table 1. Seventy healthy pregnant women were grouped according to their BMI and matched for GDM status, maternal age, and ethnicity. Fasting serum levels of nine metabolic hormones (insulin, C-peptide, glucagon, GIP, GLP-1, ghrelin, leptin, resistin, and visfatin) were measured in all participants. Detectable hormone levels were present in all participants except glucagon and visfatin, which were detectable in 50% and 27%, respectively. Visfatin was not included in further analyses. There were

significant differences in BMI, fasting glucose, HOMA-IR, insulin, C-peptide, leptin, GIP, and resistin between the two groups (Table 1), with obese women having a more disturbed metabolic profile. Fasting serum glucose was positively correlated with insulin and C-peptide concentrations (Table 2). HbA_{1c} was not correlated with fasting serum glucose or any of the nine metabolic hormones measured.

The Microbiomes of Overweight and Obese Women Are Different at 16 Weeks' Gestation

Stool samples were collected from all women to assess whether the composition of the gut microbiota was associated with maternal BMI. The ratio between the main phyla *Firmicutes* to *Bacteroidetes* was 3:1, with obese women tending to have a slightly higher abundance of *Firmicutes* (69.0% [59.0–81.4%]) than overweight women (64.2% [57.6–72.9%], $P = 0.107$). The relative abundances of two other dominant gut phyla tended to differ between overweight and obese women at 16 weeks' gestation (Fig. 1*A*) with a higher relative abundance of *Tenericutes* in the overweight group ($P < 0.05$) and higher abundance of *Actinobacteria* in the obese group ($P = 0.084$). At the family level, *Lachnospiraceae* ($\rho = 0.42$, $P < 0.0001$) and *Rikenellaceae* ($\rho = 0.28$, $P < 0.0001$) were positively correlated with maternal BMI. Microbiota composition was not significantly affected by either maternal age ($P = 0.705$) or gestational age ($P = 0.176$). Furthermore, the microbiome composition at 16 weeks' gestation was not different between women in whom GDM did or did not develop at 28 weeks' gestation ($P = 0.200$) (Supplementary Fig. 1).

We observed that overweight pregnant women tended to have a higher microbial richness (number of OTUs) and evenness (relative prevalence of the various OTUs within the gut) than obese pregnant women (Shannon index $P = 0.098$) (Fig. 1*B*); however, there was no difference in the total number of different taxa between the groups (Supplementary Fig. 2, Chao1 index). Examination of the bacterial community structure between the two groups as measured by β-diversity, principal component analysis (Supplementary Fig. 3), and pMANOVA showed no significant differences between overweight and obese women (pMANOVA $P = 0.604$); however, the hypothesis-based CCA showed that BMI was significantly associated with gut microbiota composition at the phylum level in early pregnancy ($P = 0.009$) (Fig. 1*C*).

Table 2—Metabolic hormones and their correlation with BMI and fasting glucose levels in overweight and obese pregnant women at 16 weeks' gestation

Hormone	BMI	Fasting glucose
Insulin	**0.71*****	**0.43*****
C-peptide	**0.27***	**0.35****
Glucagon	0.14	−0.13
GLP-1	−0.03	0.22
GIP	**−0.27***	0.03
Ghrelin	−0.21	0.10
Leptin	**0.37****	**0.23***
Resistin	**−0.34****	0.001
Visfatin	−0.21	0.29

Data are Spearman correlation coefficients. Significant correlations shown in boldface. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Correlations Between Maternal Metabolic Hormones and Gut Microbiota

Gut Microbiota and Gestational Glucose Metabolism

Insulin, C-peptide, HOMA-IR, and fasting glucose were all significantly and positively correlated with increasing BMI (Table 2). To assess associations between maternal glucose metabolism and gut microbiota, insulin, C-peptide, HOMA-IR, and fasting glucose levels were correlated with intestinal bacteria abundance (Table 3). At the phylum level, insulin levels and HOMA-IR were positively correlated with the relative abundance of *Actinobacteria* and negatively correlated with *Tenericutes*, resembling the trend observed with increasing BMI. Here, *Actinobacteria* was primarily composed of two main families: *Bifidobacteriaceae* and *Coriobacteriaceae*. Women with increased serum levels of insulin and C-peptide exhibited a higher abundance of *Coriobacteriaceae* ($P < 0.0001$) but a similar abundance of *Bifidobacteriaceae*. The ratio of *Bifidobacteriaceae* to *Coriobacteriaceae* was 1:2. Further analysis within *Coriobacteriaceae* indicated that abundance of the genus *Collinsella* was positively associated with insulin ($P = 0.003$), HOMA-IR ($P = 0.012$), and C-peptide ($P = 0.020$) levels and different between overweight and obese pregnant women ($P = 0.027$) (Fig. 2); however, *Collinsella* abundance was not correlated with fasting glucose ($P = 0.783$) or BMI ($P = 0.340$) after correction for multiple testing. Furthermore, the abundance of the family *Ruminococcaceae* from the phylum *Firmicutes* was enriched in women with higher C-peptide concentrations ($\rho = 0.41$, $P < 0.0001$). A positive correlation between the *Ruminococcaceae* and insulin levels was observed from the bootstrapped Spearman rank correlation coefficient produced using 100 permutations ($\rho = 0.28$, $P < 0.0001$) but not with 1,000 permutations ($\rho = 0.26$, $P > 0.05$).

The relationship between the gut microbiome composition and incretin hormone levels was also assessed. Fasting serum GIP concentrations were negatively correlated with BMI in early pregnancy. Higher fasting GIP levels were correlated with a higher abundance of the genus *Coprococcus* (family *Lachnospiraceae*) ($\rho = 0.47$, $P < 0.0001$) but a lower abundance of *Ruminococcaceae* ($\rho = -0.39$, $P < 0.0001$), with both families belonging to phylum *Firmicutes*. There were no significant associations of GLP-1 with the gut microbiota and maternal BMI.

Gut Microbiota and Energy Metabolism

To further investigate the relationship between maternal gut microbiota and metabolism, correlations to serum adipokine levels were assessed (Table 3). Leptin was found to be positively associated with BMI (Table 2) and fasting

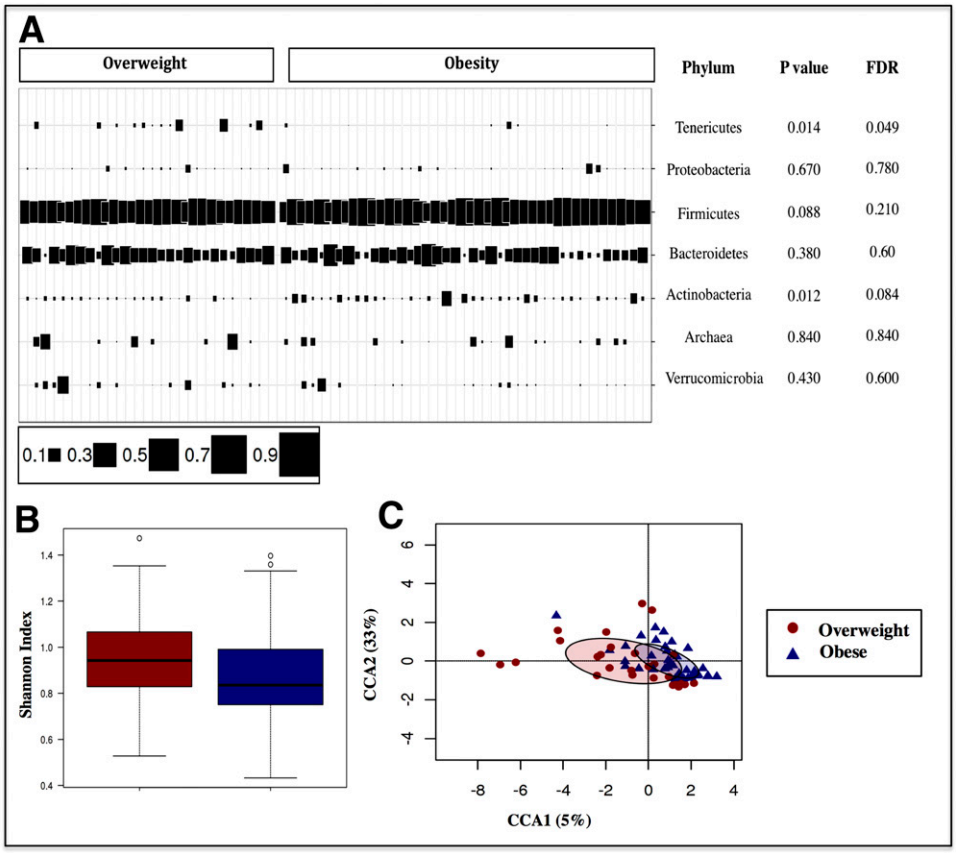

Figure 1—Comparison of the gut community composition between overweight and obese pregnant women. *A*: Phylum-level distribution of bacteria in fecal samples of overweight and obese pregnant women. The size of each box represents the mean relative abundance of bacterial phyla. *P* and FDR values indicate statistical significances of differences in phylum composition between overweight and obese pregnant women. *B*: Comparison of gut microbiota diversity within overweight (red) and obese (blue) pregnant women estimated by Shannon index ($P = 0.098$). Box plots show the 25th and 75th percentile with a line at the median. *C*: A CCA plot shows clustering of bacterial populations at the phylum level according to BMI categories. The percentage of variation is shown. This hypothesis-driven technique suggests that BMI significantly affects gut microbiota composition ($P = 0.009$).

glucose. Higher leptin levels were strongly correlated with *Firmicutes* family members *Lachnospiraceae* ($\rho = 0.45$, $P < 0.0001$) (Fig. 3) and *Ruminococcaceae* ($\rho = 0.41$, $P < 0.0001$). In contrast to leptin, resistin concentrations were negatively correlated with maternal BMI (Table 2) and *Ruminococcaceae* ($\rho = -0.40$, $P < 0.0001$) (Table 3). Ghrelin, the hunger hormone, exhibited a trend for negative correlation with maternal BMI in early pregnancy (Table 2). Of note, ghrelin levels were positively correlated with the family *Bacteroidaceae* ($\rho = 0.33$, $P < 0.0001$) and negatively with the family *Prevotellaceae* ($\rho = -0.34$, $P < 0.0001$) (Table 3), with both families belonging to the phylum *Bacteroidetes*.

The genus *Collinsella*, which was positively correlated to insulin levels, was also positively correlated with maternal triglycerides ($\rho = 0.31$, $P = 0.009$) and VLDL cholesterol levels ($\rho = 0.33$, $P = 0.006$) (Fig. 2*C*). In contrast, *Collinsella* exhibited a trend for negative correlation with maternal HDL cholesterol ($\rho = -0.31$, $P = 0.09$) (Supplementary Fig. 4). No associations were found with LDL cholesterol ($P = 0.383$).

DISCUSSION

This study is the first in our knowledge to report a relationship between maternal metabolic hormonal milieu and gut microbiome in early pregnancy. In obese pregnant women, the concentrations of fasting glucose, insulin, and leptin at 16 weeks' gestation resemble those in the third trimester of nonobese, nondiabetic pregnant women (13,14). Moreover, obesity is associated with a higher proportion of the major bacterial phylum *Actinobacteria* and a lower proportion of the phylum *Tenericutes*.

This study showed a positive correlation of the genus *Collinsella* (family *Coriobacteriaceae*, phylum *Actinobacteria*) with insulin, C-peptide, and HOMA-IR in pregnancy. *Collinsella* is well suited to colonize mucosal surfaces, metabolizes amino acids, and may directly interact with the host (15). Outside pregnancy, higher abundance of *Collinsella* has been reported in type 2 diabetes (16,17), which is reduced by weight loss (18). Whether limiting gestational weight gain in pregnancy lowers the abundance of *Collinsella* and whether this could contribute to lowering insulin resistance is not clear.

Table 3—Correlations between the relative abundance of bacterial taxa at various taxonomic levels and maternal hormones

	Phyla level	*P* value	FDR	*r*	Class level	*P* value	FDR	*r*	Family level	*P* value	FDR	*r*
Glucose metabolism												
Insulin	*Actinobacteria*	0.007	0.04	0.32	*Coriobacteriia*	<0.0001	<0.0001	0.33	*Coriobacteriaceae*	<0.0001	<0.0001	0.33
	Tenericutes	0.008	0.04	−0.30	*Bacilli*	<0.0001	<0.0001	0.31				
					Mollicutes	0.01	0.05	−0.30				
C-peptide	—	—	—	—	*Coriobacteriia*	0.006	0.09	0.31	*Coriobacteriaceae*	<0.0001	<0.0001	0.45
									Ruminococcaceae	<0.0001	<0.0001	0.41
HOMA–IR	*Actinobacteria*	0.008	0.055	0.33	*Coriobacteriia*	0.004	0.06	0.33	*Coriobacteriaceae*	0.004	0.036	0.33
	Tenericutes	0.011	0.055	−0.31	*Mollicutes*	0.011	0.06	−0.31				
Glucagon	—	—	—	—	*Actinobacteria*	0.04	0.65	−0.28	—	—	—	—
GIP	—	—	—	—	—	—	—	—	*Ruminococcaceae*	<0.0001	<0.0001	−0.39
Energy metabolism												
Leptin	—	—	—	—	—	—	—	—	*Lachnospiraceae*	<0.0001	<0.0001	0.45
									Ruminococcaceae	<0.0001	<0.0001	0.40
Ghrelin	—	—	—	—	—	—	—	—	*Prevotellaceae*	<0.0001	<0.0001	−0.34
									Bacteroidaceae	<0.0001	<0.0001	0.33
Resistin	*Firmicutes*	0.001	0.01	−0.34	*Clostridia*	0.002	0.03	−0.35	*Ruminococcaceae*	<0.0001	<0.0001	−0.40

Data are Spearman correlation coefficients for each taxonomy level.

Incretins GIP and GLP-1 stimulate insulin secretion and are secreted by cells in the gastrointestinal tract. Levels of the incretin hormone GIP are positively associated with the abundance of the genus *Coprococcus*. *Coprococci* are considered to be butyrate producers (19). Butyrate is an important short-chain fatty acid (SCFA) that can serve as a second messenger as well as a source of energy (20). Oral administration of sodium butyrate in mice significantly increased GLP-1 and GIP levels (21), supporting the importance of butyrate in the stimulation of gut hormones.

In the current study, maternal triglycerides and VLDL cholesterol were positively correlated with the genus *Collinsella*. Outside pregnancy, *Collinsella* abundance has been positively correlated with serum cholesterol (22) and bile acid deconjugation (15). Additionally, in infants, a slower increase in *Collinsella* and *Bifidobacterium* abundance is associated with lower body fat at a later age (23). Thus, there is a possible link between *Collinsella* abundance and fat metabolism both inside and outside pregnancy. This link is further supported in rodent models, where *Collinsella* abundance is strongly associated with non–HDL cholesterol (24) and hepatic triglycerides (25). Therefore, manipulating the abundance of *Collinsella* may affect both lipid and glucose metabolism in pregnancy.

Leptin levels were positively correlated with the abundance of the families *Lachnospiraceae* and *Ruminococcaceae*. These families are dominant species in the human gut. Some members are reportedly responsible for breaking down indigestible polysaccharides (i.e., dietary fiber) into SCFAs such as butyrate; however, an abnormal increase in these taxa could lead to an excessive calorie uptake by the host through increased availability of SCFAs, contributing to obesity (26). Leptin synthesis and secretion are increased from early gestation mainly due to placental production (3). Leptin opposes ghrelin activity. Ghrelin is a fast-acting hormone that stimulates food intake (27) and was higher in overweight women than in obese women. Ghrelin is positively correlated with the abundance of *Bacteroidaceae* but negatively with *Prevotellaceae*. Bacteria belonging to family *Bacteroidaceae* are involved in carbohydrate fermentation and are underrepresented in nonpregnant obese subjects (28). Members of family *Prevotellaceae* are associated with a vegetarian diet and with chronic inflammatory conditions (29). When members of both families co-occur in the gut, one or the other predominates because they have an antagonistic relationship (29). In fact, an inverse association between *Bacteroidetes* and *Prevotella* has been reported with obesity and type 2 diabetes in nonpregnant individuals (30,31) and now in pregnancy.

In human studies outside pregnancy, progression of glucose intolerance is accompanied by changes in the proportion and diversity of the gut microbiota (16,32). An obese-type microbiome often is reported to be less diverse (33).

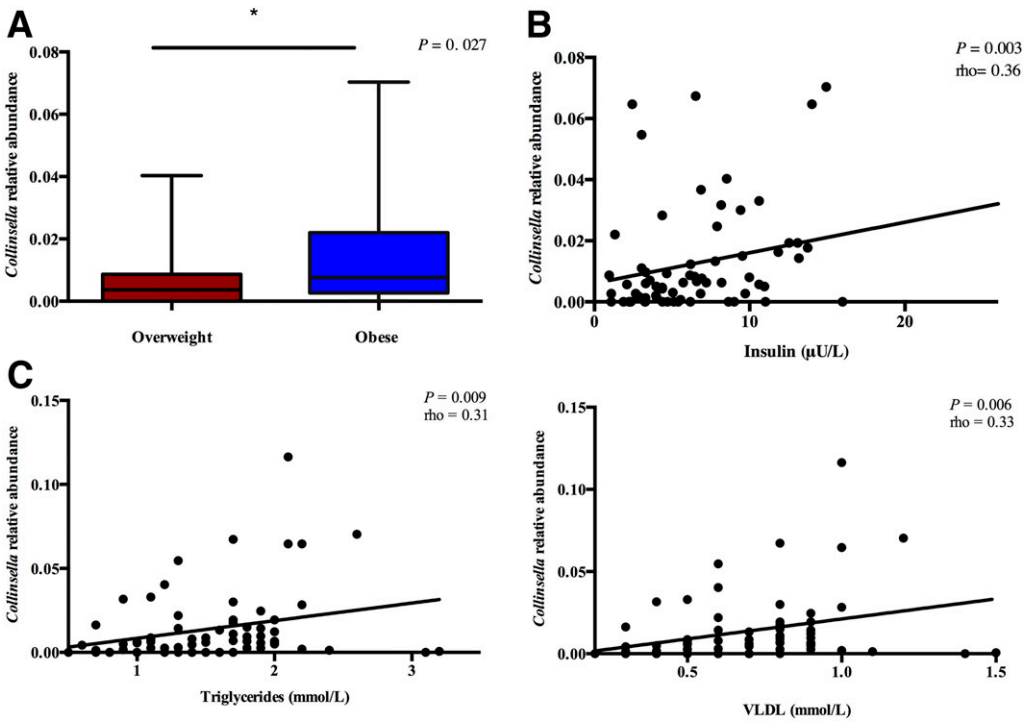

Figure 2—Genus *Collinsella* and its associations with insulin and maternal lipids profile. *A*: Comparison of genus *Collinsella* between overweight and obese pregnant women estimated by Mann-Whitney *U* test ($P = 0.027$). *B*: Positive correlation between the fasting insulin concentration of overweight and obese pregnant women at 16 weeks' gestation and genus *Collinsella* ($\rho = 0.36$, $P = 0.003$). *C*: Positive correlation between maternal triglycerides ($\rho = 0.31$, $P = 0.009$) and VLDL concentration ($\rho = 0.33$, $P = 0.006$) with genus *Collinsella*. *$P < 0.05$.

Only limited information is available on the effect of maternal obesity on the shifts in fuel metabolism and changes in microbiota composition in pregnancy. Here, obese pregnant women tended to have a less diverse gut microbiome compared with the overweight group; however, no significant differences in bacterial richness and evenness between individuals (α-diversity) were detected. This is similar to the results reported by the only maternal microbiome sequencing study to date, where the α-diversity of first-trimester gut microbiota was independent of prepregnancy BMI (4); however, the differences in composition of the maternal microbiota in early pregnancy between groups of overweight and obese women (β-diversity) show that subtle differences may exist between these groups. To definitively establish whether the relationship between microbiome and BMI is linear, a cohort of normal-weight women should be investigated.

In early gestation, the maternal microbiome shows a predominance of *Firmicutes* over *Bacteroidetes*, similar to the pattern observed in obese nonpregnant subjects (34). Here, obese pregnant women have a predominance of *Firmicutes* over *Bacteroidetes* (ratio 3.1:1) compared with overweight pregnant women (ratio 2.7:1). Santacruz et al. (35) reported similar results in early gestation, with a higher abundance of *Bacteroides* in normal-weight compared with overweight pregnant women; however, Collado et al. (36) associated *Bacteroides* with excessive weight gain over the first trimester. Moreover, the enrichment in *Firmicutes* species has been correlated with an increased expression of key enzymes involved in polysaccharide digestion, through which more energy may be harvested from the same diet (37).

Here, the significantly decreased abundance of *Tenericutes*, which is a less-prominent phylum, in the obese group may be explained by a relative increase in phylum *Actinobacteria* and/or *Firmicutes* abundance. This pattern of increased abundance of *Firmicutes* and *Actinobacteria* with decreased abundance of *Bacteroidetes* has previously been reported in nonpregnant obese, insulin-resistant subjects (33,38,39). Moreover, consumption of a high-fat diet in rodents for 2 weeks reduces the relative abundance of *Tenericutes* (40). At the family level, the abundance of *Lachnospiraceae*, from phylum *Firmicutes*, and *Rikenellaceae*, from phylum *Bacteroidetes*, were positively correlated with BMI. Abundance of some members of the *Lachnospiraceae* family have been linked with obesity in both humans and mice (41). This may be due to their role in SCFA production (42). In addition, in obese and diabetic mice, the family *Rikenellaceae* is more abundant (43) and highly enriched in mice fed a high-fat diet (44), relating *Rikenellaceae* with the host's phenotype and diet. Again, the patterns observed in early pregnancy mirror those outside pregnancy. Confirmation of these microbiome-hormonal associations in a larger cohort will be necessary.

No associations were found between GLP-1 and BMI or gut microbiota, even though these relationships have

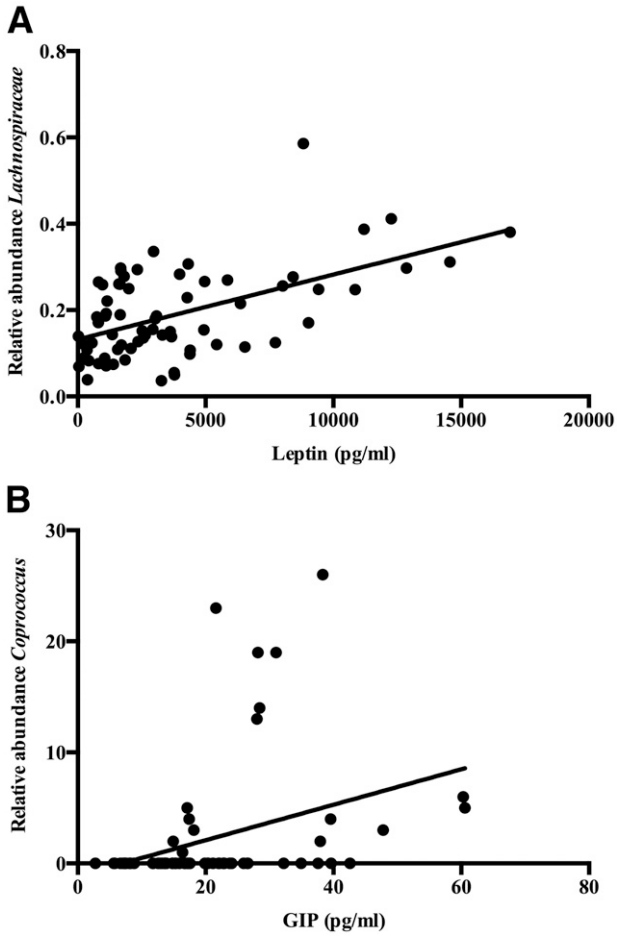

Figure 3—*A*: Positive correlation between leptin and family *Lachnospiraceae* ($\rho = 0.45$, $P < 0.0001$). *B*: Positive correlation between genus *Coprococcus* (family *Lachnospiraceae*) and GIP ($\rho = 0.47$, $P < 0.0001$).

been well established outside pregnancy (45). The lack of association in this study could be due to samples being taken during the fasting state, whereas GLP-1 secretion is stimulated upon ingestion of glucose and rapidly cleared from the circulation. Also in the current study, total GLP-1 rather than active GLP-1 was measured.

The microbiome-hormonal correlations in the current study were assessed in overweight and obese pregnant women only. To definitively establish whether the relationship between microbiome and BMI is linear, a cohort of normal-weight women should be investigated. Confounding factors such as diet and antibiotic treatment may further affect both the metabolic profile and the gut microbiota composition (16). The current study excluded women treated with agents known to alter glucose metabolism, and antimicrobial treatment was carefully recorded.

It is not clear from the study whether the microbiome determines the hormone levels or vice versa or whether the regulation is in fact bidirectional. Animal models may help to establish this; however, modulation of the gut microbiota by the ingestion of prebiotics (as a food source for beneficial bacteria) and/or administration of antibiotics in nonpregnant subjects reportedly affects adiposity and metabolic hormone levels (45,46), indicating that gut microbiome composition affects hormonal levels. Proliferation of the probiotic bacteria *Bifidobacterium* species modulates inflammation in obese mice and increases incretin secretion, thereby decreasing insulin resistance (47). In pregnancy, probiotic supplementation in normal-weight pregnant women significantly reduces the incidence of GDM (48) and lowers maternal cholesterol (49) and triglyceride (50) levels. These data support the notion that gut microbiota modulation by selective prebiotics or probiotic bacteria constitutes an interesting target for modulating maternal metabolism. The current results suggest that suppression of *Collinsella* is an additional target for improving glucose homeostasis and lipid metabolism in early pregnancy; however, if probiotics belonging to the genus *Bifidobacterium* are administered during pregnancy, the ratio between *Coriobacteriaceae* and *Bifidobacteriaceae* may change in favor of *Bifidobacterium* species. This could possibly alleviate impairments in both glucose and lipid metabolism. The SPRING trial, in which overweight and obese pregnant women are supplemented with *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12, may shed light on this (8).

This study brings clear insights into the relationships between microbiota and maternal metabolic regulatory hormones and their potential causal associations in overweight and obese pregnancy; however, as the gut microbiota composition changes throughout pregnancy, these microbial-hormonal associations need to be examined in the third trimester to provide insights into whether the changes at 16 weeks are predictive for the progression to diabetes. Pregnancy is also accompanied by immune changes allowing for fetal survival and immune cells to interact constantly in the gut. The host's immune system can also contribute to gut microbiota composition and thereby affect maternal hormones; however, no studies of the relationship among the immune system, the gut microbiota, and metabolism in pregnancy currently exist.

In summary, a high abundance of family *Ruminococcaceae* in early pregnancy may be related to adverse metabolic health. In addition, a high abundance of the family *Coriobacteriaceae* and genus *Collinsella* could serve as markers of impaired glucose metabolism, and a higher abundance of the families *Lachnospiraceae*, *Prevotellaceae*, and *Bacteroidaceae* is associated with maternal energy metabolism.

Conclusions

This study shows that a relationship exists between the gut microbiome composition and the metabolic hormone milieu in early pregnancy, which could influence the metabolic health of the mother and infant. The study brings a better understanding of the composition of the gut microbiota in early obese pregnancy and suggests that proportion and diversity of certain microbiota members

can serve as potential markers for a high risk in insulin resistance and abnormal lipid metabolism.

Acknowledgments. The authors thank the participants; the SPRING Trial Group (Katie Foxcroft, Anne Tremellen, Jacinta Tobin, Shelley Wilkinson, Chris McSweeney, Peter O'Rourke, and Barbara Lingwood); and Alicia Kang, Erin Shanahan, Naoki Fukuma (The University of Queensland Diamantina Institute), and Nicola Angel (Australian Centre for Ecogenomics, The University of Queensland) for expert technical advice.

Funding. L.F.G.-A. is supported by a Departamento Administrativo de Ciencia, Tecnología e Innovación scholarship and H.L.B. is supported by the Australian Diabetes Society Skip Martin Research Fellowship. The SPRING trial is supported by the National Health and Medical Research Council (grant 1028575) and a Royal Brisbane and Women's Hospital Foundation grant.

Duality of Interest. The SPRING trial has received probiotics and placebo products from Chr. Hansen A/S. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. L.F.G.-A. contributed to the data research and analysis and writing and editing of the manuscript. H.L.B., H.D.M., and L.K.C. provided intellectual input and contributed to the editing of the manuscript. M.M. provided intellectual input and technical expertise and contributed to the editing of the manuscript. M.D.N. provided intellectual input and contributed to the research design and writing and editing of the manuscript. M.D.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Kristensen J, Vestergaard M, Wisborg K, Kesmodel U, Secher NJ. Pre-pregnancy weight and the risk of stillbirth and neonatal death. BJOG 2005;112:403–408
2. Lain KY, Catalano PM. Metabolic changes in pregnancy. Clin Obstet Gynecol 2007;50:938–948
3. King JC. Maternal obesity, metabolism, and pregnancy outcomes. Annu Rev Nutr 2006;26:271–291
4. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012;150:470–480
5. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? Diabetes Care 2010;33:2277–2284
6. Thum C, Cookson AL, Otter DE, et al. Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? J Nutr 2012;142:1921–1928
7. Laker RC, Wlodek ME, Connelly JJ, Yan Z. Epigenetic origins of metabolic disease: the impact of the maternal condition to the offspring epigenome and later health consequences. Food Science and Human Wellness. 2013;2:1–11
8. Nitert MD, Barrett HL, Foxcroft K, et al. SPRING: an RCT study of probiotics in the prevention of gestational diabetes mellitus in overweight and obese women. BMC Pregnancy Childbirth 2013;13:50
9. Yu Z, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 2004;36:808–812
10. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335–336
11. DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 2006;72:5069–5072
12. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol 2009;26:1641–1650
13. Valsamakis G, Margeli A, Vitoratos N, et al. The role of maternal gut hormones in normal pregnancy: fasting plasma active glucagon-like peptide 1 level is a negative predictor of fetal abdomen circumference and maternal weight change. Eur J Endocrinol 2010;162:897–903
14. Kyriakakou M, Malamitsi-Puchner A, Militsi H, et al. Leptin and adiponectin concentrations in intrauterine growth restricted and appropriate for gestational age fetuses, neonates, and their mothers. Eur J Endocrinol 2008;158:343–348
15. Clavel T, Desmarchelier C, Haller D, et al. Intestinal microbiota in metabolic diseases: from bacterial community structure and functions to species of pathophysiological relevance. Gut Microbes 2014;5:544–551
16. Zhang X, Shen D, Fang Z, et al. Human gut microbiota changes reveal the progression of glucose intolerance. PLoS One 2013;8:e71108
17. Lambeth SM, Carson T, Lowe J, et al. Composition, diversity and abundance of gut microbiome in prediabetes and type 2 diabetes. J Diabetes Obes 2015;2:1–7
18. Walker AW, Ince J, Duncan SH, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J 2011;5:220–230
19. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett 2009;294:1–8
20. Hijova E, Chmelarova A. Short chain fatty acids and colonic health. Bratisl Lek Listy 2007;108:354–358
21. Lin HV, Frassetto A, Kowalik EJ Jr, et al. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PLoS One 2012;7:e35240
22. Lahti L, Salonen A, Kekkonen RA, 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;1:e32
23. Dogra S, Sakwinska O, Soh SE, et al.; GUSTO Study Group. Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. MBio 2015;6:e02419-14
24. Martínez I, Wallace G, Zhang C, et al. Diet-induced metabolic improvements in a hamster model of hypercholesterolemia are strongly linked to alterations of the gut microbiota. Appl Environ Microbiol 2009;75:4175–4184
25. Claus SP, Ellero SL, Berger B, et al. Colonization-induced host-gut microbial metabolic interaction. MBio 2011;2:e00271-10
26. Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TM, Comelli EM. Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. Nutr Diabetes 2014;4:e121
27. Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes Rev 2007;8:21–34
28. Wexler HM. Bacteroides: the good, the bad, and the nitty-gritty. Clin Microbiol Rev 2007;20:593–621
29. Ley RE. Gut microbiota in 2015: *Prevotella* in the gut: choose carefully. Nat Rev Gastroenterol Hepatol 2016;13:69–70
30. Fugmann M, Breier M, Rottenkolber M, et al. The stool microbiota of insulin resistant women with recent gestational diabetes, a high risk group for type 2 diabetes. Sci Rep 2015;5:13212
31. Furet JP, Kong LC, Tap J, et al. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. Diabetes 2010;59:3049–3057
32. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012;490:55–60
33. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature 2009;457:480–484
34. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. FEBS Lett 2014;588:4223–4233
35. Santacruz A, Collado MC, García-Valdés L, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 2010;104:83–92
36. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr 2008;88:894–899

37. Cani PD. Gut microbiota and obesity: lessons from the microbiome. Brief Funct Genomics 2013;12:381–387
38. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006;444:1022–1023
39. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–1031
40. Kang SS, Jeraldo PR, Kurti A, et al. Diet and exercise orthogonally alter the gut microbiome and reveal independent associations with anxiety and cognition. Mol Neurodegener 2014;9:36
41. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nat Rev Genet 2012;13:260–270
42. Kameyama K, Itoh K. Intestinal colonization by a *Lachnospiraceae* bacterium contributes to the development of diabetes in obese mice. Microbes Environ 2014;29:427–430
43. Geurts L, Lazarevic V, Derrien M, et al. Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue. Front Microbiol 2011;2:149
44. Kim KA, Gu W, Lee IA, Joh EH, Kim DH. High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. PLoS One 2012;7:e47713
45. Everard A, Cani PD. Gut microbiota and GLP-1. Rev Endocr Metab Disord 2014;15:189–196
46. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012;488:621–626
47. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091–1103
48. Luoto R, Laitinen K, Nermes M, Isolauri E. Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. Br J Nutr 2010;103:1792–1799
49. Asemi Z, Samimi M, Tabasi Z, et al. Effect of daily consumption of probiotic yoghurt on lipid profiles in pregnant women: a randomized controlled clinical trial. J Matern Fetal Neonatal Med 2012;25:1552–1556
50. Hoppu U, Isolauri E, Koskinen P, Laitinen K. Maternal dietary counseling reduces total and LDL cholesterol postpartum. Nutrition 2014;30:159–164