Association between gut microbiota composition and glycoalbumin level during pregnancy in Japanese women: Pilot study from Chiba Study of Mother and Child Health

Kenichi Sakurai1*, Tamotsu Kato2, Hiromi Tanabe1, Naoko Taguchi-Atarashi2, Yumi Sato3, Akifumi Eguchi4, Masahiro Watanabe4, Hiroshi Ohno2, Chisato Mori4,5

1Department of Nutrition and Metabolic Medicine, Center for Preventive Medical Sciences, Chiba University, Chiba, 2Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences, Yokohama, 3Department of Nutrition and Metabolic Medicine, Graduate School of Medical and Pharmaceutical Sciences, 4Department of Sustainable Health Science, Center for Preventive Medical Sciences, and 5Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan

Keywords
Gut microbiota, Pregnancy, Serum glycoalbumin

*Correspondence
Kenichi Sakurai
Tel: +81-43-226-2017
Fax: +81-43-226-2018
E-mail address: sakuraik@faculty.chiba-u.jp

J Diabetes Investig 2020; 11: 699–706
doi: 10.1111/jdi.13177

ABSTRACT
Aims/Introduction: Gut microbiota have various effects on human health. Some previous reports have shown that gut microbiota change during pregnancy and affect metabolism, but others have shown that microbiota do not change. Here, we examined the gut microbiota and glycoalbumin levels of 45 healthy Japanese women during pregnancy.

Materials and Methods: We carried out 16S rRNA gene sequencing analyses of maternal stool samples and compared the gut microbiota composition of samples from women in early and late pregnancy. We also examined the association between gut microbiota and maternal characteristics, including glycoalbumin.

Results: Microbiota composition in early and late pregnancy did not differ, according to principal coordinate analysis of weighted and unweighted UniFrac distances. Shannon indices were not different between early and late pregnancy. The proportion of one phylum, TM7, significantly decreased in late pregnancy compared with early pregnancy, but the proportions of other major phyla did not change. The Shannon index of late pregnancy was negatively associated with pregestational body mass index and positively correlated with glycoalbumin level, with adjustment of covariates.

Conclusions: We concluded that Japanese women did not show obvious differences in gut microbiota during pregnancy, except for TM7, and that the diversity of gut microbiota might affect maternal metabolism. As this study had limited statistical power, further large-scale studies are required.

INTRODUCTION
Gut microbiota are reported to have various effects on human health and diseases1. They play an important role in human health by producing vitamins and short-chain fatty acids2. Some gut bacteria produce B vitamins, such as folate. Others produce short-chain fatty acids through fermentation, such as butyrate, which is reported to regulate the immune system3 and metabolism4. An imbalance in gut microbiota is called dysbiosis, and is characterized by decreased diversity of gut microbiota, a reduced number of beneficial bacteria and an increased number of pathobionts or bacterial species associated with disease5. Dysbiosis is reported to be associated with obesity and metabolic diseases6–8.

Several studies have investigated gut microbiota during pregnancy. Most of those studies reported that gut microbiota is altered during pregnancy9–13. Koren et al.10 reported that gut microbiota alterations are associated with dramatic changes in metabolic status during pregnancy. They reported an increase in Proteobacteria and Actinobacteria from the first to the third
trimester in Finnish women. In their study, microbiota from the third trimester affected the adiposity and glucose tolerance of germ-free mice to which the stool was transferred. Another report showed that Bacteroidetes is dominant in pregnant women in South China, and that Vercomicrobia declines and Tenericutes increases during late pregnancy. The same study noted a significant structural shift in gut microbiota in pre-eclampsia patients. In contrast, DiGiulio et al. reported no dramatic changes in gut microbiota during pregnancy. Other studies have shown differences in the microbial diversity or abundance of some phyla. It is unclear how gut microbiota are affected by pregnancy and whether the changes are associated with metabolic status in pregnant women.

Gut microbiota composition differs among different areas or populations, and is influenced by ethnicity and diet. For example, gut microbiota during pregnancy is related to dietary fat and fiber intake in Finnish pregnant women. To understand the role of gut microbiota in pregnancy, more information is required regarding various populations of different ethnicities and those that consume different diets.

Thus, the aim of the present study was to examine the changes in gut microbiota during pregnancy in healthy Japanese women, and the association between gut microbiota and maternal metabolic status according to glycoalbumin, total cholesterol and triglyceride levels in early and late pregnancy.

**METHODS**

**Study design and participants**

The participants were members of the Chiba Study of Mother and Child Health (C-MACH), a hospital-based birth cohort study. Participants were recruited at three hospitals in Chiba and Saitama prefectures in Japan. Approximately 400 pregnant women participated between February 2014 and June 2015, and all provided blood samples. Of those women, 66 participated also consented to collection of their stool samples; however, 15 participants were excluded because they did not provide stool samples during either early or late pregnancy, and 13 were excluded because their anthropometric or blood chemical data were lacking. We analyzed pairs of samples from early and late pregnancy from 45 women.

**Ethics**

The study was carried out according to the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Japanese Ministry of Health, Labor and Welfare. The study protocol was approved by the Biomedical Research Ethics Committee of the Graduate School of Medicine, Chiba University. In addition, written informed consent was obtained from the participants.

**Questionnaires and biochemical data**

Self-administered questionnaires were carried out during early and late pregnancy, as described previously. In the present study, laboratory procedures were carried out according to the literature, with minor modifications. DNA extraction and PCR were carried out, as described previously. DNA extraction from stool samples and 16S rRNA gene sequencing were carried out, as described previously. DNA extraction was carried out using phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation. RNase treatment and polyethylene glycol precipitation were carried out. DNA extraction from stool samples and 16S rRNA gene sequencing were carried out, as described previously. The blood of participants was collected around 12 and 32 weeks of gestation. The blood was separated into serum and stored at −80°C in Chiba University Center for Preventive Medical Sciences Biobank before the measurement. The maternal serum levels of glycoalbumin, total cholesterol, and triglycerides during early and late pregnancy were measured using Lactica GA-I (Asahi Kasei Pharma Corporation, Tokyo, Japan), Cholestest CHO (Sekisui Medical Co., Ltd., Tokyo, Japan) and Pureauto S TG–N (Sekisui Medical Co., Ltd.), respectively, at SRL (SRL, Inc., Shinjuku, Tokyo, Japan).

**Gut microbiota composition**

Stool samples were collected to analyze the composition of the gut microbiota in early (~12 weeks) and late (~32 weeks) pregnancy. Stool samples were collected at home and frozen at −18°C. The samples were then transferred to Chiba University Center for Preventive Medical Sciences Biobank and kept frozen at −80°C until DNA extraction.

DNA extraction from stool samples and 16S rRNA gene sequencing were carried out, as described previously. Stool samples were blended with methanol and filtrated. The filtrate was centrifuged at 15,000 g and the supernatant was stored for later analysis. Fecal DNA extraction was carried out according to the literature, with minor modifications. The pellet was suspended and incubated with lysozyme (Fujiﬁlm Wako Pure Chemical Corporation, Osaka, Japan) and achromopeptidase (Fujiﬁlm Wako Pure Chemical Corporation), sequentially. The suspension was added to sodium dodecyl sulfate and proteinase K (Merck, Darmstadt, Germany), then incubated. The bacterial DNA was puriﬁed using phenol/chloroform/isooamyl alcohol extraction and ethanol precipitation. RNase treatment and polyethylene glycol precipitation were carried out.

The V1-2 variable region (27Fmod-338R) was sequenced on an Illumina MiSeq (Illumina, San Diego, CA, USA). The 16S rRNA V1–V2 amplicon was ampliﬁed using universal bacterial 16S rRNA gene primers: forward primer TCGTCGCGAGGGCTGAGCTACGTAGTGTATAAGACAGAGATGTGTATAAGAGACAGAGTTTGATYMTGGCTCAG and reverse primer GTCTCGTGGGCTCGGAGATGTGATCTCGTGGGCTCGGAGATGTGATC TATAAGAGACAGATTACCAGCGGGCTCGTGCG. We attached dual indexes and Illumina sequencing adapters to polymerase chain reaction products using the Nextera XT Index Kit (Illumina). After puriﬁcation of the amplicon, mixed samples were prepared by pooling approximately equal amounts of polymerase chain reaction amplicons from each sample. A sample library with 20% denatured PhiX spike-in (SeqMatic LLC, Fremont, CA, USA) was sequenced by MiSeq using MiSeq Reagent Kit v2 (2 × 250 bp).

Taxonomic assignments and estimation of the relative abundance of sequencing data were carried out using the analysis pipeline of the QIIME software package. Chimera checking was carried out usingUCHIME. An operational taxonomic unit was deﬁned at 97% similarity. The operational taxonomic unit was assigned a taxonomy based on a comparison with the Greengenes database using RDP Classiﬁer. We summarized
the proportions of identified taxa in each sample, and calculated the amount of bacterial diversity. The Shannon index, a measurement of within-sample community diversity, was used to evaluate alpha diversity. The similarity of samples was calculated using weighted or unweighted UniFrac distances as implemented in R software\(^27\) (R Foundation for Statistical Computing, Vienna, Austria) and visualized for the beta-diversity analysis using principal coordinate analysis plots. Differences in microbial communities between early and late pregnancy were assessed by permutational analysis of variance (PERMANOVA) of weighted or unweighted UniFrac distances using the adonis function from the R package vegan\(^28\). Effect sizes (\(R^2\)) and statistical significance (\(P\)-value) were determined by 1,000 permutations.

**Statistical analysis**

Data are presented as medians (1st and 3rd quartiles). The differences in the medians between values of different groups were evaluated using Wilcoxon rank-sum test. Spearman’s rank correlation coefficient was calculated using the maternal microbiota composition and other characteristics. We carried out statistical calculations using R version 3.4.0\(^27\). Partial correlation coefficients were calculated using the \texttt{ppcorr}\(^29\). As the sample size of the present study was relatively small, correlation coefficients between maternal characteristics or blood data and each phylum were calculated without adjustment for multiple tests.

**RESULTS**

**Participant characteristics**

The median characteristics of the mothers were as follows: age 34.0 years (1st and 3rd quartile values of 31.0 years and 36.0 years); pregestational weight 52.0 kg (49.0 kg and 56.0 kg); height 159.0 cm (155.0 cm and 162.0 cm); pregestational body mass index (BMI) 20.4 kg/m\(^2\) (19.1 kg/m\(^2\) and 22.2 kg/m\(^2\)) and gestational bodyweight gain 8.5 kg (5.9 kg and 10.6 kg). In early pregnancy, maternal serum glycoalbumin, total cholesterol and triglyceride values were 13.8% (13.2 and 15.5%), 174.0 mg/dL (152.0 and 193.0 mg/dL) and 81.0 mg/dL (60.0 and 110.0 mg/dL), respectively (Table 1). In late pregnancy, maternal serum glycoalbumin, total cholesterol and triglyceride values were 12.9% (12.5 and 13.6%), 265.0 mg/dL (244.0 and 295.0 mg/dL) and 81.0 mg/dL (60.0 and 110.0 mg/dL), respectively (Table 1). Pregestational BMI was almost the same as that reported in national survey data, but gestational weight gain was slightly lower in national survey data than in early pregnancy (Figure 2). In late pregnancy, the relative abundance of Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria was 64.7% (57.4 and 70.8%), 18.5% (11.6 and 26.3%), 13.6% (8.5 and 21.5%) and 0.8% (0.5 and 1.5%), respectively (Table 2). The proportion of phylum TM7 was significantly lower in late pregnancy than in early pregnancy (Figure 2). No other phyla showed significant alterations.

**Association of gut microbiota with maternal characteristics and blood chemistry**

The Shannon index for samples taken in early pregnancy did not show any correlation with maternal age \((r = -0.092, P = 0.549)\), pregestational BMI \((r = -0.210, P = 0.167)\) or gestational weight gain \((r = 0.180, P = 0.238)\). Although the Shannon index for late pregnancy also had no correlation with maternal age \((r = -0.006, P = 0.968)\) or gestational weight gain \((r = 0.064, P = 0.677)\), it showed a significant negative correlation with pregestational BMI \((r = -0.458, P = 0.002)\). The Shannon index showed no correlation with blood chemistry data in early pregnancy. It also showed no significant correlation with total cholesterol or triglyceride levels, but there was a positive correlation with serum glycoalbumin levels in late pregnancy (Table 3). The correlation between the Shannon index and glycoalbumin in late pregnancy was significant after adjustment for maternal age, pregestational BMI and gestational weight gain (Table 3).

### Table 1 | Participant characteristics

| Characteristic          | n  | Age (years) | 34.0 [31.0, 36.0] | Height (cm) | 159.0 [155.0, 162.0] | Pregestational weight (kg) | 52.0 [49.0, 56.0] | Pregestational BMI | 20.39 [19.07, 22.22] | Gestational weight gain (kg) | 9.05 [7.85, 12.05] | Glycoalbumin, early (%) | 13.8 [13.2, 14.5] | Glycoalbumin, late (%) | 12.9 [12.5, 13.6] |
|-------------------------|----|-------------|-----------------|-------------|---------------------|--------------------------|------------------|------------------|--------------------------|---------------------------|-------------------------|------------------------|----------------------|------------------------|----------------------|
| Total cholesterol, early (mg/dL) | 174.0 [152.0, 193.0] | Total cholesterol, late (mg/dL) | 265.0 [244.0, 295.0] | Triglyceride, early (mg/dL) | 81.0 [60.0, 110.0] | Triglyceride, late (mg/dL) | 208.0 [166.0, 244.0] |

Values are shown as median [1st, 3rd quartile]. BMI, body mass index.
Next, we examined the association between the proportions of five specific phyla and maternal characteristics during each period. After adjustment for multiple tests, there were no significant correlations, so we expressed the data without adjustment for multiple testing. None of the phyla showed an association with maternal pregestational BMI, gestational weight gain or serum levels of total cholesterol and triglyceride during early pregnancy. The serum level of glycoalbumin showed a negative correlation with the proportion of TM7 phylum in early pregnancy (Figure 3).

None of the phyla showed an association with maternal pregestational BMI, gestational weight gain or serum levels of total cholesterol and glycoalbumin during late pregnancy. The serum levels of triglyceride showed a negative correlation with the proportion of Firmicutes in late pregnancy (Figure 3).

To analyze the correlation between TM7 and glycoalbumin in detail, we evaluated partial correlation coefficients using maternal age and pregestational BMI. The partial correlation coefficient between TM7 and glycoalbumin was not statistically significant in early pregnancy \((r = -0.211, P = 0.17)\). In addition, changes in the proportion of TM7 phylum from early to late pregnancy did not correlate with changes in maternal serum glycoalbumin levels from early to late pregnancy \((r = -0.215, P = 0.17)\).

Table 2: Proportions of four major phyla in early and late pregnancy

| Phylum          | Early       | Late        | P-value |
|-----------------|-------------|-------------|---------|
| Actinobacteria  | 11.3 [5.8, 20.2] | 13.6 [8.5, 21.5] | 0.663   |
| Bacteroidetes   | 15.0 [6.8, 23.2] | 18.5 [11.6, 26.3] | 0.147   |
| Firmicutes      | 69.2 [62.6, 76.7] | 64.7 [57.4, 70.8] | 0.075   |
| Proteobacteria  | 0.8 [0.4, 1.5] | 0.8 [0.5, 1.5] | 0.831   |

Values are shown as median [1st, 3rd quartile]. Phyla detected in more than half of the participants at any time are shown in this table.

Table 3: Correlation between Shannon index and maternal characteristics

|                  | Crude  | Adjusted† |
|------------------|--------|-----------|
|                  | Rho    | P         | Rho    | P         |
| Early            |        |           |        |           |
| Glycoalbumin     | 0.056  | 0.717     | 0.122  | 0.443     |
| Total cholesterol| -0.151 | 0.321     | -0.238 | 0.129     |
| Triglyceride     | -0.238 | 0.116     | -0.008 | 0.962     |
| Late             |        |           |        |           |
| Glycoalbumin     | 0.423  | 0.004     | 0.345  | 0.025     |
| Total cholesterol| -0.276 | 0.067     | -0.224 | 0.155     |
| Triglyceride     | -0.327 | 0.029     | -0.215 | 0.171     |

†Adjusted by maternal age, pregestational body mass index and gestational weight gain. Statistically significant values are in bold.

Figure 1: Principal coordinate analysis based on (a) unweighted and (b) weighted UniFrac distance. The dots represent individual participants, with red indicating stool samples taken in early pregnancy, and blue indicating samples taken in late pregnancy. The horizontal axis shows the first component, and the vertical axis shows the second component.

Figure 2: Proportion of the phylum, TM7, in gut microbiota during early and late pregnancy. The Wilcoxon rank-sum test was carried out. The proportion of TM7 was decreased in late pregnancy compared with early pregnancy \((P = 0.022)\).

Figure 3: Proportion of the phylum, TM7, in gut microbiota during early and late pregnancy. The Wilcoxon rank-sum test was carried out. The proportion of TM7 was decreased in late pregnancy compared with early pregnancy \((P = 0.022)\).
0.179, \( P = 0.24 \)). To analyze the correlation between Firmicutes and triglyceride in detail, we evaluated partial correlation coefficients using maternal age, pregestational BMI and gestational weight gain (until 32 weeks of gestational age). The partial correlation coefficient between Firmicutes and triglyceride was not significant (\( r = -0.265, P = 0.090 \)).

**DISCUSSION**

In the present study, we examined microbiota changes during pregnancy, and their association with maternal characteristics and blood chemistry related to glucose/lipid metabolism in pregnant Japanese women. Principal coordinate analysis and constexpr did not show significant changes in microbiota composition between early and late pregnancy. There were no obvious differences among four major phyla between early and late pregnancy, although the proportion of the TM7 phylum decreased in late pregnancy compared with that in early pregnancy. The Shannon index showed a significant negative correlation with pregestational BMI in late pregnancy and a positive correlation with glucose/lipid metabolism during pregnancy.

Several previous reports showed that the microbiota composition changes during pregnancy\(^9,12,14,16\). In the present study, unlike that in a previous study\(^10\), Proteobacteria and Actinobacteria did not change during pregnancy, but the proportion of the TM7 phylum did change. Previous studies reported that the gut microbiota in different populations are distinct from one another\(^16-18,34\), and that long-term dietary habits affect human gut microbiota\(^35\). The differences between previous studies and the present study might be due to different ethnicities and dietary habits of the study populations. Although pregestational weight, BMI and gestational weight gain reportedly correlate with microbiota composition during pregnancy\(^36,37\), we did not detect any correlation of microbiota composition with these variables. The participants showed relatively small pregestational BMI values, which might contribute to the differences between previous reports and the present results. Sample size was relatively small, so statistical power was not enough to detect a significant difference. Larger studies of Japanese pregnant women are required.

The diversity of gut microbiota plays an important role in health. Patients with inflammatory bowel disease have less diverse gut microbiota than do healthy individuals\(^38\). Decreased gut microbiota diversity is also associated with metabolic disorders, such as obesity and glucose intolerance\(^39,40\). In the present study, the Shannon index, which reflects the diversity of gut microbiota, had a significant negative correlation with pregestational BMI, and a positive correlation with serum glycoalbumin levels. Gut microbiota might directly affect adiposity or blood glucose levels. Another possibility is that maternal nutrition affects both gut microbiota and the blood glucose level. However, there was only a narrow range of glycoalbumin levels in the participants of the present study. This narrow distribution might have affected the trend in correlations. Although several other parameters (e.g., fasting plasma glucose levels, hemoglobin A1c levels and triglyceride/high-density lipoprotein cholesterol ratios) also reflect glucose metabolism or insulin resistance, we did not obtain those parameters. Thus, this result should be verified to clarify the relationship between gut microbiota diversity and glucose metabolism during pregnancy.

The proportion of the TM7 phylum during early pregnancy was negatively associated with glycoalbumin levels in a simple correlation, but the relationship was not significant after

---

**Figure 3** | Heat map showing correlations between the proportion of maternal gut phyla and their characteristics during (a) early and (b) late pregnancy. The color intensity represents the magnitude of the correlation evaluated using Spearman’s rank correlation coefficients, with blue indicating a negative correlation, and red indicating a positive correlation. The upper and lower panels show early and late pregnancy, respectively. *\( P < 0.05 \). BMI, body mass index.
adjustment for confounding factors. Because the proportion of TM7 was small, we compared glycoalbumin levels with or without detection of TM7, in addition to correlation analysis. This analysis showed a significant difference between both groups (Figure S1). Previous studies reported that the composition of gut microbiota can affect glucose metabolism. TM7 has not been reported to be associated with glucose metabolism; instead, the intestinal environment that favors TM7 might also affect maternal glucose metabolism. Further studies are required to clarify the relationship of the TM7 phylum to glucose metabolism.

The proportion of the Firmicutes phylum during late pregnancy was negatively associated with serum triglyceride levels in a simple correlation, but the relationship was not significant after adjustment for confounding factors. Gut microbiota might affect serum lipid profiles during pregnancy. In one study, pregnant women who received supplementation with probiotics showed lower serum triglyceride levels. In contrast, Hoppu et al. did not detect any probiotic effects in the serum lipid profiles of pregnant women. The association between gut microbiota and lipid profiles during pregnancy remains unclear. In the present study, the association between Firmicutes and serum triglyceride levels was not significant after adjustment for pregestational BMI and gestational weight gain. Thus, the results might have been affected by those confounding factors. In addition, the study participants were not fasting when blood sampling was carried out, which might have affected the results. Further studies are required to clarify the relationship between gut microbiota and lipid profiles during pregnancy.

The present study was subject to several limitations. Although dietary habits can affect gut microbiota, we did not evaluate the diets of the participants. The study was carried out in a small region of Japan and evaluated a relatively small number of participants. A large-scale study spanning multiple regions is required to confirm the present results in the overall Japanese population.

In conclusion, the proportion of the TM7 phylum within the gut microbiota of Japanese women changed during pregnancy. Gut microbiota might affect maternal metabolic status during pregnancy. The present study contributes to understanding the role of gut microbiota during pregnancy, but further studies are required.

ACKNOWLEDGMENTS

We express our sincere appreciation for the cooperation and support of all study participants and members of C-MACH. The authors thank Professor Hideoki Fukuoka (Waseda University) for his contribution toward planning and conducting C-MACH. This work was partly supported by JSPS KAKENHI (grant numbers 16H01781, 17K00577), AMED-CREST (grant number 18gm0710009b0004), Chiba Foundation for Health Promotion & Disease Prevention and Yakult Bio-Science Foundation.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Selber-Htniwi S, Rukundo B, Ahmadi M, et al. Human gut microbiota: toward an ecology of disease. Front Microbiol 2017; 8: 1265.
2. Adak A, Khan MR. An insight into gut microbiota and its functionalities. Cell Mol Life Sci 2019; 76: 473–493.
3. LeBlanc JG, Chain F, Martin R, et al. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. Microb Cell Fact 2017; 16: 79.
4. Kimura I, Inoue D, Hirano K, et al. The SCFA receptor GPR43 and energy metabolism. Front Endocrinol (Lausanne) 2014; 5: 85.
5. Levy M, Kolodziejczyk AA, Thaiss CA, et al. Dysbiosis and the immune system. Nat Rev Immunol 2017; 17: 219–232.
6. Levy RE, Turnbaugh PJ, Klein S, et al. Microbial ecology: human gut microbes associated with obesity. Nature 2006; 444: 1022–1023.
7. Mazidi M, Rezaie P, Pengne AP, et al. Gut microbiome and metabolic syndrome. Diabetes Metab Syndr 2016; 10(2 Suppl 1): S150–S157.
8. 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.
9. Nuriel-Ohayon M, Neuman H, Koren O. Microbial changes during pregnancy, birth, and infancy. Front Microbiol 2016; 7: 1031.
10. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012; 150: 470–480.
11. Liu J, Yang H, Yin Z, et al. Remodeling of the gut microbiota and structural shifts in preeclampsia patients in South China. Eur J Clin Microbiol Infect Dis 2017; 36: 713–719.
12. Smid MC, Ricks NM, Panzer A, et al. Maternal gut microbiome biodiversity in pregnancy. Am J Perinatol 2018; 35: 24–30.
13. Ferrocino I, Ponzo V, Gambino R, et al. Changes in the gut microbiota composition during pregnancy in patients with gestational diabetes mellitus (GDM). Sci Rep 2018; 8: 12216.
14. DiGiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci USA 2015; 112: 11060–11065.
15. Goltsman DSA, Sun CL, Proctor DM, et al. Metagenomic analysis with strain-level resolution reveals fine-scale variation in the human pregnancy microbiome. Genome Res 2018; 28: 1467–1480.
16. Rothenberg SE, Wagner CL, Hamidi B, et al. Longitudinal changes during pregnancy in gut microbiota and
methylmercury biomarkers, and reversal of microexposure correlations. Environ Res 2019; 172: 700–712.

17. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature 2012; 486: 222–227.

18. Nishijima S, Suda W, Oshima K, et al. The gut microbiome of healthy Japanese and its microbial and functional uniqueness. DNA Res 2016; 23: 125–133.

19. Roytio H, Mokkala K, Vahlberg T, et al. Dietary intake of fat and fibre according to reference values relates to higher gut microbiota richness in overweight pregnant women. Br J Nutr 2017; 118: 343–352.

20. Sakurai K, Miyasato H, Eguchi A, et al. Chiba study of Mother and Children’s Health (C-MACH): cohort study with omics analyses. BMJ Open 2016; 6: e010531.

21. Sato Y, Sakurai K, Tanabe H, et al. Maternal gut microbiota is associated with newborn anthropometrics in a sex-specific manner. J Dev Orig Health Dis 2019; 10: 659–666.

22. Kim SW, Suda W, Kim S, et al. Robustness of gut microbiota of healthy adults in response to probiotic intervention revealed by high-throughput pyrosequencing. DNA Res 2013; 20: 241–253.

23. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010; 7: 335–336.

24. Edgar RC, Haas BJ, Clemente JC, et al. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 2011; 27: 2194–2200.

25. McDonald D, Price MN, Goodrich J, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J 2012; 6: 610–618.

26. Wang Q, Garrity GM, Tiedje JM, et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 2007; 73: 5261–5267.

27. Core Team R R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 2017.

28. Oksanen J, Blanchet FG, Kindt R, et al. vegan: community ecology package. R package version 2.2–0. 2014.

29. Kim S, ppcor: An R package for a fast calculation to semi-partial correlation coefficients. Commun Stat Appl Met 2015; 22: 665–674.

30. Morisaki N, Nagata C, Jwa SC, et al. Pre-pregnancy BMI-specific optimal gestational weight gain for women in Japan. J Epidemiol 2017; 27: 492–498.

31. Suzuki S. Gestational weight gain in Japanese women with favorable perinatal outcomes. J Clin Med Res 2017; 9: 64–66.

32. Ogura K, Miyatake T, Fukui O, et al. Low-density lipoprotein particle diameter in normal pregnancy and preeclampsia. J Atheroscler Thromb 2002; 9: 42–47.

33. Hiramatsu Y, Shimizu I, Omori Y, et al. Determination of reference intervals of glycated albumin and hemoglobin A1c in healthy pregnant Japanese women and analysis of their time courses and influencing factors during pregnancy. Endocr J 2012; 59: 145–151.

34. De Filippo C, Cavallieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA 2010; 107: 14691–14696.

35. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011; 334: 105–108.

36. Collado MC, Isolauri E, Laitinen K, et al. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr 2008; 88: 894–899.

37. Stanislawski MA, Dabelea D, Wagner BD, et al. Pre-pregnancy weight, gestational weight gain, and the gut microbiota of mothers and their infants. Microbiome 2017; 5: 113.

38. Zuo T, Ng SC. The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease. Front Microbiol 2018; 9: 2247.

39. Zhang X, Shen D, Fang Z, et al. Human gut microbiota changes reveal the progression of glucose intolerance. PLoS ONE 2013; 8: e71108.

40. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. Nature 2013; 500: 541–546.

41. Pedersen HK, Gudmundsdottir V, Nielsen HB, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature 2016; 535: 376–381.

42. Karlsson FH, Tremaroli V, Nookaev I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 2013; 498: 99–103.

43. Utzschneider KM, Kratz M, Dammman CJ, et al. Mechanisms linking the gut microbiome and glucose metabolism. J Clin Endocrinol Metab 2016; 101: 1445–1454.

44. de Brito Alves JL, de Oliveira Y, Carvalho NNC, et al. Gut microbiota and probiotic intervention as a promising therapeutic for pregnant women with cardiometabolic disorders: present and future directions. Pharmacol Res 2019; 103: 145–154.

45. Ahn HY, Kim M, Ahn YT, et al. The triglyceride-lowering effect of supplementation with dual probiotic strains, Lactobacillus curvatus HY7601 and Lactobacillus plantarum KY1032: reduction of fasting plasma lysophosphatidylcholines in nondiabetic and hypertriglyceremic subjects. Nutr Metab Cardiovasc Dis 2015; 25: 724–733.

46. Hoppu U, Isolauri E, Koskinen P, et al. Maternal dietary counseling reduces total and LDL cholesterol postpartum. Nutrition 2014; 30: 159–164.
SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Serum glycoalbumin levels with or without TM7 in early pregnancy. Wilcoxon rank-sum test was carried out. Serum glycoalbumin levels were lower in participants with TM7 than in participants without TM7 ($P = 0.029$).