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SHORT COMMUNICATION

Flash on gut microbiome in gestational diabetes: a pilot study

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SUMMARY

Pregnancy induces a deep modification of women's gut microbiota composition. These changes may influence hormonal and metabolic factors, increasing insulin resistance and leading to hyperglycaemia in susceptible women. Data on 29 women in pregnancy showed insignificant reductions in the Bacteroidetes/Firmicutes ratio in women with (n. 14) and without (n. 15) gestational diabetes (GDM). Gut microbiota compositions at the genera and species level were further analysed in ten pregnant women with and ten without GDM (9 samples were excluded due to low DNA quality/quantity), showing differences in functionally specific patterns affecting host energy dietary polysaccharide metabolism pathways. According to our results, gut microbiome alteration may play a role in GDM pathogenesis through an increase of gut permeability and higher intestinal energetic balance.

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The gut microbiota is involved in many processes of human health. Intestinal dysbiosis can alter the development and function of the immune system and the integrity of the muco-epithelial intestinal barrier and metabolism and can contribute to inducing metabolic disorders such as obesity (Kalliomaki *et al.,* 2008), type 1 and type 2 diabetes (Harjutsalo *et al.,* 2008), (Amar *et al.,* 2011) and insulin resistance (Zhang *et al.,* 2013). Pregnancy induces several changes, including a deep modification of women's gut microbiota composition (Koren *et al.,* 2012). These changes may have deep and still unknown influences on the foetus as a part of the maternal-foetal axis. Moreover, hormonal and metabolic factors increase insulin resistance, leading to hyperglycaemia in susceptible women (McIntyre *et al.,* 2010).

In this pilot study, we compared faecal gut microbiota compositions at the phylum, genera and species levels between women with GDM and control healthy pregnant women in their third trimester. Fourteen women with GDM and fifteen matched healthy pregnant women were enrolled after providing written informed consent. This study was approved by "Sapienza" University of Rome, DR n. 3210/16 del 16/12/2016.

Inclusion criteria: Caucasian women aged ≥18 years. Exclusion criteria: antibiotics/probiotics/symbiotics/metformin use during pregnancy, obesity, twin pregnancy, pre-gestational diabetes, and inflammatory bowel diseases. All women underwent an oral glucose tolerance test (OGTT) at the 24-28th gestational week, and the diagnosis of GDM was assessed according to IADPS criteria (Duran *et al.,* 2014). At the first visit, the main demographic and clinical parameters were recorded. Maternal outcomes included weight gain, gestational week and type of delivery, hypertension. Foetal outcomes included ponderal index (weight*100/length^3) and weight percentile. Composite adverse foetal outcome included one of the following: large for gestational age (LGA) (>90th percentile), hypocalcaemia, hypoglycaemia, jaundice, and respiratory disorders. At the time of diagnosis, women with GDM and controls received a medical nutrition therapy (MNT) prescription (Associazione Medici Diabetologi, 2016).

When MNT was unable to achieve glycaemic targets, only insulin treatment was allowed in cases of GDM. At 34-36 gestational weeks, participants provided a fresh stool sample that was stored within 2-6 hours at -20°C until processing. DNA was extracted using a QIAamp DNA mini kit (Qiagen, Italy) according to the manufacturer's instructions. 16S rRNA genes were PCR-amplified from genomic DNA using the primers Bact934F (5'-GGARCATGTGGTTTAATTCGATGAT-3') and Bact1060R (5'-AGCTGACGACAACCATGCAG-3') for Bacteroidetes and Firm934F (5'-GGAGYATGTGGTTTAATTCGAAGCA-3') and Firm1060R (5'- AGCTGACGACAACCATGCAC-3'). After excluding nine samples for low DNA quality/quantity, DNA sequencing was performed using the Ion Torrent PGM (Life Technology, Italy) as previously described (Drago *et al.,* 2016). Results are expressed as medians (25-75th centiles) or means±SDs according to distribution; biodiversity indexes (Shannon, Simpson, and Chao's indexes) were calculated using the Vegan 2.4.3 package for R Software V.3.3.1

Key words:
Hyperglycaemia, pregnancy, microbiome, microbiota, gut, GDM.

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Table 1 - *Clinical-anthropometric characteristics and maternal-neonatal outcomes of women enrolled in the study.*

	GDM (n.10)	*Controls (n.10)*	*p*
BMI (kg/m²)	24.6 (21.2-27.8)	22.3 (19.1-26)	ns
Age (years)	36.2±4.4	33.4±3.5	ns
Week of delivery	38 (38-39.3)	40 (39-40.7)	0,01
ponderal index (g/cm³)	2.71 (2.47-2.85)	2.71 (2.30-2.74)	ns
Newborn weight (g)	3380 (3006-3582.5)	3325 (3192.5-3502.5)	ns
Composite adverse foetal outcomes n°	2 (1 jaundice, 1 LGA)	2 (1 jaundice, 1 LGA)	ns
Weight centiles	58.5 (24.7-71.7)	49.5 (28.7-65.5)	ns
Weight gain (Kg)	9.6±4.3 or 9.25 (6.3-13.1)	13.5±5.5 or 13.5 (9.8-19.2)	ns

for Windows. Kruskal-Wallis and Wilcoxon rank-sum tests were used for 'α diversity' and microbial taxa. Adjustment for multiple testing was evaluated with Benjamini-Hochberg FDR correction. Chi-square and/or Fisher tests were used for categorical variables (percentage). A p-value <0.05 was statistically significant.

Clinical characteristics of GDM and control women, in whom the gut microbiota was analysed with both techniques, are shown in *Table 1*. At the '*phylum*' level, quantitative PCR analysis showed no significant reduction in Bacteroidetes and Firmicutes in GDM *vs* Controls: Bacteroidetes: 0.7525 (0.0453-1.0595) *vs* 1.1050 (0.9763-1,2735); p=0.08; Firmicutes 1.0950 (1.0182-1.8920) *vs* 1.2795 (1.0560-1.3935); ns, respectively. The Bacteroidetes/Firmicutes ratios (B/F ratios) were similar in the two groups (GDM vs Controls: 0.53 (0.18-1) *vs* 0.88 (0.55-0.99); p=0.6). Due to the quality and/or quantity of the extracted DNA, further analysis at the *genus* and *species* levels was performed only on n.10 GDM and n.10 matched controls. Stool microbiota composition at the *genus* and *species* level are shown in *Figure 1*, *panel A*. Although no significant differences in biodiversity indexes were observed (data not shown), women with GDM showed higher relative abundance levels of *Bacteroides caccae, Bacteroides massiliensis,*

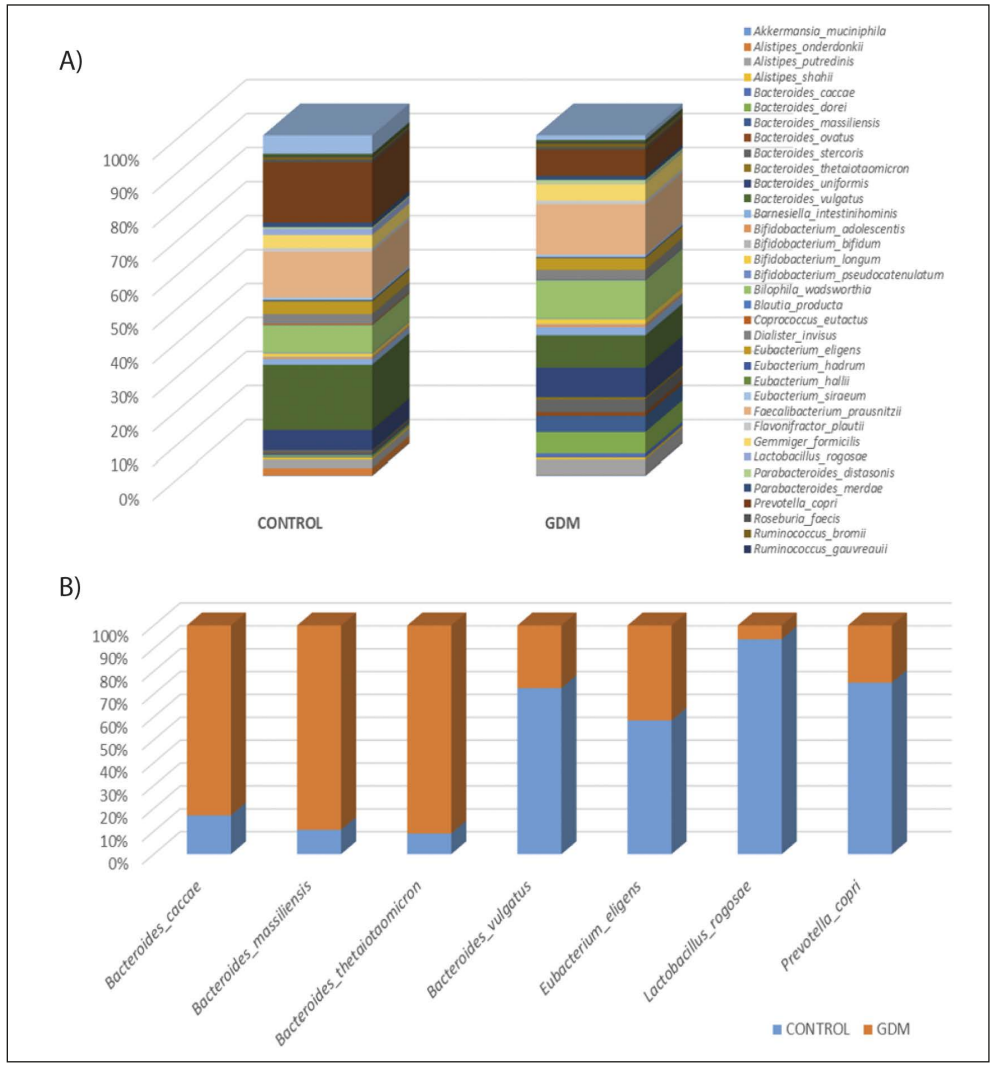

Figure 1 - *Relative abundance (%) of genera and species. A) Histograms depicting the main bacterial genera and species in control and GDM groups, differences are statistically significant. B) Relative abundance of bacterial species significantly differs between GDM patients vs healthy controls (p≤0.05).*

and Bacteroides thetaiotaomicron ($p<0.05$) together with reductions of *Bacteroides vulgatus, Eubacterium eligens, Lactobacillus rogosae,* and *Prevotella copri* ($p<0.05$) (*Figure 1, panel B*). No differences in neonatal/maternal outcome were detected between groups. A total of 7/10 GDM women needed insulin treatment. At the *phylum* level, our data confirm a B/F ratio shifting in favour of the Firmicutes in GDM, even though the shift was not significant, as previously reported in high-risk GDM (Fugmann *et al.,* 2015) and Type 2 DM (Qin *et al.,* 2012). At the *genera* and *species* levels, specific *Bacteroides* spp. were more abundant in the GDM group. Functionally, this pattern affects host energy metabolism through the polysaccharide utilization loci (PUL) pathway, allowing microorganisms to metabolize fructose-based dietary polysaccharides (Wexler *et al.,* 2017). Moreover, a high-sugar/high-fat/low-fibre Western diet promotes Bacteroides to consume host-derived glycans by switching their transcriptional profile. In particular, *B. thetaiotaomicron* possesses protein O-glycosylation systems and the ability to catabolise any carbohydrates (Devaraj *et al.,* 2013); nonetheless, it is reported to contribute to intestinal mucosa integrity and permeability by acting at the level of desmosomes of epithelial villus, intestinal mucus and the glycocalyx layer (Jandhyala *et al.,* 2015). Interestingly, together with the increases in the abovementioned symbionts, our GDM individuals showed significant decreases in B. vulgatus, E. eligens, L. rogosae and P. copri (*Figure 1, panel B*). Similarly, Crusell found an aberrant microbiota composition in GDM women in the third trimester of pregnancy (Crusell *et al.,* 2018); in particular, low levels of *E. eligens*, a butyrate-producing bacteria, have been reported in a large-scale study on Swedish women with type 2 diabetes and by Kuang in GDM patients (Karlsson *et al.,* 2016; Kuang *et al.,* 2017). Butyrate, as another short-chain fatty acid (SCFA), promotes the integrity of the intestinal barrier, modulating the gut permeability and inflammatory response that precedes the development of diabetes (Mejía-León *et al.,* 2015); (Pedersen *et al.,* 2016). Additionally, SCFAs interact with the GLP-1 metabolic pathway, increasing intestinal gluconeogenesis binding Gpr43 and the Gp41 receptor. Recently, *P. copri* and *B. vulgatus* were identified as the main species driving the association between the biosynthesis of branched-chain amino acids, insulin resistance, and glucose intolerance (Pedersen *et al.,* 2016). Physiologically, during the 3rd trimester, the gut microbiome contributes to increases in energy intake and insulin resistance to promote foetal supply (Koren *et al.,* 2012).

Taken together, the differences in the microbiota observed in the present study suggest that alteration of the intestinal microbiome may play a role in the pathogenesis of GDM through an increase in intestinal permeability and a greater intestinal energy balance. Although, at the species level, we report in GDM group an increase in B. thetaiotaomicron, which is known to improve mucosal integrity rather than affecting it, we need to consider that the final effects on intestinal permeability are given by the sum of the changes of the analysed microbiota. Moreover, it is well known that dietary habits affect the intestinal microbiota in all people and standardized nutrition to study microbiota changes remains a kind of "mission impossible" in any clinical human study.

In the present study, both groups received only dietary advice. Patients included in the control group, received general advice based on the national guidelines on simple pregnancy, (https://www.epicentro.iss.it/itoss/pdf/gravidanza%20fisiologica_allegato.pdf), while GDM patients received instructions more focused on glucose level control through the diet. The lack of a diet questionnaire to evaluate the eating habits of the subjects, together with the relatively small sample size, and the analysis of a single stool sample from each participant represent three limitations of the present study. Nonetheless, our results indicate that GDM patients have some distinctive microbial features compared to controls during the 3rd trimester of pregnancy. These findings highlight the challenges for achieving a complete understanding of GDM-related microbiota and its potential manipulation.

Conflicts of interest

None.

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