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Gravidas with Class III Obesity: Evaluating the Abdominal Skin Microbiota Above and Below the Panniculus

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Abstract

Objective—We sought to compare the skin microbiota above and below the panniculus of third-trimester pregnant women with class III obesity.

Methods—We collected swabs from the anterior panniculus and from the intertriginous area under the panniculus of women with class III obesity (body mass index ≥ 40 kg/m²) carrying a live singleton at 28 weeks. DNA was extracted. PCR with primers targeting the 16S rDNA V4 region were used to prepare an amplicon library. PCR products were sequenced (Illumina MiSeq platform). The 16S rDNA sequences were processed, integrated, analyzed, and reported using QIIME and in-house developed scripts. Taxonomy was assigned using RDP Classifier (threshold 0.8) against the Greengenes 16S rDNA database.

Results—Twenty women were enrolled. One sample pair was excluded for low sequence depth. Using permutation testing, there was significantly less bacterial diversity in the samples above compared to below the panniculus (beta diversity using weighted UniFrac metrics; $p < 0.001$).

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Reprints will not be available.

DECLARATION OF INTEREST

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Conclusion—In pregnant women with class III obesity, the skin microbiota in the subpannicular fold differs from and is more diverse than that on the anterior panniculus.

Keywords

skin flora; microbiome; pregnancy; intertriginous; morbid obesity

INTRODUCTION

Obesity during pregnancy is defined as a body mass index (BMI) ≥ 30 kg/m² at the first prenatal visit, and it has become much more prevalent in the past few decades. In 1999, the overall rate of obesity in the United States was 28%; it was 34% in 2008 [1]. In a study from our center, over a 20-year period, the obesity rate in the obstetric population more than doubled from 16% in 1980 to 36% in 1999 [2]. The rate at our center now exceeds 40%. Using BMI, classes of obesity have been described—values of 30–34.9, 35–39.9, and ≥ 40 kg/m², respectively, define individuals as having class I, II, or III obesity [3]. Of reproductive age women in the United States, 8% have class III obesity [1].

Among a number of other complications, obesity is associated with higher rates of labor induction, failure to progress in labor (whether spontaneous or induced), cesarean delivery, and cesarean complications such as increased operative time, wound infection, surgical wound disruptions, and hemorrhage [4–8]. Furthermore, these obesity-related complications are dose-dependent. Marshall and colleagues reported that, compared to women with a BMI of 30–39.9, women with a BMI ≥ 50 were more likely to have a cesarean delivery (adjusted relative risk 1.8, 95% confidence interval 1.5, 2.1) [9]. Another group of authors reported that 30% of women with a BMI ≥ 50 have cesarean wound complications [10].

When performing cesarean deliveries on women of normal weight, most obstetricians favor a Pfannenstiel incision for entering the abdomen, since it is associated with less postoperative pain and a better cosmetic result [11]. This incision is transverse and involves entering the skin about 3 cm above the pubic symphysis. However, the best choice for obese women is unknown. Increasing obesity results in a panniculus—an apron-like redundancy of skin and subcutaneous fat. For cesarean delivery in obese women, some authors favor a Pfannenstiel incision made in the subpannicular fold [10,12,13], while others advocate for a vertical abdominal incision due to theoretic concern about making an incision under the panniculus in “a warm, moist, anaerobic environment...[that] promotes the proliferation of numerous microorganisms, producing a veritable cesspool [14].” Despite this colorful assertion, no data exist to support such a concern in relation to cesarean delivery and post-cesarean wound infections. Therefore, our objective was to evaluate the bacterial communities comprising the skin flora above and below the panniculus of pregnant women with class III obesity.

METHODS

We conducted a prospective cohort study of women with a BMI ≥ 40 kg/m² who were carrying a live singleton fetus at 28 weeks gestational age. Exclusion criteria were labor, antibiotics taken during the last 28 days, prior panniculectomy, chronic hypertension treated

with more than one medication, collagen vascular disease, human immunodeficiency virus infection or other cause of immune compromise, serum creatinine >1.1, chronic subcutaneous medication use (e.g. insulin or heparin), and age <19 years. Women with gestational diabetes were not excluded unless using subcutaneous insulin. All women were enrolled and had samples collected in June and July 2013 during a prenatal care visit at the University of Alabama at Birmingham (Birmingham, AL, USA). The institutional review board of the University of Alabama at Birmingham approved the study.

Cotton swabs moistened with 0.15 M NaCl + 0.1% Tween 20 were used by trained research nurses to collect samples, one from the anterior surface of the panniculus (3–4 cm inferior to the umbilicus) and another from the intertriginous area under the panniculus. These swab samples were immediately stored at –80°C. Samples were thawed, and microbial genomic DNA was isolated using the Fecal DNA isolation kit from Zymo Research (catalog #D6010) following the manufacturer's instructions. Once the sample DNA was prepared, PCR was used with unique bar coded primers to prepare the 16S rDNA V4 region amplicon library [15–18]. PCR products were purified by gel electrophoresis, quantified, and sequenced using an Illumina MiSeq platform [19,20].

The proprietary software from Illumina “MiSeq reporter” was used for demultiplexing the raw sequences, and FASTQ files were generated. Illumina sequencing generated paired-end reads of 251 bases. Overall read quality was assessed before and after filtering using FASTQC. Due to low quality of the last base, it was trimmed to make the read length 250 bases for all reads. Using the sequence overlap between the paired ends, they were merged together to generate a high quality single end read using the program USEARCH (module “fastq_mergepairs”) [21]. Read pairs with an overlap of less than 50 bases or with too many mismatches (>20) in the overlapping region were discarded. Low quality reads (average quality Q<20) and sequences containing “N” were discarded. Chimeric reads were filtered using the “identity_chimeric_seqs.py” module of USEARCH. The QIIME data analysis package was used for subsequent 16S rDNA data analysis as mentioned below. We excluded any subject having one or both samples with less than 10,000 identified sequences after quality control measures. Sequences were grouped into operational taxonomic units (OTUs) using the clustering program UCLUST at a similarity threshold of 97%. RDP classifier [22] (taxonomy classification program), trained with the latest version of the Greengenes database [23] (v13_8), was used for OTU taxonomy classification at a confidence threshold of 0.8. OTU tables were generated which included all OTUs, their taxonomic identification, and relative abundance information. OTUs whose average relative abundance was less than 0.0005% were filtered out. OTUs were grouped together based on taxonomic similarity to create relative abundance profiles at different hierarchical levels of classification (e.g. phylum, class, order, family, and genus). Multiple sequence alignment of OTUs was performed with PyNAST; the phylogenetic tree was constructed with FastTree [24,25]. The beta diversity calculations were done using weighted UniFrac metrics and visualized using principal coordinates analysis (PCoA) [26].

In addition to the skin swabs, the depth of the subpannicular fold was assessed by measuring the apposed skin in the midline both with the patient supine and standing (Figure 1).

Demographic and historical information, including time of last bath or shower, were also obtained. We did not evaluate delivery route or delivery complications.

Demographic statistics were summarized using medians, ranges and proportions. Permutation testing (using weighted UniFrac metrics) was used to assess the differences in overall microbiota of the two groups of samples (from above and below the panniculus). The test statistic reported was the difference between the average beta diversity of the samples above and that of the samples below the panniculus. The real value was compared against 9999 random samples with permuted labels.

The 10 most prevalent genera were compared between sites (over vs. under) using paired t-tests. The Bonferroni correction was used to adjust for multiple comparisons. Two-sided tests were used. We considered p values <0.05 to be statistically significant. A sample size calculation was not undertaken.

RESULTS

Twenty (20) women were enrolled and had paired samples collected. The sequencing produced a mean of 160K paired-end reads (range 8876–561,467) per sample. After the merging and QC steps, we obtained, on average, 131K merged, 250 base reads (range 7352–475,223) for analysis. One pair of samples which had read depth less than 10K reads was discarded. The lowest read depth in the remaining 19 pairs of samples was 37K reads, and this threshold was used for read depth normalization across all samples using the rarefaction function in QIIME. After sequence clustering and filtering, we identified 27,343 different OTUs.

The 10 most commonly occurring genera accounted for 72% of the total microbial population of all samples. Of these, five were from the phylum Firmicutes, two were from the phylum Actinobacteria, two were from the phylum Proteobacteria, and one was from the phylum Bacteroidetes. These top 10 genera, with their mean relative abundance in the two sampling sites (over and under the panniculus), are presented in Table I. Samples below the panniculus had a significantly higher abundance of genus *Corynebacterium* ($p=0.007$), while samples above the panniculus had a significantly higher abundance of the genus *Paenibacillus* ($p=0.042$) and an unclassified genus from the family *Comamonadaceae* ($p=0.046$) (all p values Bonferroni adjusted). These same ten genera, along with an “other” group representing all other OTUs collectively that were identified, are presented in a figure as supplemental digital content (S1). Based on permutation testing, the microbiota was statistically more diverse in the below samples compared to above the panniculus (permutation test using weighted UniFrac distance $p=0.001$).

The PCoA plot using weighted UniFrac metrics also shows a distinct clustering pattern, identifying seven women whose samples from below the panniculus had microbiota that were significantly different than other samples (figure presented as supplemental digital content S2). Demographic data were compared between women in that group of seven women with the distinct microbiota of the skin under the panniculus and the rest of the cohort (Table II). Increased time since bathing was the only factor significantly associated

with women being in that group of seven that had a distinct skin microbiota under the panniculus. Presented as supplemental digital content S3 is a heatmap, along with hierarchical clustering of the samples, based on relative abundance of the top 20 genera. This figure shows a similar clustering pattern to that shown in S2 and demonstrates that the seven women with a distinct microbiome under the panniculus have a higher relative abundance of *Corynebacterium* and *Brevibacterium* and lower relative abundance of *Paenibacillus*.

DISCUSSION

In pregnant women with class III obesity, using next generation sequencing technology, we have demonstrated that there is significantly different bacterial diversity in the microbiota of the skin in the subpannicular fold compared to the anterior surface of the panniculus. In addition, we identified a subgroup of women whose skin microbiota below the panniculus was significantly different than the other women in the study and whose results drove the differences between the “under” samples compared to the “over” samples. Relating these findings to wound infections or appropriateness of various preoperative maneuvers to decrease the likelihood of wound infections (e.g. skin preparation, prophylactic antibiotics) is beyond the scope of our current study. However, our data show that the bacteria on the skin at the site of an abdominal incision made to perform a cesarean delivery might be different in certain women, depending on whether the incision is made on the anterior surface of the panniculus or in the subpannicular fold.

Our finding that *Corynebacterium* species are more abundant in the subpannicular fold than on the anterior surface of the panniculus is consistent with those of other investigators who evaluated various skin sites in healthy, non-pregnant adults and reported that *Corynebacterium* species are more abundant in moist areas than in dry areas [27,28]. However, we are unaware of other data specifically evaluating the skin microbiota of the intertriginous area in the subpannicular fold. Our finding that the dry skin on the anterior surface of the panniculus was dominated by the genus *Paenibacillus*, a member of the Firmicutes phylum, differs from those of others. Grice and colleagues reported that Firmicutes were more prevalent in intertriginous areas (e.g. gluteal crease) than on dry areas (e.g. buttock) [27]. In contrast, we found that *Paenibacillus* dominated most samples and was significantly more abundant in samples from the anterior surface of the panniculus.

We were able to classify around 77% of the top 100 most abundant OTUs (which represents >85% of all sequences) down to the genus level. Since our study was an exploratory evaluation of the differences between the microbiota of the skin above and below the panniculus, we did not attempt to further classify these OTUs (e.g. down to the species level). Certainly, in a given clinical situation, it might be necessary to classify OTUs more specifically to the species and/or strain level. Further techniques involving metagenomics would be required, and our study is an example of the fact that 250 base pairs is not sufficient to classify OTUs to the species level. Therefore, caution is advisable when interpreting results from next generation sequencing evaluations of the microbiota of various tissues.

The relatively small number of patients in the current study potentially limits the generalizability of our findings, and they should be interpreted with caution until replicated. In addition, the lack of non-obese and non-pregnant controls limits our ability to draw conclusions about the relative contributions of obesity and pregnancy to our findings. Also, fungi and viruses may be present on the skin; we evaluated only bacteria, since fungi and viruses rarely are thought to contribute to surgical site infections. Finally, our results reflect one point in time. We cannot comment on changes over time in the skin microbiota in these patients. However, strengths of this study include use of a next generation sequencing technique to characterize the bacteria present in samples and use of a prospectively collected cohort of patients as their own controls for comparing skin site differences.

The findings in our current study may have implications related to the risk of surgical site infections with various abdominal incision types for cesarean delivery of obese patients. That possibility, coupled with the current prevalence of obesity, the increased risk of cesarean delivery in these patients, and the increased risk of post-cesarean complications in obese women, begs the question of whether post-cesarean wound infection is more likely if the abdominal incision is made in the subpannicular fold or on the anterior surface of the panniculus. This question would best be answered by a randomized trial of abdominal incision for cesarean delivery in women with class III obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Image depicting the subpannicular fold

This line drawing of a sagittal image of a pregnant woman with class III obesity shows the subpannicular fold (arrow). Swab samples were collected 3–4 cm inferior to the umbilicus from the anterior surface of the panniculus (right side of image) and also from the intertriginous area under the panniculus (referenced by the arrow). The apposed skin making up the subpannicular fold was measured in the midline.

Table 1

Listed here in order are the ten most abundant microbes (genus level) in the abdominal skin microbiome in samples overall and their mean relative abundance above (over) and below (under) the panniculus of studied patients

Rank	Phylum	Genus	Mean relative abundance (Over)	Mean relative abundance (Under)	p	Bonferroni adjusted p
1	<i>Firmicutes</i>	<i>Ptenibacillus</i>	46.0%	31.6%	0.004	0.042
2	<i>Actinobacteria</i>	<i>Corynebacterium</i>	3.3%	22.8%	0.001	0.007
3	<i>Firmicutes</i>	<i>Lactobacillus</i>	8.1%	4.0%	0.061	0.607
4	<i>Proteobacteria</i>	<i>Family Comamonadaceae; Unclassified genus</i>	4.0%	2.5%	0.005	0.046
5	<i>Firmicutes</i>	<i>Staphylococcus</i>	1.4%	4.6%	0.026	0.255
6	<i>Firmicutes</i>	<i>Streptococcus</i>	2.7%	1.5%	0.009	0.091
7	<i>Proteobacteria</i>	<i>Acinetobacter</i>	2.2%	1.8%	0.375	1.000
8	<i>Actinobacteria</i>	<i>Brevibacterium</i>	0.1%	3.2%	0.042	0.421
9	<i>Bacteroidetes</i>	<i>Prevotella</i>	1.7%	1.2%	0.119	1.000
10	<i>Firmicutes</i>	<i>Allotaculum</i>	1.1%	1.1%	0.947	1.000

Table II

Demographic data compared between the group of seven women with distinct microbiota of the skin under the panniculus and the rest of the cohort

Characteristic	Group of seven	Rest of cohort	P
Nulliparous	1 (14%)	3 (25%)	1.0
Age (years)	28.3 ± 6.1	28.8 ± 4.6	0.83
Prepregnancy weight (lbs)	249 ± 18.6	263 ± 33.6	0.33
Weight at sample collection (lbs)	283 ± 41.2	286 ± 33.9	0.90
Weight gain (lbs)	34.1 ± 41.5	22.5 ± 16.9	0.40
Height (inches)	65.2 ± 2.5	64.3 ± 2.4	0.40
BMI (kg/m ²)	46.8 ± 5.9	48.7 ± 6.1	0.51
Race			0.26
Black	7 (100%)	9 (75%)	
White	0 (0%)	3 (25%)	
Payment source= Medicaid	7 (100%)	12 (100%)	1.0
Marital status			1.0
Single	6 (86%)	10 (83%)	
Married	1 (14%)	1 (8%)	
Divorced	0 (0%)	1 (8%)	
Prior cesarean	4 (57%)	4 (33%)	0.38
Other infraumbilical laparotomy	0 (0%)	1 (8%)	1.0
One or more prior vaginal deliveries	4 (57%)	6 (50%)	1.0
Chronic hypertension	1 (14%)	2 (17%)	1.0
Gestational diabetes	2 (29%)	2 (17%)	0.60
Smoker	1 (14%)	2 (17%)	1.0
Drug abuse	0 (0%)	0 (0%)	1.0
Last bathing type			0.33
Bath	1 (14%)	5 (42%)	
Shower	6 (86%)	7 (58%)	
Last bathing timing			0.04
Evening before visit	5 (71%)	2 (17%)	
Morning of visit	2 (29%)	10 (83%)	
Subpannicular fold—supine (cm)	7.0 ± 2.1	5.3 ± 3.0	0.21

Characteristic	Group of seven	Rest of cohort	P
Subpannicular fold—standing (cm)	13.6 ± 3.9	12.3 ± 3.3	0.44
Delta subpannicular fold (cm)	6.6 ± 3.7	7.0 ± 3.1	0.81

Data are presented as n (%) or mean ± standard deviation and analyzed with Fisher's exact test or the unpaired Student's t-test. Abbreviations used in the table: lbs=pounds, BMI=body mass index; kg=kilograms of weight, m=meters of height, cm=centimeters.