The Association Between Obesity and Weight Loss after Bariatric Surgery on the Vaginal Microbiota

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Research

Keywords: obesity, overweight, body mass index, BMI, vaginal microbiota, bariatric surgery

Posted Date: June 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-31292/v1

Version of Record: A version of this preprint was published at Microbiome on May 28th, 2021. See the published version at https://doi.org/10.1186/s40168-021-01011-2.

Abstract

Background: Obesity and vaginal microbiome (VMB) dysbiosis are each risk factors for adverse reproductive and oncological health outcomes in women. Here we investigated the relationship between obesity, vaginal bacterial composition, local inflammation and bariatric surgery.

Methods: Vaginal bacterial composition assessed by high-throughput sequencing of bacterial 16S rRNA genes and local cytokine levels measured using a multiplexed Magnetic Luminex Screening Assay were compared between 67 obese and 42 non-obese women. We further assessed temporal changes in the microbiota and cytokines in a subset of 27 women who underwent bariatric surgery.

Results: The bacterial component of the vaginal microbiota in obese women was characterised by a lower prevalence of a *Lactobacillus*-dominant VMB and higher prevalence of a high diversity (*Lactobacillus* spp., and *Gardnerella*- spp. depleted) VMB, compared with non-obese subjects ($p<0.001$). Obese women had higher relative abundance of *Dialister* species ($p<0.001$), *Anaerococcus vaginalis* ($p=0.021$) and *Prevotella timonensis* ($p=0.020$) and decreased relative abundance of *Lactobacillus crispatus* ($p=0.014$). Local vaginal IL-1β, IL-4, IL-6, IL-8, IFNγ, MIP-1α, and TNFα levels were all higher among obese women, however only IL-1β and IL-8 correlated with VMB species diversity. In a subset of obese women undergoing bariatric surgery, there were no significant overall differences in VMB following surgery, however 75% of these women remained obese at six months. Prior to surgery there was no relationship between body mass index (BMI) and VMB structure, however post-surgery women with a *Lactobacillus*-dominant VMB had a significantly lower BMI than those with a high diversity VMB.

Conclusions: Obese women have a significantly different vaginal microbiota composition with increased levels of local inflammation compared to non-obese women. Bariatric surgery does not change the VMB, however, those with the greatest weight loss six-months post-surgery are most likely to have a *Lactobacillus*-dominant VMB.

Background

Obesity has become a worldwide problem, with projections of 1 in 5 women being obese and 1 in 10 morbidly obese by 2025 [1]. The gynaecological complications of obesity include menstrual disorders [2, 3], anovulation [4], polycystic ovarian syndrome [5, 6], infertility [7-9], early pregnancy loss [10-12], preterm birth [13], obstetric complications [13, 14] and increased risk for gynaecological malignancies (endometrial and ovarian) [15-18]. The mechanism by which obesity promotes several of these outcomes is not fully understood.

There is growing recognition that microbiota (i.e. the assemblage of microorganisms found at a specific environment [19]) and their functions influence disease pathophysiology [20-22]. Shifts in microbiota composition at mucosal surfaces can lead to pathobiont overgrowth and activation of innate immune responses that in turn are modified by microbial products such as short-chain fatty acids, lipids and bioamines [23-26]. The vaginal microbiota is commonly dominated by *Lactobacillus* spp. [27, 28], which

offer protection against colonisation of pathogenic bacteria through production of lactic acid, lowering of vaginal pH, production of antimicrobial compounds and modulation of both the immunological and physical properties of cervicovaginal mucosa [29, 30]. High-throughput sequencing approaches have aided characterisation of the vaginal microbiota in health and disease states, leading to a better understanding of the factors which affect vaginal community composition including age and ethnicity [31], menstrual cycle phase [32], oestrogen levels and menopause status [33], intercourse [34], pregnancy [35, 36] and hygiene practices [37].

Bacterial vaginosis (BV), characterised by a shift from *Lactobacillus* spp. dominance towards high relative abundance of anaerobes, has long been associated with a range of adverse outcomes [38]. Molecular-based characterisation of vaginal bacteria composition has extended these findings to permit identification of specific taxa that modify risk of preterm birth [39-42] and preterm premature rupture of membranes [43], sexually transmitted infections [44], human papilloma virus and cervical disease [45-47].

Obesity and specific vaginal microbiome (VMB) compositions are each risk factors for adverse reproductive and oncological health outcomes in women, but there is limited evidence describing the relationship between vaginal microbiota composition and obesity and the impact that body weight may have on local inflammation, immune response and health outcomes. Brookheart and colleagues found that overweight and obese women have higher Nugent scores and a greater occurrence of BV [48]. Conversely using data from the USA National Health and Nutrition Examination Survey 2001-2004, Koumans and co-workers concluded that body mass index (BMI) was not an independent risk factor for BV[49]. Two Korean studies have examined the relationship between VMB, assessed by metataxanomic analysis, and obesity. Oh and colleagues found that obesity associates with cervical microbiota dominated by *Lactobacillus iners* [50]. Si and co-workers reported that discordant twin obesity associated with increased bacterial diversity and prevalence of *Prevotella* [51].

In this study, we investigated how obesity, defined as BMI ³30kg/m^2, associates with bacterial structure and composition of the vaginal microbiome compared to non-obese women and local inflammation. In a secondary analysis, we investigated the effects of bariatric surgery on vaginal bacterial diversity and local inflammation.

Results

In the metataxonomic analysis, a total of 3,045,614 reads were captured from 166 samples (109 baseline, 57 longitudinal samples) with an average number of reads per sample of 18,021. To avoid sequencing bias, operational taxonomic units (OTUs) were randomly sub-sampled to the lowest common read count of 1,885 with coverage of greater than 95% (Good's coverage index) being maintained for all samples. A total of 265 taxa were detected in the study cohort microbiota after the removal of singletons and rare OTUs. Rare OTUs were defined as those present at less than 10 counts within the entire cohort, and along with singletons, were included in the final analysis to maintain consistent read counts across all samples.

Vaginal microbiota composition and local cytokine expression according to obesity, diabetes and insulin resistance status

Ward hierarchical clustering analysis of genera-level data identified three major groups, on the basis of relative bacterial abundance; i) *Lactobacillus*-dominant - characterised by high relative abundance of *Lactobacillus* spp., ii) *Gardnerella*-dominant - characterised by high relative abundance of *Gardnerella* spp. and low relative abundance of *Lactobacillus* species, and iii) high diversity VMB - characterised by low relative abundance of each of *Lactobacillus* and *Gardnerella* species, and increased bacterial diversity (Figure 1a). Across the whole cohort the prevalence of *Lactobacillus*-dominant and high diversity VMB groups were significantly different (Figure 1b). The frequency of the bacterial groups within patient groups subcategorised on the basis of obesity status, diabetes and insulin resistance status is presented in Table 2. Prevalence of the high diversity VMB was significantly greater in obese women (obese; 18/42 (42.8%) compared to non-obese; 10/67 (14.9%), p=0.002) whilst the prevalence of *Lactobacillus*-dominant VMB was significantly lower (Table 2). There was no difference in the prevalence of *Gardnerella*-dominant VMB between obese and non-obese women. There were overall no significant associations between the three major vaginal bacterial groups and diabetes (Table 2) or where diabetic status was subcategorised into obese or non-obese women (Supplementary Table 2).

Table 1
Patient characteristics of the whole cohort at baseline sample collection (n=109) according to obesity status.

Characteristics	Non-obese (n=67)	Obese (n=42)	Total (n=109)	P value[a]
Age (years)				0.083
Mean (SD, range)	44 (11.79, 20-75)	46 (11.26, 28-72)	44 (11.79, 20-75)	
Ethnicity, n/N (%)				**0.024**
Caucasian	48/67 (71.6)	22/42 (52.4)	70/109 (64.2)	
Asian	3/67 (4.5)	5/42 (11.9)	8/109 (7.3)	
Black	12/67 (17.9)	15/42 (35.7)	27/109 (24.8)	
Other	4/67 (6.0)	0/42 (0)	4/109 (3.7)	
Parity, n/N (%)				**<0.001**
Nulliparous	44/67 (65.7)	7/42 (16.7)	51/109 (46.8)	
Parous	23/67 (34.3)	35/42 (83.3)	58/109 (53.2)	
Smoking status, n/N (%)				0.128
Current smoker	11/67 (16.4)	2/42 (4.8)	13/109 (11.9)	
Non-smoker	56/67 (83.6)	40/42 (95.2)	96/109 (88.1)	
HVS results n/N (%)				0.062
Normal	46/67 (68.7)	36/42 (85.7)	82/109 (75.2)	
Abnormal	15/67 (22.4)	6/42 (14.3)	21/109 (19.3)	
Unknown	6/67 (8.9)	0/42 (0)	6/109 (5.5)	
Abnormal HVS results, n/N (%)				0.076
Bacterial vaginosis	3/15 (20.0)	1/6 (16.7)	4/21 (19.0)	
E.coli	1/15 (6.7)	0/6 (0)	1/21 (4.8)	
S.aureus	1/15 (6.7)	0/6 (0)	1/21 (4.8)	
Group B streptococcus (S.agalactiae)	2/15 (13.3)	3/6 (50.0)	5/21 (23.8)	
Yeast	0/15 (0)	1/6 (16.7)	1/21 (4.8)	
Mixed coliforms	0/15 (0)	1/6 (16.6)	1/21 (4.8)	
Unknown	8/15 (53.3)	0/6 (0)	8/21 (38.0)	

Menopause status, n/N (%)				0.233
Premenopausal	50/67 (74.6)	26/42 (61.9)	76/109 (70.0)	
Postmenopausal	17/67 (25.4)	16/42 (38.1)	33/109 (30.0)	
Phase of menstrual cycle (PrMP), n/N (%)				0.115
Luteal	24/50 (48.0)	7/26 (26.9)	31/76 (40.8)	
Follicular	15/50 (30.0)	8/26 (30.8)	23/76 (30.3)	
Ovulation	0/50 (0)	1/26 (3.8)	1/76 (1.3)	
Unknown	11/50 (22.0)	10/26 (38.5)	21/76 (27.6)	
Use of contraception (PrMP), n/N (%)				**0.016**
Nil	27/50 (54.0)	20/26 (77.0)	47/76 (61.9)	
Condoms	5/50 (10.0)	0/26 (0)	5/76 (6.6)	
COCP	12/50 (24.0)	1/26 (3.8)	13/76 (17.1)	
POP	1/50 (2.0)	0/26 (0)	1/76 (1.3)	
Copper IUD	0/50 (0)	0/26 (0)	0/76 (0)	
Mirena IUS	3/50 (6.0)	5/26 (19.2)	8/76 (10.5)	
Vaginal ring	0/50 (0)	0/26 (0)	0/76 (0)	
Contraceptive implant	2/50 (4.0)	0/26 (0)	2/76 (2.6)	
Contraceptive injection	0/50 (0)	0/26 (0)	0/76 (0)	
Use of HRT (PoMP), n/N (%)				0.175
Yes	5/17 (29.4)	1/16 (6.2)	6/33 (18.2)	
No	12/17 (70.6)	15/16 (93.8)	27/33 (81.8)	
Diabetes status, n/N (%)				**0.001**
Non-diabetic	65/67 (97.0)	32/42 (76.2)	97/109 (89.0)	
Diabetic	2/67 (3.0)	10/42 (23.8)	12/109 (11.0)	
Diabetic treatment, n/N (%)				0.212
Diet control only	1/2 (50.0)	3/10 (30.0)	4/12 (33.3)	
Metformin alone	0/2 (0)	3/10 (30.0)	3/12 (25.0)	
Metformin combined 2nd diabetic medication	0/2 (0)	0/10 (0)	0/12 (0)	
Other oral antiglycaemic medication	1/2 (50.0)	0/10 (0)	1/12 (8.4)	

Insulin (alone or with oral medication)	0/2 (0)	4/10 (40.0)	4/12 (33.3)	
HOMA-IR[b], n/N (%)				0.136
Insulin resistant	2/67 (3.0)	5/42 (11.9)	7/109 (6.4)	
Non-insulin resistant	5/67 (7.5)	5/42 (11.9)	10/109 (9.2)	
Unknown insulin resistance status	60/67 (89.5)	32/42 (76.2)	92/109 (84.4)	

[b] HOMA-IR was calculated according to the formula: fasting insulin (mU/L) multiplied by fasting glucose (nmol/L)/22.5. The 2nd tertile was used as the cut-off to determine insulin resistance status. Insulin resistance cut-off value: 2.98

BMI, body mass index; COCP, combined oral contraceptive pill; E.coli, Escherichia coli; HOMA-IR, homeostatic model of assessment - insulin resistance; HVS, high vaginal swab; IUD, intrauterine device; IUS, intrauterine system; PoMP, postmenopausal; POP, progesterone-only pill; PrMP, premenopausal; S.aureus, staphylococcus aureus; SD, standard deviation.

Table 2
Prevalence of genus group present according to obesity status, diabetes and insulin resistance status for the whole cohort (n=109) at baseline sampling.

	***Lactobacillus* dominant** n/N (%)	***Gardnerella* dominant** n/N (%)	**High diversity** n/N (%)	**Total** n/N (%)
Obesity status				
Non-obese (BMI <30.0kg/m^2)	41/67 (61.2)	16/67 (23.9)	10/67 (14.9)	67/67 (100)
Obese (≥30.0kg/m^2)	12/42 (28.6)	12/42 (28.6)	18/42 (42.8)	42/42 (100)
Total	53/109 (48.6)	28/109 (25.7)	28/109 (25.7)	109/109 (100)
P value[a]				**0.002**
Diabetes status				
Non-diabetic	49/97 (50.5)	25/97 (25.8)	23/97 (23.7)	97/97 (100)
Diabetic	4/12 (33.3)	3/12 (25.0)	5/12 (41.7)	12/12 (100)
Total	53/109 (48.6)	28/109 (25.7)	28/109 (25.7)	109/109 (100)
P value				0.451
Insulin resistance status[b]				
Non-insulin resistant	4/10 (40.0)	2/10 (20.0)	4/10 (40.0)	10/10 (100)
Insulin resistant	2/7 (28.6)	2/7 (28.6)	3/7 (42.9)	7/7 (100)
Total	6/17 (35.3)	4/17 (23.5)	7/17 (41.2)	17/17 (100)
P value				1.000

[a] A p-value of less than 0.05 demonstrates a significant difference in the distribution of genus group present according to obesity, diabetic and insulin resistance status. Fisher's exact test employed as small numbers were present for each group (e.g. diabetic).

[b] Where concomitant fasting serum samples were available, fasting glucose and fasting insulin levels were identified. Using these values, we were able to calculate the HOMA-IR, according to the formula: the product of fasting insulin (mU/L) multiplied by fasting glucose (nmol/L) divided by 22.5. The 2nd tertile was used as the cut-off to determine insulin resistance status. Insulin resistance cut-off value: 2.98.

BMI, body mass index

When women who had used antibiotics within two weeks of sample collection or had sexual intercourse within 48 hours of sample collection were excluded, the prevalence of both the high diversity and *Lactobacillus*-dominant VMB remained significantly different in obese women in both categories (excluding intercourse, p=0.001; excluding recent antibiotic use, p=0.003) (Supplementary Table 3). When pre- and post-menopausal women were analysed separately, premenopausal obese women had a significantly higher prevalence of a high diversity VMB (p=0.001), even when oral contraceptive (OCP) users were excluded (p=0.003). There was a significant difference in the prevalence of each genus according to subcategories of normal weight, overweight and obese women (p<0.001) (Supplementary Table 3).

Table 3
Prevalence of genus group present over serial sample collection timepoints in the total bariatric cohort and according to menopausal status, diabetes status and insulin resistance status.

	Lactobacillus dominant n/N (%)	*Gardnerella* dominant n/N (%)	High diversity n/N (%)	TOTAL n/N (%)
Total bariatric surgery cohort				
Baseline (day of surgery) (n=27)	10/27 (37.0)	5/27 (18.6)	12/27 (44.4)	27/27 (100)
3 months post-surgery (n=22)	7/22 (31.8)	6/22 (27.3)	9/22 (40.9)	22/22 (100)
6 months post-surgery (n=19)	10/19 (52.6)	2/19 (10.5)	7/19 (36.9)	19/19 (100)
Total (n=84)	31/84 (36.9)	16/84 (19.1)	37/84 (44.0)	84/84 (100)
p value[a]				0.506
Pre-menopausal women only				
Baseline (day of surgery) (n=17)	6/17 (35.3)	4/17 (23.5)	7/17 (41.2)	17/17 (100)
3 months post-surgery (n=15)	5/15 (33.3)	4/15 (26.7)	6/15 (40.0)	15/15 (100)
6 months post-surgery (n=14)	8/14 (57.1)	2/14 (14.3)	4/14 (28.6)	14/14 (100)
Total (n=58)	22/58 (37.9)	12/58 (20.7)	24/58 (41.4)	58/58 (100)
p value				0.572
Post-menopausal women only				
Baseline (day of surgery) (n=10)	4/10 (40.0)	1/10 (10.0)	5/10 (50.0)	10/10 (100)
3 months post-surgery (n=7)	2/7 (28.6)	2/7 (28.6)	3/7 (42.8)	7/7 (100)
6 months post-surgery (n=5)	2/5 (40.0)	0/5 (0)	3/5 (60.0)	5/5 (100)
Total (n=26)	9/26 (34.6)	4/26 (15.4)	13/26 (50.0)	26/26 (100)
p value				0.999
Non-diabetic women only				

Baseline (day of surgery) (n=19)	6/19 (31.6)	3/19 (15.8)	10/19 (52.6)	19/19 (100)
3 months post-surgery (n=16)	6/16 (37.5)	4/16 (25.0)	6/16 (37.5)	16/16 (100)
6 months post-surgery (n=14)	8/14 (57.1)	1/14 (7.2)	5/14 (35.7)	14/14 (100)
Total (n= 58)	21/58 (36.2)	9/58 (15.5)	28/58 (48.3)	58/58 (100)
p value				0.572
Diabetic women only				
Baseline (day of surgery) (n=8)	4/8 (50.0)	2/8 (25.0)	2/8 (25.0)	8/8 (100)
3 months post-surgery (n=6)	1/6 (16.7)	2/6 (33.3)	3/6 (50.0)	6/6 (100)
6 months post-surgery (n=5)	2/5 (40.0)	1/5 (20.0)	2/5 (40.0)	5/5 (100)
Total (n=26)	10/26 (38.5)	7/26 (26.9)	9/26 (34.6)	26/26 (100)
p value**				n/a
Women without insulin resistance[c]				
Baseline (day of surgery) (n=4)	1/4 (25.0)	1/4 (25.0)	2/4 (50.0)	4/4 (100)
3 months post-surgery (n=2)	0/2 (0)	0/2 (0)	2/2 (100)	2/2 (100)
6 months post-surgery (n=7)	4/7 (57.1)	1/7 (14.3)	2/7 (28.6)	7/7 (100)
Total (n=13)	5/13 (38.5)	2/13 (15.4)	6/13 (46.1)	13/13 (100)
p value				n/a
Women with insulin resistance				
Baseline (day of surgery) (n=5)	2/5 (40.0)	1/5 (20.0)	2/5 (40.0)	5/5 (100)
3 months post-surgery (n=1)	0/1 (0)	0/1 (0)	1/1 (100)	1/1 (100)
6 months post-surgery (n=1)	1/1 (100)	0/1 (0)	0/1 (0)	1/1 (100)
Total (n=7)	3/7 (42.9)	1/7 (14.3)	3/7 (42.9)	7/7 (100)
p value[b]				n/a

[a] A p-value of less than 0.05 demonstrates a significant difference between the proportion (%) of

genus group present (*Lactobacillus*-dominant, *Gardnerella*-dominant or high diversity VMB) over serial timepoints 0 to 6 months (McNemar's Chi-square test).

[b] Sample size too small to compute p-value using McNemar's Chi-square test.

[c] Where concomitant fasting serum samples were available, fasting glucose and fasting insulin levels were identified. Using these values, we were able to calculate the HOMA-IR, according to the formula: the product of fasting insulin (mU/L) multiplied by fasting glucose (nmol/L) divided by 22.5. The 2nd tertile was used as the cut-off to determine insulin resistance status. Insulin resistance cut-off value: 2.98.

Consistent with an increased prevalence of high diversity VMB (Figure 2a), increased richness (number of species observed) and alpha diversity was observed in obese women (diversity, p= 0.006) (Figure 2b, 2c, Supplementary Table 4). The vaginal microbiota of obese women was characterised by a greater mean proportion of anaerobic bacterial species, specifically unclassified *Dialister* spp. (unclassified) (p<0.001), *Anaerococcus vaginalis* (p=0.021) and *Prevotella timonensis* (p=0.020) (Figure 2d).

To identify vaginal microbiota biomarkers specifically associated with obesity, we performed linear discriminant analysis (LDA) effect size (LefSe) modelling on the 16S rRNA gene sequence data collected from baseline samples (Supplementary Figure 1). Vaginal microbiota of obese women was enriched with members of *Bacteroidales* and *Clostridiales*, the *Prevotella* genus and the phylum *Actinobacteria*. Conversely, non-obese women were found to have enriched levels of *Lactobacillales* associated OTUs.

Obese women had significantly increased expression of pro-inflammatory cytokines IL-1, IL-6, IL-8, MIP-1, IFN and TNF compared to non-obese women (Figure 3a, Supplementary Table 5). The anti-inflammatory cytokine IL-4 showed increased expression among obese women. As bacterial diversity increased among obese women (depicted using non-parametric Shannon Index), the expression of IL-1 and IL-8 but not the other cytokines, increased (Figure 3b).

Metabolic and vaginal microbiota compositional changes after bariatric surgery

In the subset of obese women undergoing bariatric surgery, there were no significant overall differences in VMB following surgery (Figure 4a, Supplementary Figure 3). Neither were changes observed following bariatric surgery according to menopause status, diabetes or insulin resistance status (Table 3). Prior to surgery there was no relationship between BMI and VMB structure, however post-surgery women with *Lactobacillus*-dominant VMB had a significantly lower BMI than those with a high diversity VMB (Figure 4b). This difference principally applied to pre-menopausal women (Figure 4c). Local cervicovaginal cytokine levels in the bariatric surgery cohort at baseline sampling (n=27) and 6 months post-surgery (n=21) did not show any significant changes (Supplementary Figure 2).

Discussion

In the UK, two thirds of the female population are either overweight (30%), obese (27%) or morbidly obese (4%) [52]. Obesity has been associated with a multitude of adverse health outcomes in women [2, 4, 5, 12, 18], and although the mechanisms leading to these complications of obesity in women remain unclear, the vaginal microbiota composition may be important.

In our cohort, we found three VMB groups at genus level. Approximately half of the vaginal samples were categorised as *Lactobacillus*-dominant VMB, whilst the remaining samples were categorised in equal proportion as either *Gardnerella*-dominant (with a high relative abundance of *Gardnerella* spp. and low relative abundance of *Lactobacillus* spp.), or high diversity VMB (with a low relative abundance of each of *Lactobacillus* and *Gardnerella* spp., and increased bacterial diversity). When these samples were analysed with respect to obesity status, about 70% of obese women demonstrated a *Lactobacillus*-dominant VMB. This proportion is consistent with other reported studies. Brotman and colleagues showed that a *Lactobacillus*-dominant VMB is found in 80% of premenopausal women, but only 55% of postmenopausal women [53]. Our study represents a mixture of pre- and post-menopausal women. In those who were categorised as obese, 30% of women had a *Lactobacillus*-dominant VMB. This prevalence is lower than that found in non-obese postmenopausal women [53]. Our study shows that obesity associates with vaginal microbiota composition, with significantly higher vaginal bacterial species diversity and increased abundance of *Dialister*, *Prevotella* and *Anaerococcus* among obese women. Two previous studies, both from Korean patient cohorts, have described vaginal microbiota composition in relation to obesity in a non-pregnant population. The first study reported that obese women had a greater predominance of *Lactobacillus iners* compared to non-obese women who were more likely to have a *Lactobacillus crispatus*-dominant VMB [50]. However, this study was limited to the analysis of interrelationships between cervical *Lactobacillus* species only with non-*Lactobacillus* members of the microbiota not considered. Therefore the generalisability of these findings are unclear [50]. Our findings suggest an increased prevalence of *Lactobacillus*-depleted vaginal microbiome in obese women, with a greater mean proportion of *Lactobacillus crispatus* in non-obese compared to obese women (Figure 2d). The second study, by Si et al., reported that obesity was associated with increased levels of *Prevotella*, and reduced *Lactobacillus* relative abundance consistent with our data [51].

The vaginal microbiota composition is dynamic and fluctuates throughout the life cycle with relative dominance of the niche by *Lactobacillus* spp. mediated by oestrogen-driven vaginal epithelium thickening and glycogen deposition, which is used as a primary energy source by lactic acid producing bacteria, encouraging a *Lactobacillus*-dominant VMB [54]. Accordingly, prepuberty and post-menopause vaginal microbiota composition associates with reduced glycogen levels [55] and a tendency towards a high diversity vaginal microbiota [56, 57]. In our study, vaginal microbiota composition in pre- and post-menopausal women were largely consistent with the published literature. In peri- or post-menopausal women with declining ovarian function, peripheral adipose tissue becomes the major source of

production of unopposed circulating oestrogen by aromatisation of adrenal androstenedione to excess endogenous oestrogen [58-60]. In this group of women, reduced systemic oestrogen levels cause a decrease in glycogen deposition resulting in a vaginal epithelia that resembles pre-puberty with a thinner mucus layer and increased incidence of high diversity VMB [61, 62]. However, oestrogen levels are not higher in premenopausal obese women compared to non-obese. Freeman and co-workers found that premenopausal obese and overweight women had significantly lower oestradiol levels compared with non-obese women, independent of age, race, or smoking [63]. Reduced circulating oestrogen concentrations is therefore a potential explanation for the lower prevalence of *Lactobacillus*-dominant VMB in obese women. It is also possible that due to restrictions in mobility caused by morbid obesity, female hygiene practices are affected. Local skin irritation and breakdown caused by the presence and rubbing of excess adipose tissue, together with persistent moisture may alter the local vaginal microbiota composition. The impact of women's sexual, sanitary and hygiene practices such as douching on the vaginal microbiota is still controversial [64, 65].

Sustained weight loss may be brought about by significant lifestyle changes (diet and increased physical activity) or induced by bariatric surgery, that can result in improved metabolic health. It has previously been reported that bariatric surgery can reduce cancer incidence [66], improve sex hormone profiles [67], polycystic ovarian syndrome symptoms [68], spontaneous and assisted conception rates [69-72], and reduce obstetric complications such as gestational diabetes, pregnancy-induced hypertension and macrosomia [73, 74]. Reversal or reduction in obesity brought about by bariatric surgery has been shown to be accompanied by metabolic improvement and reduction of alpha-diversity of the gut microbiota within 3 months post-operatively [75-77]. The impact of bariatric surgery on the vaginal microbiome has not however been previously assessed. In our study, in the subset of obese women undergoing bariatric surgery, there were no significant overall differences in VMB following surgery, however 75% of these women remained in the obese range. Prior to surgery there was no relationship between BMI and VMB structure, however, post-surgery women with *Lactobacillus*-dominant VMB had a significantly lower BMI than those with a high diversity VMB. Significant weight loss following bariatric surgery therefore associates with a tendency toward an 'optimal' VMB. We did not, however, find any correlation between degree of weight loss and systemic oestradiol or SHBG concentrations. Additionally, SHBG increased in every woman at 6 months post-surgery which would lead to lower bioavailable oestrogen. It is therefore unlikely any that any effect of bariatric surgery upon the VMB is principally due to changes in systemic oestradiol or SHBG concentrations.

The role of immune modulators such as adipose tissue macrophages, cytokines and adipokines in obesity leading to systemic inflammation is well described [78, 79]. Cervicovaginal changes in immune factors in response to genital tract infection have been linked to preterm birth [80] and bacterial vaginosis persistence [81]. Cervicovaginal cytokine levels (IL-1, IL-6, IL-8, TNF, MIP-1 and IFN) were increased in our

obese population. Modulation of the VMB in pregnancy has been shown to increase local expression of each of these cytokines which correlates with increasing species diversity [82]. In the present study however, there was an association between increasing bacterial species diversity and expression of only of the two pro-inflammatory cytokines IL-1 and IL-8. This data leads us to conclude that, in obesity, factors other than the VMB act to modulate cervicovaginal inflammation. We found no significant change in cervicovaginal cytokine levels following bariatric surgery, and cytokine concentrations were not different between the different genera groups. This finding again shows that much of the local cervicovaginal inflammation associated with obesity is unrelated to the vaginal microbiota.

Previous cross-sectional studies have highlighted that vaginal microbiota composition may be affected by obesity [50, 51]. A strength of the present study is the assessment of the vaginal microbiota composition in obese pre- and postmenopausal women separately, after exclusion of OCP and HRT-users and after taking into account confounding variables and hormonal serum levels. Furthermore, this is the first study to explore the temporal changes in the vaginal microbiota in a cohort of morbidly obese women undergoing surgically induced weight loss.

Although this study is one of the largest cohorts assessing the impact of obesity on the vaginal microbiota composition the number of women undergoing bariatric surgery, it was too small to draw meaningful conclusions for many comparisons of interest. Recruitment of much larger numbers would be practically difficult. Follow up for longer periods may show changes not revealed at 6-months, maximum weight loss is usually achieved 12-24 months after surgery [83, 84], although the majority of women who undergo bariatric surgery regain between 5-10% of their pre-operative weight by 2-3 years [84].

Conclusions

Obesity has significant associations with the vaginal microbiota composition and local inflammation. Local inflammation is only partly explained by the adverse VMB. Overall, bariatric surgery does not change the VMB however, despite weight loss, three quarters of these women remain categorised as obese six months post-surgery. However, those with the greatest weight loss six-months post-surgery are most likely to have a *Lactobacillus*-dominant VMB.

A clear benefit of bariatric surgery is a reduction in diabetes and hyperinsulinaemia which would be expected to translate into better long-term health. A healthier VMB following bariatric surgery-induced weight loss might also improve long term health, but since this does not associate with a reduction in local inflammation other factors are likely to be involved and require further investigation.

Materials And Methods

Study population – inclusion and exclusion criteria

We prospectively recruited non-pregnant women attending outpatient gynaecology and bariatric surgery clinics at Imperial College NHS Healthcare Trust between 2013-2016. A subset of this population was scheduled for bariatric surgery. Women were recruited irrespective of age, menopause status, ethnicity, parity, smoking, phase in menstrual cycle and contraception use. Women who were HIV, hepatitis B or C positive, had autoimmune disorders, or had a previous hysterectomy were excluded. Ethical approval was obtained from the National Research Ethics Service Committee London – Fulham (Approval number 13/LO/0126) and the NHS West of Scotland Research Ethics Service Committee (WoSRES) (REC 14/WS/1098). All patients gave informed consent.

Sample collection and processing

Cervicovaginal secretions were collected during the clinic visit from the posterior vaginal fornix with a BBL™ CultureSwab™ containing liquid Amies (Becton Dickinson, Oxford, UK) using a sterile, disposable speculum, without lubricant and immediately stored at -80°C. A second transport microbiology swab (Transwab®) containing Amies gel medium was simultaneously collected for cytokine analysis. In the subset population of women planned for bariatric surgery (Gastric band, Roux-en-Y Gastric Bypass or Vertical Sleeve Gastrectomy), we collected serial vaginal swab samples on the day of surgery and at months 3 and 6 post-surgery (Supplementary Figure 4). Serial fasting blood samples were also collected on the day of surgery and at 6-month follow-up with the aim to correlate changes in vaginal microbiota to four serum markers known to be affected by surgery-induced weight loss and hyperinsulinaemia correction. Blood samples were centrifuged at 4,472 *g* for 10 minutes and serum collected for freezing and storage in -80°C.

A comprehensive interview and questionnaire were used to obtain all relevant gynaecological, medical and surgical history. Menopause status, type of contraception or HRT use and menstrual cycle phase (follicular or luteal) were documented. Ethnicity was self-reported as Caucasian, Asian, Black or Other.

Whole-Genomic bacterial DNA was extracted from the CultureSwab™ using a QiAmp Mini DNA kit (Qiagen, Venlo, Netherlands) as described previously [36]. The second swab for cytokine analysis was thawed on ice and re-suspended in 350ml phosphate-buffered saline solution with protease inhibitor (5ml/ml; Sigma Aldrich). The suspension was centrifuged at 402*g* for 2 minutes, and supernatant collected into a new 1.5ml microcentrifuge tube. This centrifugation step was repeated to remove any remaining cellular debris. The cell-free supernatant was stored in -80°C. A set of 5 negative control swab was processed alongside each DNA extraction set. These controls did not amplify contaminant DNA and were therefore not sequenced.

Illumina MiSeq sequencing of 16S rRNA gene amplicons and data processing

The V1-V2 hypervariable regions of 16S rRNA genes were amplified by PCR using a forward and reverse fusion primer, described in detail in Supplementary Methods S1. Bacterial profiling using a MiSeq platform (Illumina, San Diego, CA, USA) was conducted at Research and Testing Laboratory (Lubbock, TX, USA). The 16S rRNA gene sequence data was analysed with bioinformatic software package Mothur [85] using the MiSeq SOP Pipeline. Sequence reads were quality checked and normalised to the lowest number of reads (n=1,855) and singleton operational taxonomic units (OTUs); samples containing fewer than 10 reads were excluded. OTU taxonomies (from Phylum to Genus) were then determined using the RDP MultiClassifier script to generate the RDP taxonomy. Taxonomy level for species of the OTUs was determined using the USEARCH algorithm with 16S rRNA gene sequences from the cultured representatives from the RDP database [86]. Rare OTUs were defined as those present at less than 10 counts within the entire cohort. Alpha and beta diversity indices were calculated from these datasets with Mothur and R statistical package using the Vegan package.

Serum biomarker and local vaginal cytokine analyses

Four serum markers including oestradiol (pmol/L), insulin (mIU/L), glucose (mmol/L) and sex hormone binding globulin (SHBG, nmol/L) were quantified using ELISA at the Imperial College Healthcare NHS Trust North West London Pathology laboratory (Supplementary Methods S2).

Levels of interleukin (IL)-4, IL-6, IL-8, IL-1β, tumour necrosis factor-alpha (TNFα), interferon-gamma (IFN-γ), and macrophage inflammatory protein-1 alpha (MIP1α) in cell-free cervicovaginal secretion supernatants were determined using the Magnetic Luminex Screening Assay multiplex kit (R&D Systems, Minneapolis, MN, USA) on a MAGPIX Analyzer (Luminex® Corporation, s-Hertogenbosch, Netherlands), as per manufacturer's instructions. Analytes were chosen based on evidence of inflammatory markers specific to adiposity [87-89].

Statistical analyses

The population was categorized into two groups of interest for the main analysis, non-obese (BMI <30kg/m^2) versus obese (BMI ³30kg/m^2). We performed further supplementary analyses to assess results for different obesity status subcategories and according to insulin resistance and diabetic status. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by the following formula: the product of fasting insulin (mU/L) multiplied by fasting glucose (nmol/L) divided by 22.5 [90]. We used the 2^{nd} tertile value of HOMA-IR as the cut-off to determine insulin resistance status (at 2.98). Differences in categorical clinical parameters between the two main groups of interest (non-obese versus obese) were assessed using Fisher's exact test for each of the listed characteristics; age, ethnicity,

parity, smoking, menopause, menstrual cycle, use of contraception, HRT use, diabetes status and treatment, and abnormal high vaginal swab (HVS) results.

Significant differences between vaginal microbiota at genera taxonomic level were assessed using the Statistical Analysis of Metagenomic Profiles (STAMP) software package [91]. Dependent on genera hierarchical clustering analysis, *Lactobacillus* spp. or *Gardnerella* spp. abundance among selected phenotypic categories was investigated by assigning each patient sample into one of three groups (*Lactobacillus*-dominant, *Gardnerella*-dominant, or high diversity VMB). Linear discriminant analysis (LDA) effect size (LEfSe) modelling was used to identify biomarkers based on obesity status, according to relative taxonomic abundance [92].

At genus taxonomic level, prevalence of each of the three categories relating to *Lactobacillus* or *Gardnerella* presence were compared between the two phenotypic categories (obese or non-obese) using Fisher's exact test. We performed further sub-analyses for different weight categories, as well as by the presence of diabetes and/or insulin resistance status. A sensitivity analysis assessed whether the exclusion of women that had antibiotics less than 2 weeks before sample collection or those disclosing sexual intercourse within 48 hours from sampling would affect the results. We further analysed the results for pre- and post-menopausal women separately and after exclusion of those taking oral hormonal contraception or hormone replacement therapy (HRT).

Expression levels of assayed cytokines were compared according to obesity status, prevalence of each genus group and diversity (non-parametric Shannon Index) at baseline sampling using two-way ANOVA. Where data fell outside the range of the standard curve for each analyte, either the minimal or maximal extrapolated or minimal or maximal value of the standard curve was used, where appropriate. Analyses were performed using Prism 8, p values <0.05 considered significant.

In the subset of women undergoing bariatric surgery, changes in serum markers introduced by weight loss were analysed. We further assessed the impact of surgically-induced weight loss on the prevalence of each of the three genus groups at baseline, month 3 and 6 using McNemar's Chi square test. We analysed the results for the full cohort and separately for pre- and post-menopausal women, and according to diabetic and insulin resistance status. Transition in vaginal microbiota across genus groups correlating with weight loss from baseline sampling to 6 months post-surgery was analysed for the total bariatric cohort and pre- and postmenopausal women separately. Cytokine and serum marker expression levels were compared between baseline sampling and 6 months after surgically-induced weight loss.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the National Research Ethics Service Committee London – Fulham (Approval number 13/LO/0126) and the NHS West of Scotland Research Ethics Service Committee (WoSRES) (REC 14/WS/1098). All patients gave informed consent.

Consent for publication

Where individual patient data is included, all patients provided informed consent.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by Genesis Research Trust (P55549 and Garfield Weston Foundation, Grant number P63522 to MK); Ovarian Cancer Action (Grant number PS5827 and PSA601to MG and MK); British Society of Colposcopy Cervical Pathology Jordan/Singer Award (P47773)(MK); Imperial College Healthcare Charity (P47907) (MK, AM); NIHR Academic Clinical Fellowship programme (AM); the Imperial Experimental Cancer Medicine Centre, the Cancer Research UK Imperial Centre, Imperial Healthcare NHS Trust NIHR BRC (Grant number P45272). None of the funders have had any influence over: study design, collection, analysis and interpretation of the data, in writing the report and in the decisions to submit this article for publication. The Division of Digestive Disease at Imperial College London receives financial support from the National Institute of Health Research (NIHR) Imperial Biomedical Research Centre (BRC) based at Imperial College Healthcare NHS Trust and Imperial College London. This article is independent research funded by the NIHR BRC, and the views expressed in this publication are those of the authors and not necessarily those of the NHS, NIHR, or the Department of Health.

Authors' contributions

The study was designed by MK, DM, OR and PB. The samples and data were acquired and collated by OR, AM, SP and MK. The data was analysed by OR, DM, AM, AS, NA, YL, PB and MK. The manuscript was drafted by OR, DM, MK, PB and revised critically for important intellectual content by all authors (OR, DM, AM, YL, AS, NA, JN, SP, MG, HG, JM, PB, MK). All authors gave final approval of the version to be published and have contributed to the manuscript.

Acknowledgements

We thank all participants of the study. Our work was supported by Imperial College Healthcare Charity, Genesis Research Trust, Ovarian Cancer Action, the British Society of Colposcopy Cervical Pathology (Jordan/Singer Award), the Imperial Experimental Cancer Medicine Centre, and the Cancer Research UK Imperial Centre. The Division of Digestive Disease at Imperial College London receives financial support from the National Institute of Health Research (NIHR) Imperial Biomedical Research Centre (BRC) based at Imperial College Healthcare NHS Trust and Imperial College London.

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Figures

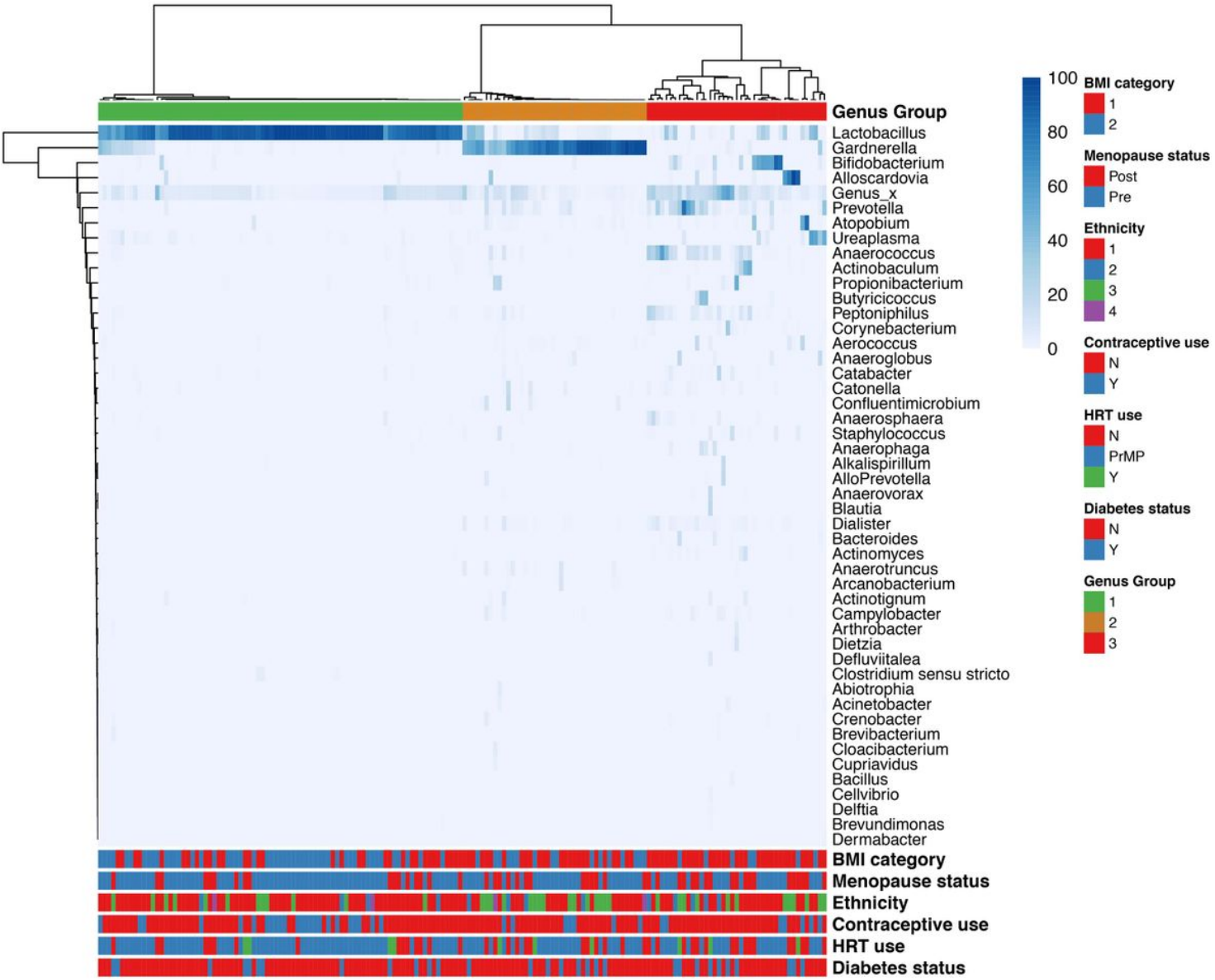

Figure 1

) Hierarchical clustering analysis of genera taxonomic level data generates three distinct groups consisting of i) Lactobacillus-dominant (characterised by high relative abundance of Lactobacillus spp., ii) Gardnerella-dominant (characterised by high relative abundance of Gardnerella spp. and low relative abundance of Lactobacillus spp.) and iii) High diversity vaginal microbiome (VMB) – characterised by low relative abundance of each of Lactobacillus and Gardnerella species, and increased bacterial diversity. Heatmap created from all samples collected (n=166), using Ward linkage with the fifty most commonly identified microbial genera shown. Cohort characteristics including BMI category, menopause status, ethnicity, contraceptive or hormone replacement therapy (HRT) use and diabetic status are also shown below the heatmap.

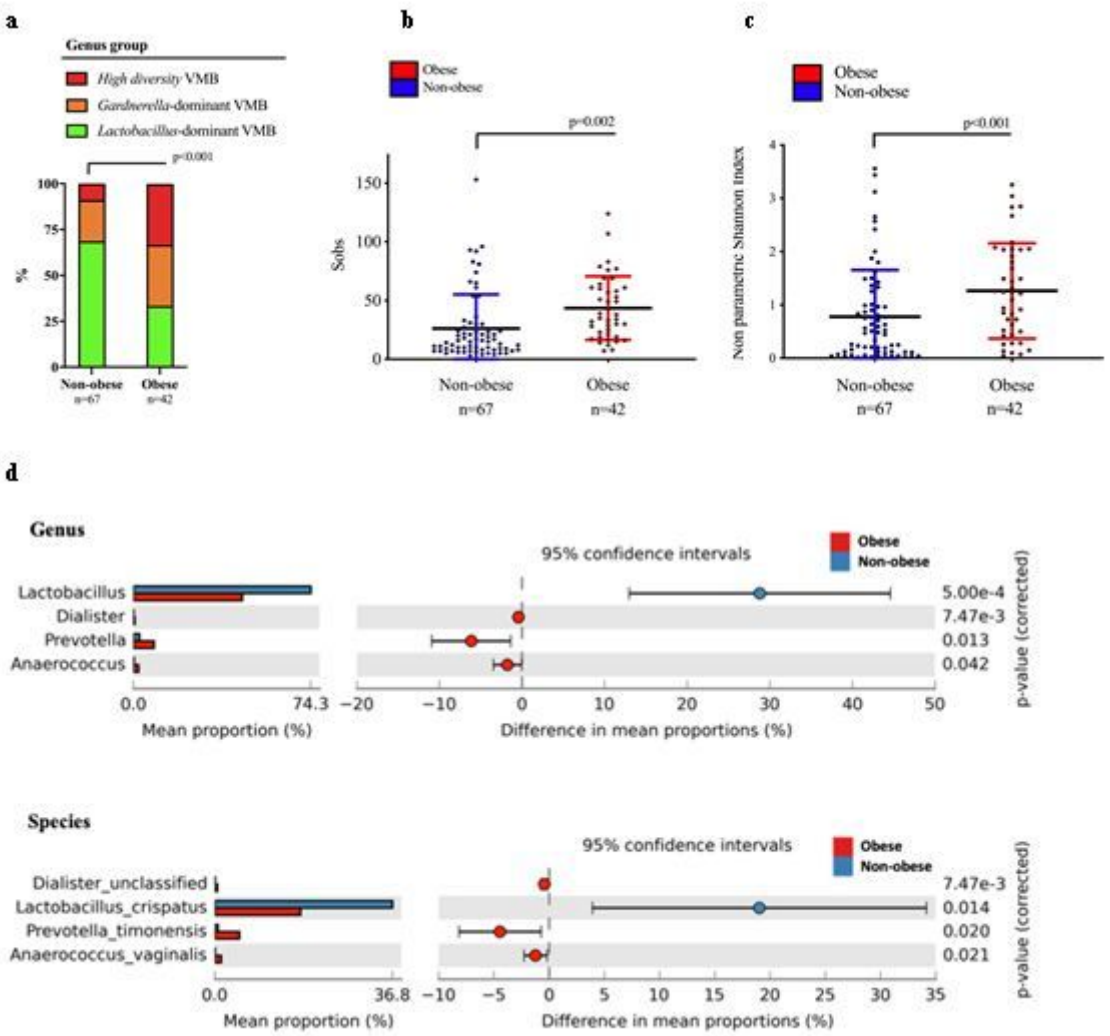

Figure 2

a) The prevalence of the Lactobacillus-dominant genus group was lower in obese compared to non-obese women (p<0.001). b) Number of species observed (Sobs) increased with obesity (p=0.002). c) Significantly increased microbial diversity was seen among obese women (p=0006). d) The vaginal microbiome of obese women was characterised at genus level by increased mean proportion (%) of Dialister, Prevotella and Anaerococcus. Results at species level showed increased mean proportion (%) of Dialister (unclassified), Prevotella timonensis and Anaerococcus vaginalis in obese women, and Lactobacillus crispatus dominated among non-obese women.

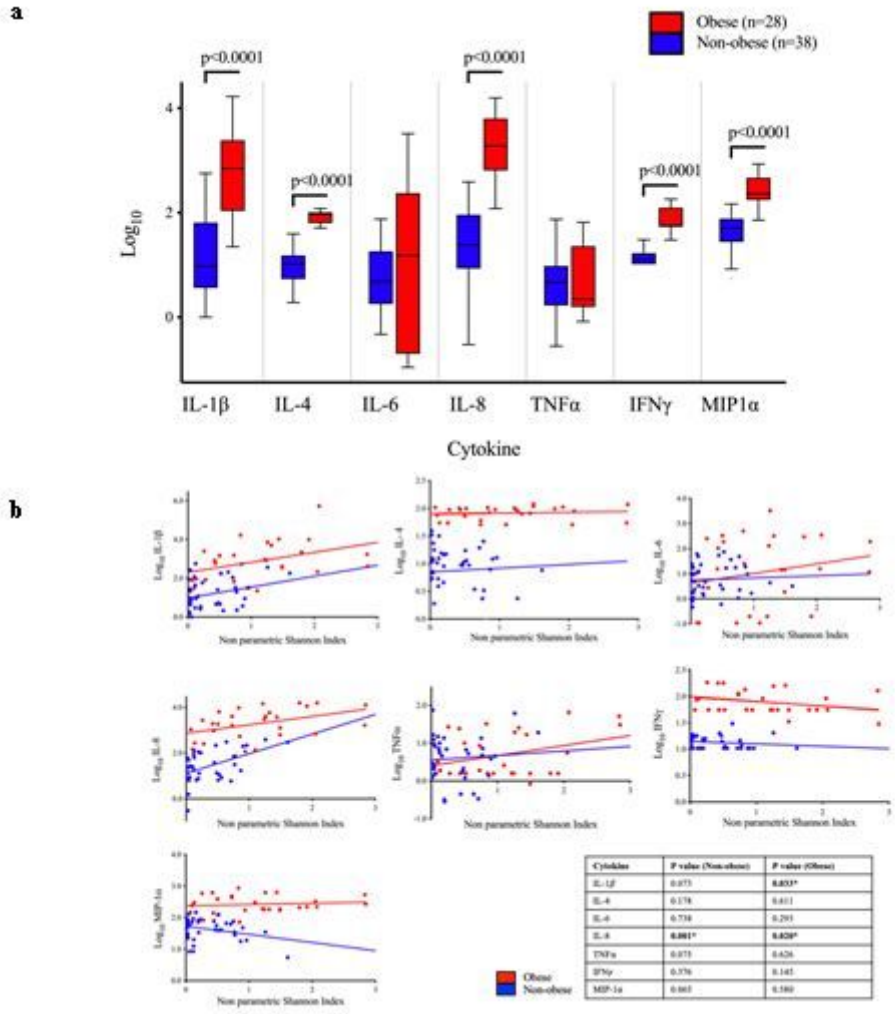

Figure 3

Pro-inflammatory local vaginal cytokine expression is increased in obesity. a) Expression levels (log10) of seven cytokines measured among non-obese and obese women in the baseline cohort. Pro-inflammatory cytokines IL-1β, IL-8, IFNγ and MIP-1α all showed significantly increased expression among obese women ($p<0.001$). Anti-inflammatory cytokine IL-4 also had increased significance in obese women ($p<0.001$). b) Expression of seven local cervicovaginal cytokine levels (log10) according to obesity status and species diversity (non-parametric Shannon indices). As the diversity of vaginal bacterial species increases, there is a significant increase in expression of pro-inflammatory cytokines IL-1β and IL-8, dependent on obesity status.

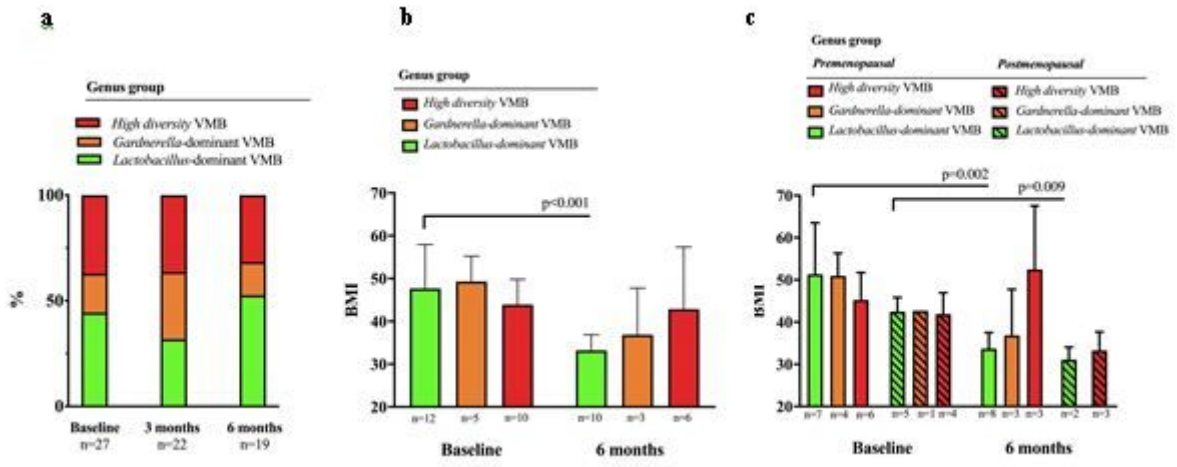

Figure 4

a) Differences in proportion of vaginal microbiota groups from baseline (day of surgery) to 6 months post-surgery in the bariatric cohort. b) The mean BMI of each of the three genus groups at baseline sampling and at 6 months post-surgery. There was a significant reduction in mean BMI in Lactobacillus-dominant VMB samples at 6 months post bariatric surgery ($p<0.001$). c) The mean BMI of each of the three genus groups at baseline and 6 months post-surgery, according to menopause status. There was a significant reduction in mean BMI in the Lactobacillus-dominant group in premenopausal ($p=0.002$) and postmenopausal women ($p=0.009$).

Supplementary Files

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- SupplementaryMaterials.docx