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Original article

Vaginal Glycogen, Not Estradiol, Is Associated With Vaginal Bacterial Community Composition in Black Adolescent Women

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A B S T R A C T

Purpose: The purpose of this study was to characterize the composition of vaginal bacterial communities in a cohort of black adolescent women and to determine how the species composition of these communities correlates with levels of estradiol, glycogen, and stress.
Methods: Twenty-one black adolescent women were sampled longitudinally. The composition of their vaginal communities was determined by analyzing the sequences of the V1–V3 regions of 16S rRNA genes, and they were grouped based on patterns in species abundances. The relationships between estradiol, glycogen, psychosocial stress, and the composition of these communities were assessed.
Results: Vaginal communities could be distinguished and classified into three groups that differed in the abundances of *Lactobacillus*. Eighty-one percent of study participants had communities dominated by species of *Lactobacillus*. Glycogen levels were higher in communities dominated by one or multiple species of *Lactobacillus* compared with those having low proportions of *Lactobacillus*. Estradiol and psychosocial stress measurements did not differ among the three groups, whereas estradiol and glycogen exhibited a weak positive relationship that was not statistically significant.
Conclusions: The findings of this pilot study suggest that glycogen levels are associated with vaginal community composition in young black women; however, estradiol and psychosocial stress are not. In addition, the results suggest there is no simple relationship between levels of estradiol and the production of vaginal glycogen.

IMPLICATIONS AND CONTRIBUTION

The positive association between *Lactobacillus* dominance and glycogen levels suggests that factors affecting vaginal glycogen levels may influence clinical conditions such as bacterial vaginosis and alter susceptibility to other urogenital infections. Future studies might elucidate these factors and explore whether glycogen levels could serve as a surrogate that predicts community composition.

Sexually transmitted infections (STIs) are highly prevalent among adolescent women [1]. In 2016 alone, young women aged 10–19 years comprised nearly one fifth of chlamydia and gonorrhea cases [2], and these STIs increase HIV risk in adolescents [3]. Rates are especially high among young black women [2]. Although various physiological, behavioral, and psychosocial

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factors are thought to contribute [4–6], it is unclear why young black women have such high risk. Poor sexual and reproductive health outcomes in women have been linked to the bacterial composition of the vaginal microbiome of older women, but we know very little about the vaginal microbiomes of black adolescent women [7,8].

In premenopausal women older than 18 years, high proportions of *Lactobacillus* are associated with a lower prevalence of HIV and other STIs [9]. *Lactobacillus* species produce lactic acid, which is thought to inhibit colonization by pathogenic organisms [10–13]. However, approximately 25% of reproductive age women have vaginal communities that are depleted of *Lactobacillus* [11]. Moreover, the prevalence of communities dominated by *Lactobacillus* is greater in Asian and white women (80.2% and 89.7%, respectively) compared with black and Hispanic women (61.9% and 59.6%, respectively) [14]. In addition to these observed differences between women, point estimates of vaginal bacterial community composition can change rapidly [15–17], sometimes resulting in low proportions of lactobacilli that may expose women to windows of risk for acquiring infections. Currently, the factors accounting for the compositional differences observed between women and temporal changes within women are poorly understood. Thus, there is a need to understand drivers of changes in vaginal community composition, especially in young black women.

The abundances of *Lactobacillus* species have been positively associated with circulating estrogen and vaginal glycogen content (reviewed ~~in the study~~ by Hickey et al.[11]). It has also been proposed that other factors such as psychosocial stress may influence vaginal community composition and account for the racial/ethnic differences in vaginal communities seen among adult women [18]. Thus, the main objective of this study was to determine whether vaginal bacterial community composition in black adolescent women is associated with stress, estradiol, and glycogen. To explore this, we sampled the vaginas of 21 women, aged 14 years, at baseline and then monthly for 6 months. We determined the species composition of their vaginal bacterial communities, assessed levels of estrogen and glycogen, measured vaginal pH and Nugent scores, and assessed psychosocial stress. With these data, we addressed three questions: first, what kinds of vaginal bacterial communities do young black women have? Second, do the levels of estradiol, glycogen, and stress differ among women with different kinds of communities? And third, what are the relationships between estradiol, glycogen, stress, and key species of the vaginal communities?

Materials and Methods

Study design

Twenty-five self-identified black women (aged 14.01–14.99 years) were recruited from neighborhood clinics in Indianapolis, where most participants received primary care, to participate in a longitudinal study to assess the relationships between stress, estrogen, and vaginal community composition. This study was approved by the Institutional Review Board at Indiana University. We focused on 14-year-olds because this age is developmentally meaningful interval in which hygiene practices (such as pad or tampon use, douching, and pubic hair removal) become common, the nature of sexual interpersonal relationships changes rapidly, and studying this group fills gaps in existing research that cannot otherwise be addressed. Written informed consent of both participants and a parent were obtained before enrollment. Exclusion criteria at enrollment included structural abnormalities of the vagina, chronic medical conditions that could alter the vaginal microbiome, pregnancy, immune deficiency conditions, and antibiotic use within the previous 90 days. Two participants were excluded due to antibiotic use, and two were excluded due to health complications or noncompliance. In total, 21 participants provided seven monthly, self-collected vaginal swab and saliva samples and self-assessments of psychosocial stress, menses, and sexual behaviors. All study participants were postmenarcheal.

Estrogen and glycogen measurements

Saliva samples were used to measure estradiol levels because the method of sample collection is noninvasive, well-accepted assays exist [19,20], and levels of salivary estradiol are well correlated with serum estradiol concentrations [20,21]. For 3 days up to and including the scheduled day of vaginal swab sampling, once-per-day salivary samples were self-collected by passive drool into polypropylene cryotubes and frozen shortly after collection. Salivary estradiol concentrations were determined with a commercially available enzyme-linked immunosorbent assay kit (17β-estradiol high-sensitivity ELISA kit ADI-901-174; Enzo Life Sciences). The standard curve was prepared per manufacturer's instructions but extended to eight standards with 7.8 pg/mL as the lowest value. The intra-assay variation was 4.2%, and the interassay variation was 7.8%. During extraction, samples were concentrated four times in assay buffer, and each was measured in duplicate using the protocol available at http://hdl.handle.net/2022/21883.

Glycogen in vaginal swab samples was quantified using the EnzyChrom Glycogen Assay Kit (BioAssay Systems) according to the manufacturer's instructions. To measure vaginal pH, subjects inserted a gloved finger into the vagina for 10 seconds then rolled the finger over a commercially available pH stick (pH-EcoCare Comfort; Merete Medical GmbH). The pH measurements were confirmed by a trained research associate and recorded in .5 increments. Self-obtained vaginal swabs (eSwab Copan Diagnostics Inc.) were used to assess Nugent score and provide vaginal samples for STI testing and microbial community analyses. Nugent score is a Gram stain scoring system used to diagnose bacterial vaginosis (BV) [22]. To assess Nugent scores, vaginal swabs were rolled onto glass microscope slides, air-dried in the field, and transported to the Infectious Diseases Laboratory at Indiana University. Slides were then stained according to standard procedures and scored 0–3 (normal), 4–6 (intermediate), and 7–10 (abnormal/BV) [22]. STI testing for chlamydia and gonorrhea was conducted using Abbott RealTime CT/NG assay on the Abbott m2000 platform (Abbott Molecular, Des Plaines, IL). *Trichomonas vaginalis* testing was performed using a validated real-time polymerase chain reaction assay on the Abbott m2000 platform. The remainder of swab samples were stored at −80°C until DNA sequencing was done at the University of Idaho.

Measurement of psychosocial factors

Psychosocial factors were assessed using audio computer-assisted self-interview [23], obtained at baseline, month 3, and month 6. The standardized scales (all previously validated in adolescent populations) addressed depression (the Patient

Health Questionnaire [PHQ] [24]), stress (Perceived Stress Scale [PSS] [25]), and anxiety (the Brief Symptom Inventory [BSI] [26]).

Determination of menstrual cycle phase

Throughout the study, self-reported menses data were used to determine the start and end date of menstrual periods for subjects not on hormonal birth control. We assumed an average luteal phase of 14 days, one day for ovulation, and the remaining days to be follicular. Based on these criteria, we divided menstrual period into phases and matched sample collection dates accordingly.

Microbial community analysis

Total genomic DNA was extracted from vaginal swab samples using chemical and mechanical lysis and purified using QIAamp DNA mini kits (Qiagen) as described previously [27]. The V1–V3 regions of 16S rRNA genes were amplified using a two-step polymerase chain reaction protocol, first amplifying the gene region using universal primers 27F and 534R and then adding sample barcodes and sequence adapters. Amplicons were sequenced using an Illumina MiSeq platform in the Genomics Resources Core facility at the University of Idaho. High-quality reads were obtained from all samples except one, resulting in 146 samples total. Forward and reverse reads were paired using FLASH [28], processed through DADA2 [29] to identify unique sequences, and these were classified to genus and species levels using SPINGO [30]. A total of 422 taxa were identified. Of those, 44 taxa were present at a minimum of 1% in at least two individuals or at least 5% in one individual. Using this filter, the remaining taxa (378) were considered uncommon and therefore grouped into an "other" category that was included in subsequent analyses. The relative abundances of the taxa found in these communities are reported in Table S1.

To group communities on the basis of similarities and differences in composition, we performed complete-linkage hierarchical clustering on alt-Gower distances computed from taxon relative abundance data. Silhouette information was used to define nine clusters. Clusters were assigned to groups A, B, and C based on whether they were dominated by one species of *Lactobacillus* (group A), dominated by multiple species of *Lactobacillus* (group B), or had low proportions of lactobacilli (group C).

Statistics

Linear and linear mixed-effects models were used for multiple analyses, including (1) modeling the means of the response variables estradiol, glycogen, vaginal pH, and psychosocial stress (BSI, PHQ, and PSS) between groups; (2) modeling the means of estradiol between menstrual cycle phase; and (3) characterizing the linear relationship between estradiol and glycogen. Response variables were transformed where appropriate to avoid violating model assumptions. Statistical significance of these models was determined using analysis of variance. Statistical comparisons were performed by testing general linear hypotheses and multiple comparisons of the means using Tukey's test. A Kruskal–Wallis rank-sum test was used to evaluate group significant differences in Nugent score, with post-hoc analysis of multiple comparisons using a Dunn's test with Bonferroni adjustment. Finally, Pearson correlation coefficients were used to explore relationships between metadata and key taxa. More details on these analyses can be found in the Supplementary Material.

Results

Study participants and metadata collection

We determined the relationships of stress, estrogen, and vaginal community composition in 21 black women who averaged 14.6 ± .3 years of age at the time of enrollment (Table S2). The mean vaginal pH and Nugent score across all individuals were 5.2 ± .9 and 1.3 ± 2.5, respectively. This vaginal pH is higher than that reported for older reproductive age women. Vaginal glycogen levels across all samples showed substantial variability (mean glycogen 324.7 ± 399.1 μg/mL). Mean salivary estradiol across samples was 7.3 ± 4.5 pg/mL. Finally, across all measurements, the mean PHQ score was 5.9 ± 4.8 (range 0–25), the mean BSI score was .5 ± .7 (range 0–3.7), and the mean PSS score was 18.5 ± 6.5 (range 7–38). These scores indicate that on average, the women of this cohort experienced mild to moderate depression, low anxiety, and moderate to high stress.

Vaginal bacterial community composition

To characterize the composition of vaginal communities, we sequenced the V1–V3 regions of 16S rRNA genes. Most participants (81%; 17 of 21) had communities that were dominated by *Lactobacillus*. Figure 1 shows a heat map of relative abundance data for the 20 most abundant taxa. The dendrogram in Figure 1 was used to identify nine clusters of communities that differed in composition. We observed that we could further group communities (i.e., combine clusters) based on the abundances of *Lactobacillus* species. Seven of the nine clusters had more than 50% total *Lactobacillus*, leaving two clusters in which the relative abundances of lactobacilli were <50%. The use of a 50% threshold to define "dominated" and "not dominated" was adopted from Klatt et al. [31] who used the same criterion in a study of how the abundance of lactobacilli was positively correlated with the efficacy of tenofovir. Of the seven clusters that were dominated by *Lactobacillus*, four were dominated by a single species of *Lactobacillus*, and the remaining three had mixtures of *Lactobacillus* species. These became groups A and B, respectively. All clusters that had communities with low proportions of *Lactobacillus* were aggregated into group C. In addition to low proportions of lactobacilli, these communities had higher proportions of *G vaginalis* and mixtures of other bacteria such as *Atopobium vaginalis*, *Corynebacterium* spp., *Prevotella* spp., *Peptoniphilus* spp., *Streptococcus* spp., and *Anaerococcus prevotii* (Figure 1). The mean relative abundances of key taxa in groups A–C are shown in Table S3.

Vaginal community composition is known to change over time in older, reproductive age women. To determine whether similar trends occur in young black women, we created bar plots representing vaginal bacterial community composition over time for each subject (Figure 2, Figures S1 and S2). Figure 2 shows plots for four subjects that illustrate the variability in community composition found within and between women of the cohort. Communities with high stability were seen in women of different groups. For example, Subject 35 (group A) maintained high proportions of *L crispatus* over time, whereas Subject 11 (group C) maintained 50% or more *G vaginalis* over time. In contrast, Subject 31 had high proportions of *G vaginalis* (group C) during the

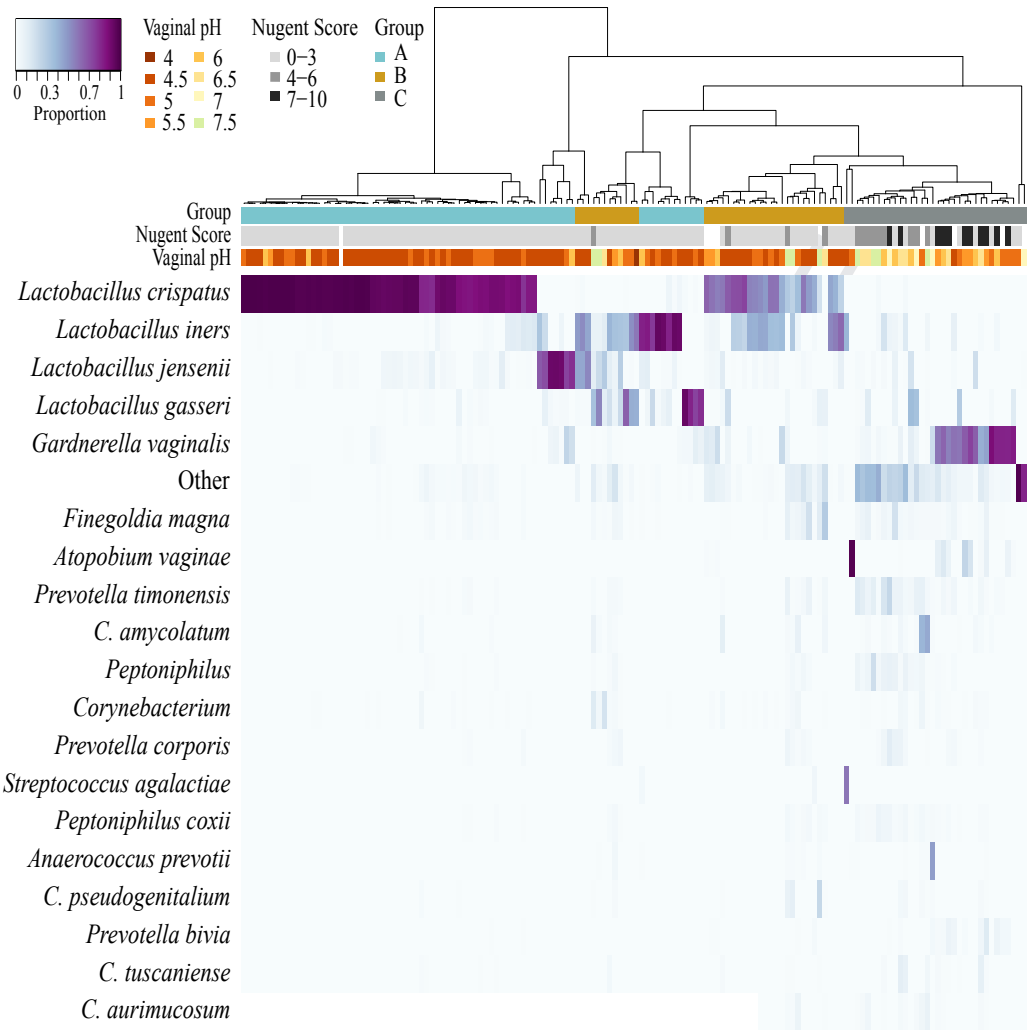

Figure 1. Heatmap based on the relative proportions of the 20 most abundant taxa in vaginal communities of black adolescent women. The columns of the heatmap include 146 samples collected from 21 young women over a 6-month period. The corresponding dendrogram represents complete-linkage hierarchical clustering of samples based on alt-Gower distances. The colored bar immediately below the dendrogram indicates which clusters were combined to form three groups (A, B, and C).

first 3 months, then transitioned once to a community dominated by *L. gasseri* (group A). Subject 24 started with a community dominated by *L. jensenii* (group A) and transitioned to different groups five times over the course of the study.

Differences between groups

We determined if levels of estradiol, glycogen, vaginal pH, and Nugent score differed among groups A, B, and C. Vaginal pH and Nugent score were included in the analysis because they are well-accepted correlates of vaginal community composition. We fit linear mixed effects models accounting for variation due to subject and performed nonparametric analyses where appropriate. The distributions of log-transformed estradiol and glycogen levels for each group are depicted by the boxplots in Figure 3A,B, respectively. There were no significant differences in estradiol levels among groups ($\chi^2 = 4.4$, $p = .1$). Estradiol levels are known to vary over the menstrual cycle; therefore, we compared estradiol measurements between menstrual cycle phase to test our ability to detect differences in estradiol levels (Figure S3). Mean estradiol concentrations were lowest in follicular samples and highest in periovulatory samples; these differences were not significant ($\chi^2 = 5.1$, $p = .08$). Although we were unable to discriminate between estradiol levels, we did find that vaginal glycogen differed significantly between groups A and C ($z = -4.1$, $p < .001$).

Figure 3C,D show the distribution of Nugent scores and log-transformed vaginal pH for each group. As expected, group C had significantly higher Nugent scores and vaginal pH than did samples in groups A (Nugent: $z = -7.6$, pH: $z = 6.5$; $p < .001$ for both) and B (Nugent: $z = -5.0$, pH: $z = 3.8$, $p < .001$ for both). Moreover, groups A and B, both marked by high proportions of *Lactobacillus*, had similar Nugent scores yet differed significantly in vaginal pH (Nugent: $z = -1.9$, $p = .2$; pH: $z = 2.9$; $p = .01$).

To gain insight to whether measurements of psychosocial factors differed between groups, we used linear mixed-effects models, incorporating variation due to subject when necessary, to test for differences in the mean values of perceived stress, anxiety, and depression. Summary statistics for the models are shown in Table 1. No significant differences in perceived stress

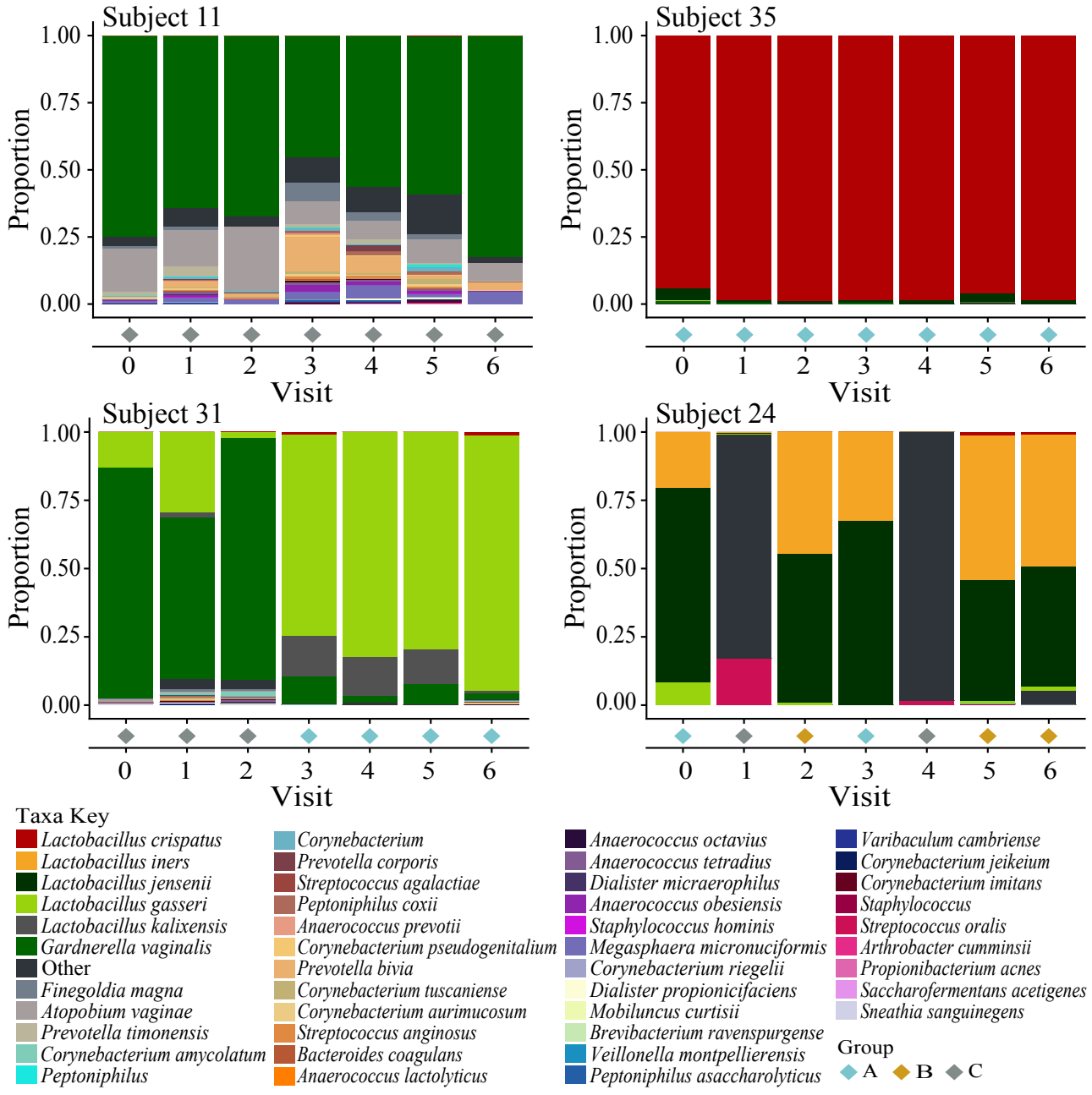

Figure 2. Examples of changes in community composition over time. The stacked bar charts represent proportions of bacteria in each community over seven monthly visits where zero was the baseline visit. Colors for each taxon are shown in the legend below the figure. The community group for each visit is highlighted by a colored diamond below each chart, and the corresponding legend is listed in the bottom right. The profiles of these subjects were chosen to illustrate the temporal variability in community composition within subjects.

($F = .04$, $p = 1$), anxiety ($\chi^2 = .7$, $p = .7$), or depression ($\chi^2 = 3.2$, $p = .2$) were identified.

Correlations between estradiol, glycogen, stress, and key taxa

We investigated the relationships between estradiol, glycogen, stress, and key taxa in the vaginal community by plotting Pearson correlation coefficients (Figure S4). As expected, vaginal pH and Nugent score were positively correlated ($r = .63$), and both pH and Nugent score were negatively correlated with glycogen ($r = -.39$ and $-.34$, respectively; Figure S4, panel A). Vaginal glycogen was positively correlated with *L. crispatus* ($r = .17$) and *L. jensenii* ($r = .26$; Figure S4, panel B). Estradiol was positively correlated with *L. iners* ($r = .37$) but no other

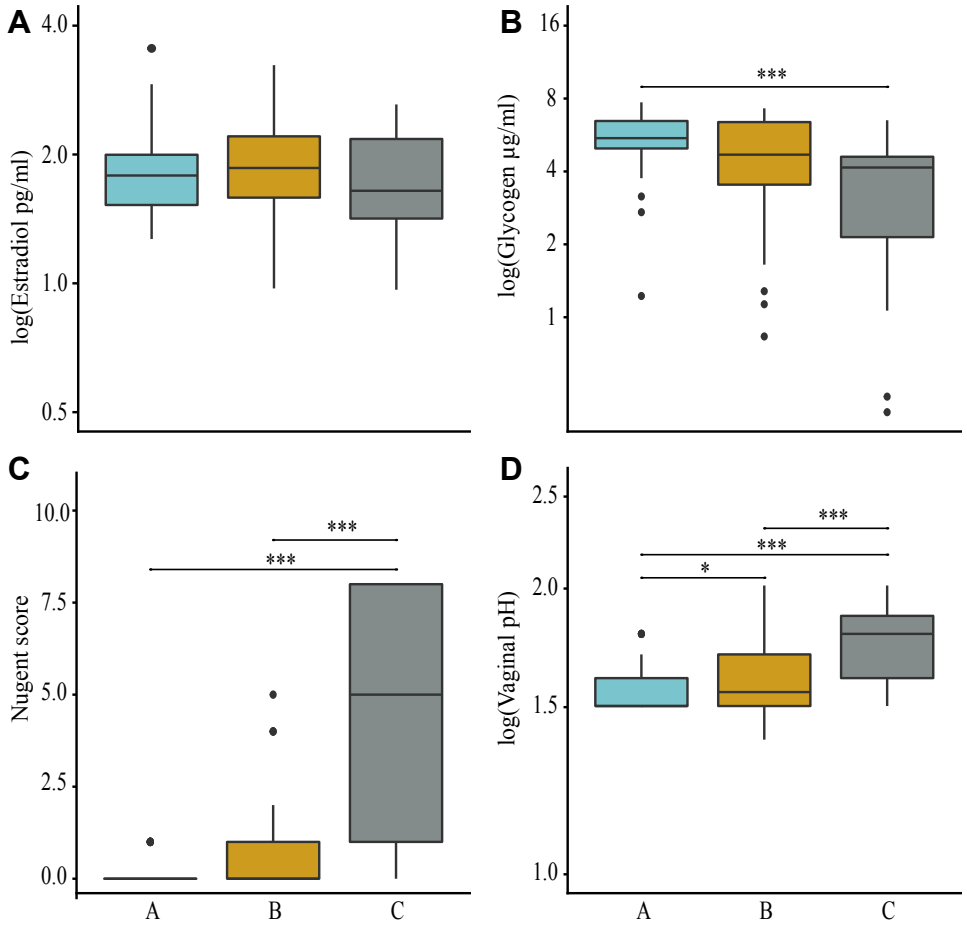

Figure 3. Differences in estradiol, glycogen, Nugent score, and vaginal pH measurements among groups. The boxplots represent log-transformed estradiol (panel A), log-transformed glycogen (panel B), Nugent scores (panel C), and log-transformed vaginal pH (panel D) for samples in groups A, B, and C. Statistical significance ($^{*}p < .05$, $^{***}p < .001$) is indicated above the bars.

Lactobacillus species (Figure S4, panel B). Furthermore, we did not observe a statistically significant correlation between levels of estradiol and glycogen.

To further evaluate the relationship between estradiol and glycogen, we fit a linear mixed-effects model incorporating variation due to subject. Figure 4 shows a scatterplot of log estradiol versus glycogen content in all samples. Our model suggests a positive relationship between estradiol and glycogen ($m = 1.4 \times 10^{-4}$); however, in agreement with the correlation plots, this relationship was not statistically significant ($p = .2$; Figure 4).

Table 1
Stress, anxiety, and depression in community groups A, B, and C

Variable	Measure	Group						Statistic[a]	*p* value[b]
		A		B		C			
		N	Mean (SD)	N	Mean (SD)	N	Mean (SD)		
PHQ score[c]	Depression	29	2.4 (.2)	19	2.0 (.3)	15	1.9 (.3)	$\chi^2 = 3.2$	.2
BSI score[d]	Anxiety	29	.5 (.1)	19	.5 (.1)	15	.6 (.2)	$\chi^2 = .7$	.7
PSS score[e]	Stress	29	4.3 (.1)	19	4.2 (.2)	15	4.2 (.2)	$F = .04$	1

[a] The test statistic listed results from the linear mixed-effects models conducted for PHQ and BSI, and the linear model conducted for PSS to test differences in their means between groups A, B, and C. The means and standard error (SE) that are reported above result from the modeled means (betas) and SE. Variation due to subject was incorporated where appropriate. Type II Wald chi-square tests were calculated for PHQ and BSI, and the F-test was calculated for PSS.

[b] The *p* value listed in this table is associated with the test statistic to the left.

[c] PHQ (Personal Health Questionnaire 9) is a self-report questionnaire consisting of 10 questions (nine asking about specific symptoms, and the final asking how impactful those symptoms are to assess the severity of depression).

[d] BSI (Brief Symptom Inventory)—the anxiety subscale used here—is a self-report questionnaire consisting of six questions designed to clinically assess the level of anxiety in individuals.

[e] PSS (Perceived Stress Scale) is a self-report questionnaire used to evaluate the degree to which particular situations in one's life are deemed stressful.

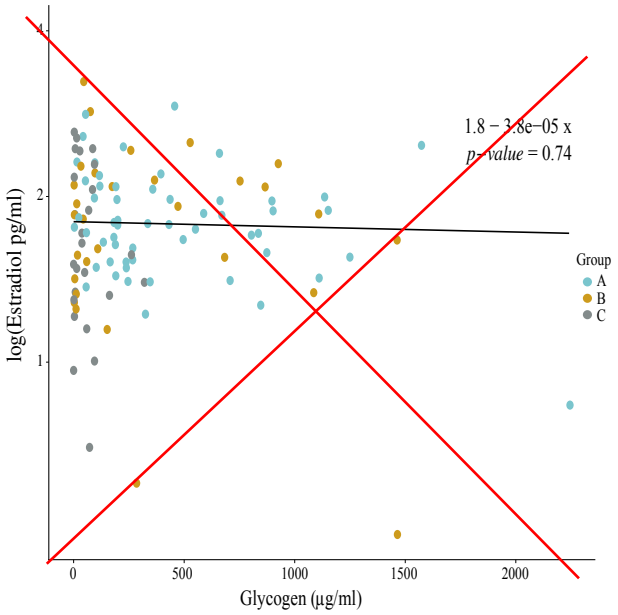

Figure 4. The relationship between log-transformed estradiol and glycogen concentrations in black adolescent women sampled longitudinally. The amounts of salivary estradiol in each sample were log-transformed and modeled over corresponding glycogen measurements including subject as a random effect. The resulting linear model equation and *p* value (shown in the upper right corner) were obtained by computing an analysis of variance on the linear model. Each dot represents the log estrogen concentration and the corresponding glycogen value for a given sample. Dots are colored according to community group as shown in the legend to the right of the graph.

Discussion

We evaluated the relationships between mental health (perceived stress, depression, and anxiety), salivary estradiol, vaginal glycogen, and vaginal community composition in a small cohort of 14-year-old black women. We showed that most of the cohort (81%) had communities that were dominated by species of *Lactobacillus*. The kind and abundances of *Lactobacillus* species served as a basis for the classification of communities into three groups: A, B, and C. Consistent with reports in older reproductive age women [11], some women had stable vaginal communities, whereas others transitioned from one community group to another. Importantly, differences in groups were marked by differences in vaginal glycogen levels but not salivary estradiol or any of the mental health measures.

Few studies have used culture-independent methods to characterize vaginal community composition in adolescent women. A cross-sectional study found young women, aged 13–18 years, to have either vaginal communities that were dominated by *L. iners* or *L. crispatus* or contained a mixture of *L. crispatus*, *L. jensenii*, and *L. gasseri* or were heterogeneous in composition with low proportions of *Lactobacillus* [7]. Another study showed *Lactobacillus* species to be prominent members in the adolescent vaginal microbiome early in puberty, even before menarche [8]. The high prevalence of *Lactobacillus* in our cohort of young black women (81%) is in agreement with previous studies but is higher than that reported for older black women (61.9%) [14]. As noted previously, reproductive age black women are less likely than white and Asian women to have *Lactobacillus* dominant communities [11,14] and more likely to have communities that have been associated with BV [32]. The fact that more than three quarters of this cohort of young black women had mostly *Lactobacillus* in their communities suggests there could be an age-associated transitional period in these vaginal communities. To understand this, we need more extensive longitudinal studies that evaluate the normal development of the vaginal microbiome in black women over time. This will broaden our understanding of what is healthy and what leads to the health disparities observed later in the lives of black women. In future studies, the variability seen within ethnic groups might be lessened if host genetics were used to classify individuals instead of self-reported ethnicity.

Mental health such as chronic psychosocial stress has been associated with recurrent vulvovaginal candidiasis [33] and increased odds of vaginal conditions such as BV [34]. The correlation between chronic stress and BV has been demonstrated to be even more prominent in pregnant women [18]. Culhane et al. [18] found that pregnant women who experienced high stress were 2.2 times more likely to have BV than those who experienced low stress. These associations between stress and BV likely result from stress-induced reduction in proteins involved in immune homeostasis that is associated with a decrease in the abundance of vaginal lactobacilli [35]. Although we sampled vaginal microbial communities that varied in terms of the abundance of *Lactobacillus* and other species, we did not observe significant differences in psychosocial stress, depression, or anxiety among the women studied. The sample size of our pilot study could have contributed to our inability to detect differences in stress, and this is the most likely explanation for the discrepancies between the findings of our study and others [18,34]. However, it is also plausible that differences in the cohorts that were sampled in addition to variation in the perception of stress among cohorts of women could have played a role. Future research to evaluate the influence of psychosocial stress on vaginal community composition might include measuring a common biomarker of stress such as cortisol in addition to self-reported measures.

The positive association between estrogen, glycogen, and *Lactobacillus* over a woman's lifespan [11] has led to an assumption that there is a simple linear relationship between estrogen and the levels of glycogen in the vagina. Our results suggest otherwise and are in general agreement with those of Mirmonsef et al. [36] who sampled older reproductive-age women over time and found no relationship between estrogen levels and vaginal glycogen. In an effort to understand this, one might assume that the rate of glycogen production (and release from cells) is counterbalanced by the rate of glycogen metabolism by members of the vaginal community. The resulting pseudo-steady state could result in some relatively constant level of glycogen in vaginal secretions in instances where the rate of glycogen production exceeds the rate of consumption. In contrast, the steady-state concentration of glycogen would be near zero if the rate of glycogen consumption is equal to or greater than the rate of glycogen production. This reasoning could also be extended to explain how high levels of glycogen can persist in low estrogen environments [37]. The relationship between the levels of estrogen and glycogen in the vagina might be further complicated by the fact that both the rates of glycogen production by the host and the rates of glycogen consumption by vaginal bacteria probably vary among women and over time within a woman. A second scenario emerges from the results of a study done by Pessina et al. [38] to evaluate the effects of

steroidal hormones on the structure of the vaginal tissues of rats, which showed that sub-physiological levels of estradiol thickened the vaginal epithelium more than physiological doses. Given this, it could well be that a positive linear relationship between estradiol and glycogen may only exist only at low concentrations of estradiol. As the estradiol level increases, its effect on glycogen production could be diminished or perhaps even saturated. The result would be a nonlinear relationship between estradiol levels and glycogen production in which glycogen is mainly influenced by the number of glycogen-producing cells in the thickened vaginal epithelium.

In this study, we sampled a relatively small number of individuals (21) infrequently over time, and this limited our ability to resolve temporal changes in the vaginal microbiome and potential correlates of change (e.g., estradiol and stress) or to assess the effects of potentially confounding factors such as sexual activity or methods of birth control. In addition, the sampling regimen precluded knowing the ovulatory status of subjects in a menstrual cycle. Because adolescents have a high frequency of anovulatory cycles [39], this might impact the vaginal environment in ways not commonly observed in adult women and confounded efforts to demonstrate an association between estradiol and microbiome composition. Although these limitations exist, this is the first study done to evaluate estradiol, glycogen, and stress as potential drivers of vaginal community composition in black adolescent women. Our results support previous findings that vaginal glycogen content is positively correlated with *Lactobacillus* dominance in vaginal secretions from older reproductive age women [40] and confirm that there is no simple relationship between levels of estradiol and the production of vaginal glycogen.

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Authors' contributions:

J.D.F. and L.J.F. designed the research and provided feedback on data analysis and interpretation. E.M.C. and V.J.V. both helped with data collection and analysis. B.J.R. helped with statistical data analysis and interpretation. K.L.N. performed data collection, data analysis, and interpretation and wrote the article. All authors contributed to editing the article before submission.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jadohealth.2019.01.010.

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