Exploring Associations Between Stress and the Vaginal Microbiome in Healthy Young Females

Alice Zhang, BA[1], Jyontika Kapoor, BA[1], Christen Deveney, PhD[2], Adela G. Cope, MD[5,6], Marina R.S. Walther-Antonio, PhD[5,6,7], Nicholas Chia, PhD[7,8], Cassandra W. Pattanayak, PhD[3], Marc J. Tetel, PhD[4]
[1]Data Science Program, [2]Psychology Department, [3]Quantitative Analysis Institute, [4]Neuroscience Department, Wellesley College, Wellesley, MA, USA;
[5]Department of Obstetrics and Gynecology, [6]Department of Surgery, [7]Microbiome Program, Center for Individualized Medicine, Mayo Clinic, Rochester, MN 55905; [8]Argonne National Lab, Lemont, IL, USA.

BACKGROUND

The human body hosts trillions of microorganisms known as the human microbiome. These microbes inhabit various body sites (*e.g.,* gut, skin, mouth) and are critical in human health and disease (1,2). Disruptions in the gut microbiome have been associated with many diseases, including diabetes, obesity, and cancers (2). Additionally, the gut-brain axis is implicated in mental health and neurological disorders, including anxiety, depression, and Alzheimer's disease (1, 3-8). While the vaginal microbiome is essential for maintaining vaginal health, imbalances in its composition have been linked to diseases such as endometrial and ovarian cancers (9-11). While the gut microbiome exhibits high microbial diversity, the vaginal microbiome is primarily characterized by the dominance of the *Lactobacillus* genus (9,10). Prior work from our group has shown that vaginal microbiota are related to factors such as the menstrual cycle, birth control, and diet (12). Compared to the gut, the vaginal microbiome is understudied and lacks the longitudinal, high-resolution studies needed to understand its temporal dynamics and stability (2). **The present longitudinal study seeks to understand how lifestyle variables and stress relate to the composition of the vaginal microbiome over 10 weeks.**

METHODS

Data were collected over a 10-week period on the Wellesley College campus. Students (n=78) aged 18 and older with a vagina, who submitted at least one type of data, were included in the study analyzing the relationship between hormonal factors (e.g., menstruation, hormonal birth control), stress, diet, activity, and the gut and vaginal microbiomes (Fig. 1).

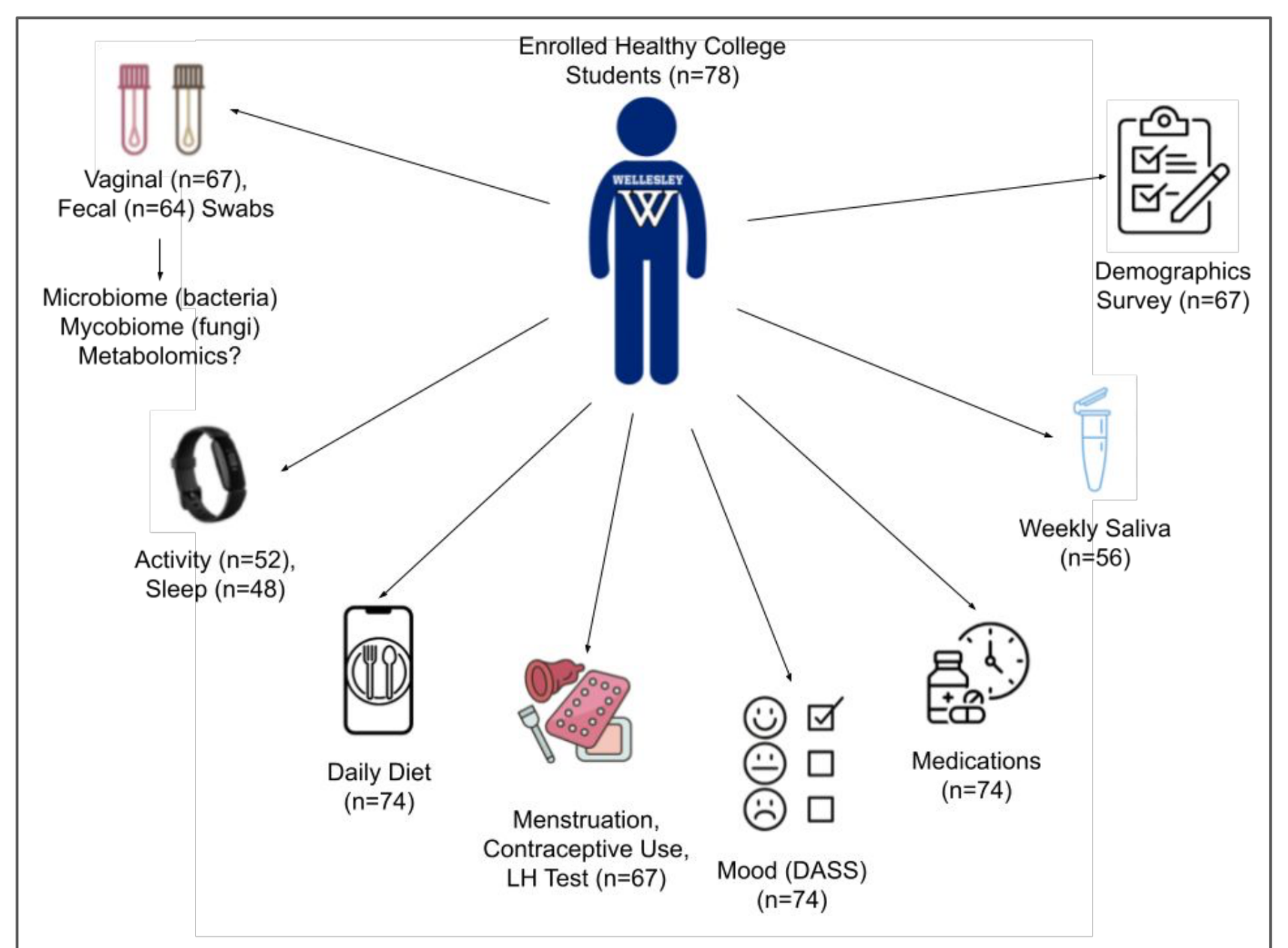

Figure 1. Samples and data provided by participants. Microbial and fungal samples, diet, activity, and menstruation data were collected daily, while mood data and saliva samples (for cortisol assays) were collected weekly. Anal and vaginal swab samples were analyzed by 16S rRNA sequencing (Univ. Minnesota Genomics Center).

Linear regression was used to analyze aggregate outcomes by treating each participant as a single data point, with values averaged across time. Separately, linear random intercept, fixed slope mixed effects models were used to analyze Shannon diversity, genus-level abundance, and individual lifestyle variables at the sample level, accounting for repeated measures over time within individuals. Quadratic terms were included to model nonlinear patterns of microbial diversity over time.

	Did Not Menstruate	Menstruate	Total
No Birth Control	2	31	33
Local P	4	5	9
Systemic P	3	5	8
Systemic E&P	3	13	16
Total	12	54	66

Table 1. Participant menstruation status (menstruated ≥1 day vs. no menstruation during the study period) and type of hormonal birth control used throughout study (E=estrogens; P=progestins).

Hormonal birth control was categorized into four types: 1) none, 2) progestin-only local release contraceptives (local P), 3) progestin-only systemic release contraceptives (systemic P), and 4) estrogens and progestins combined systemic release contraceptives (systemic combined (E&P)). Participants were classified by whether or not they menstruated (Table 1).

RESULTS

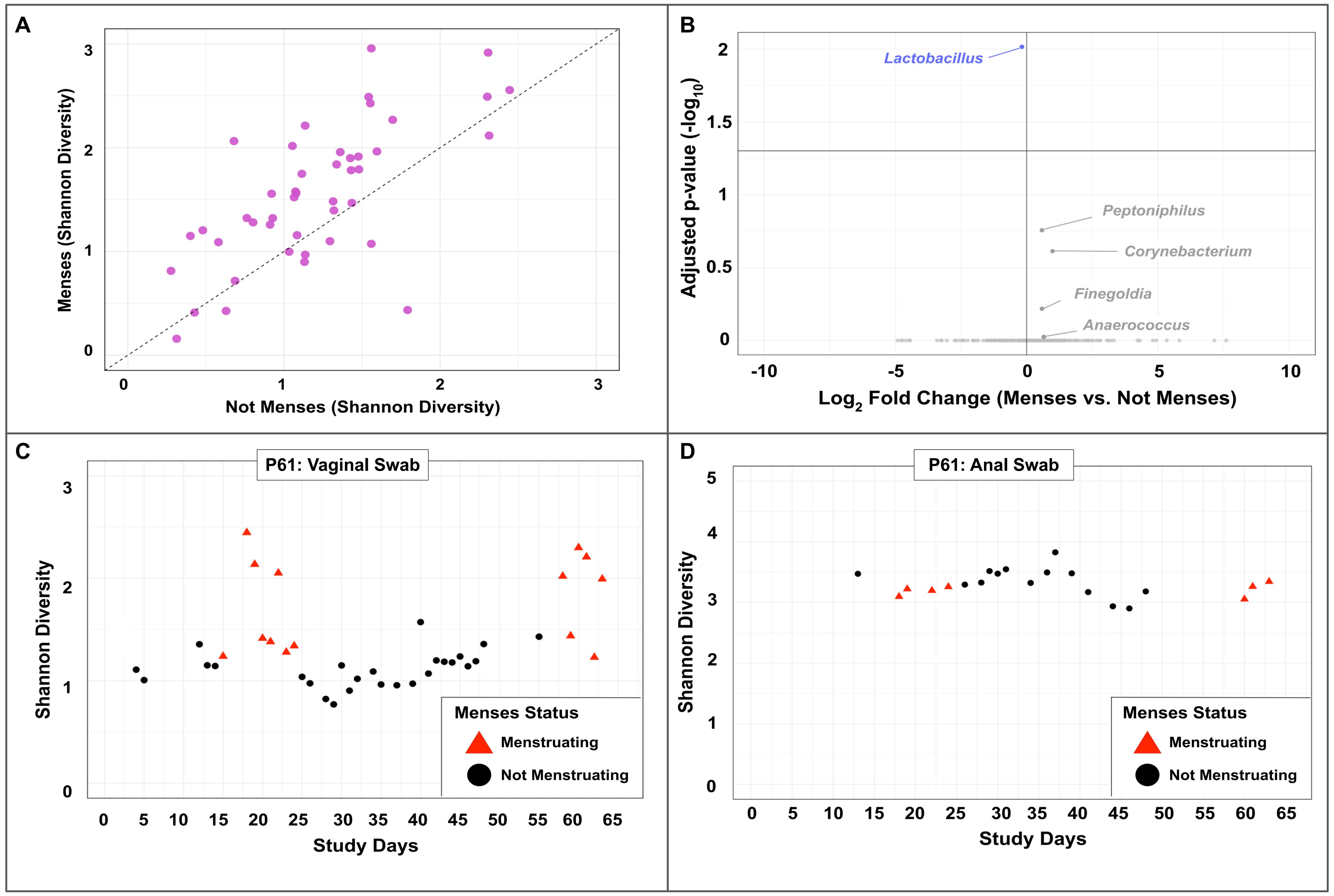

Figure 2. Menstruation is associated with instability in the vaginal microbiome. The analysis included participants who provided data for a at least one day during menstruation and one day outside of menstruation. (A) Average Shannon diversity increased during menstruation ($p < 0.0001$). (B) *Lactobacillus* was significantly decreased during menses. Log fold change is shown, using non-menstruating abundances as a reference; negative values indicate a decrease in abundance, with greater magnitude reflecting larger decreases. Patterns over time in vaginal microbiome Shannon diversity in relation to menstruation varied by individual; (C) focuses on an individual (participant 61) with a significant increase in vaginal Shannon diversity during menstruation. (D) Gut Shannon diversity for participant 61 is shown.

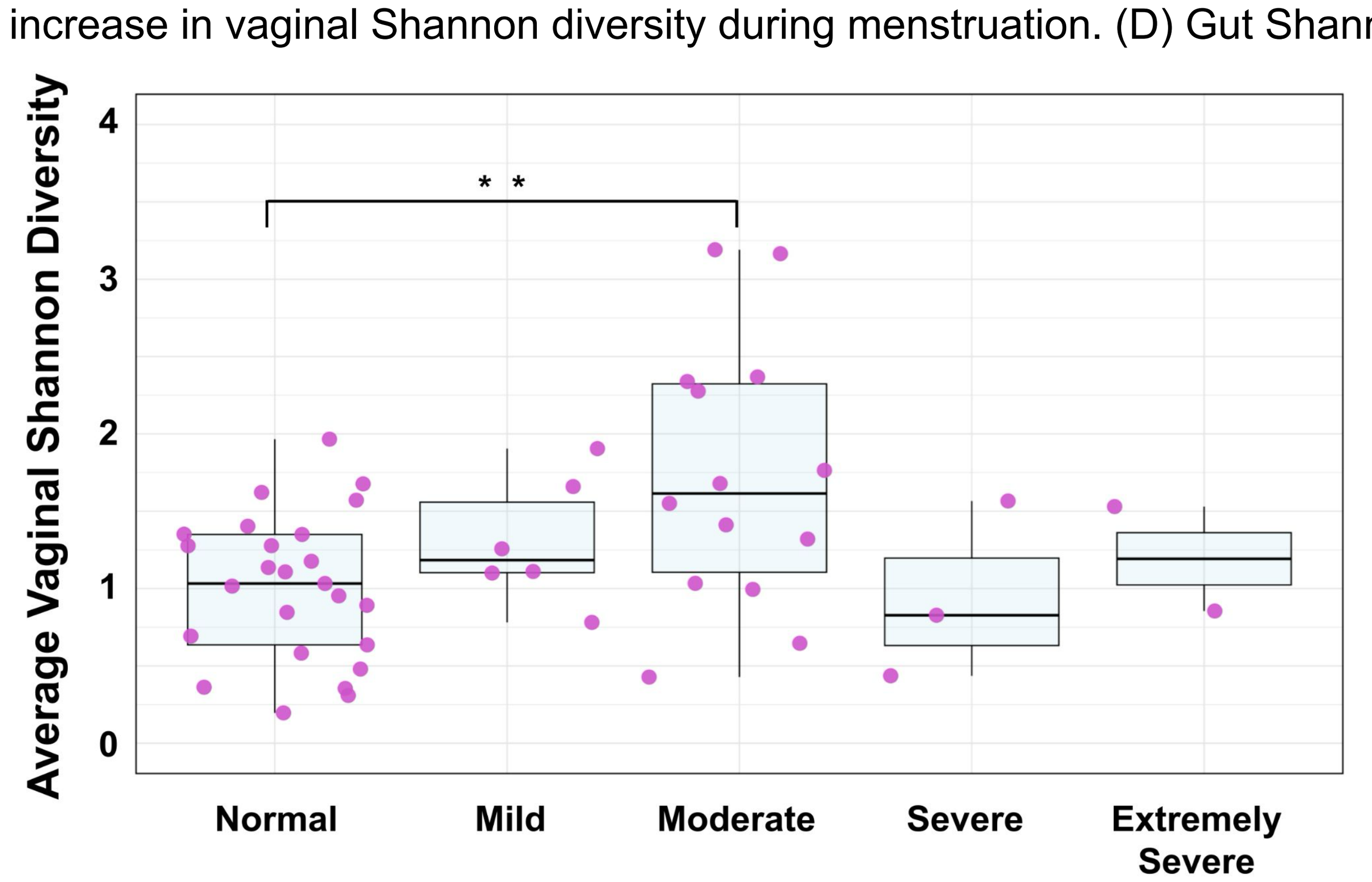

Figure 3. Stress scores and Shannon diversity per participant across the 10-week study were averaged; participants reporting moderate stress had significantly higher vaginal Shannon diversity compared to those with normal stress levels ($p = 0.00817$). Participants (n = 61) who submitted at least one complete DASS stress survey and a corresponding vaginal sample were included.

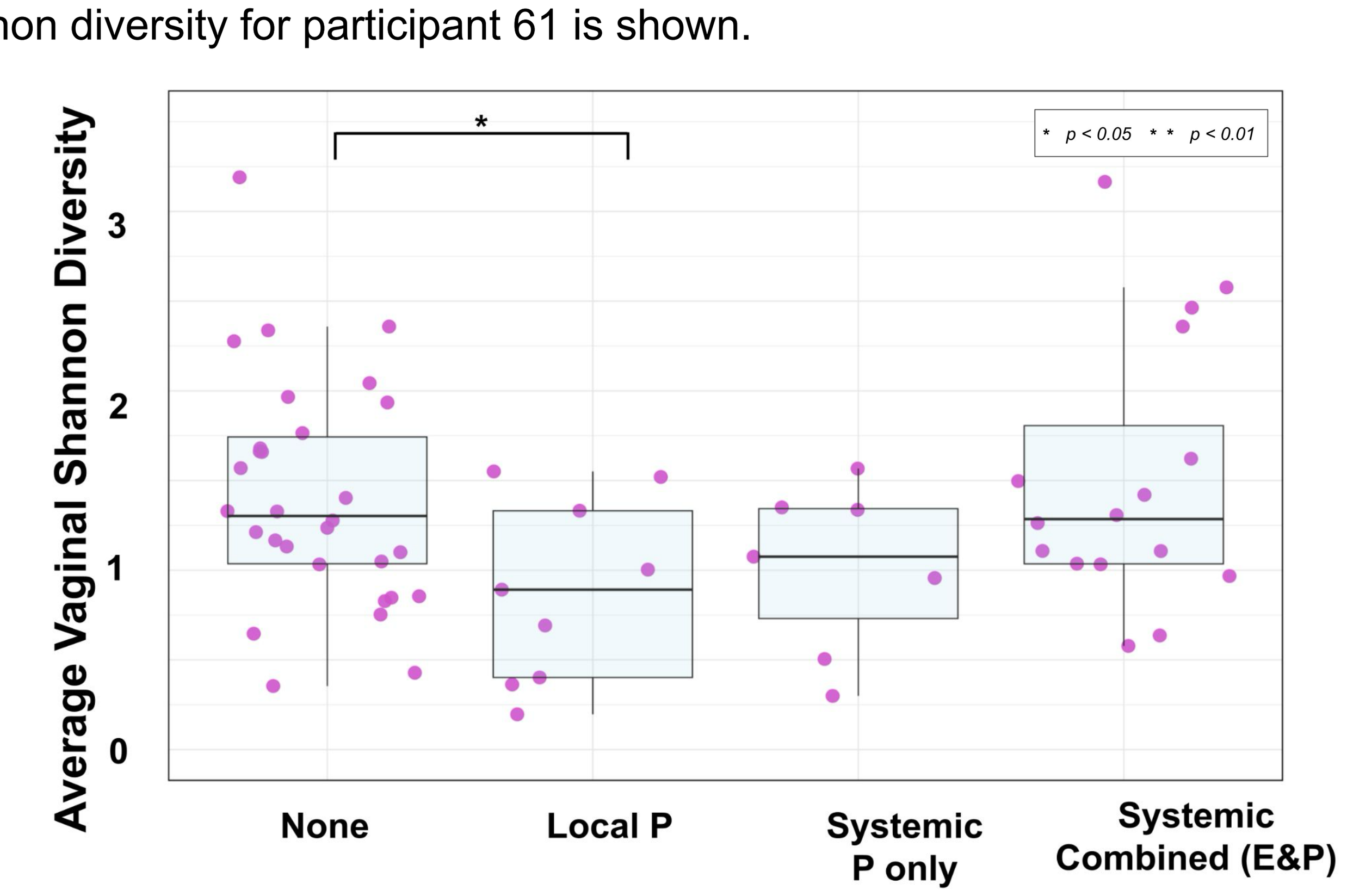

Figure 4. Participants not on birth control had higher vaginal microbial diversity compared to participants using hormonal birth control. On average over 10 weeks, participants not using hormonal birth control had a higher Shannon index than those using progestin-only local release birth control ($p = 0.0319$).

CONCLUSIONS & LIMITATIONS

Conclusions

- Shannon diversity in the vaginal microbiome fluctuated over time, with individuals exhibiting distinct temporal patterns; in contrast, diversity in the gut microbiome remained relatively stable.
- Higher average Shannon diversity in the vaginal microbiome was associated with menstruation and moderate stress.
- Lower average Shannon diversity in the vaginal microbiome was associated with progestin-based, local-release hormonal birth control, and no birth control.

Limitations

- Missing data: the extent of missing data varied substantially by participant.
- Sample size: lack of participants at the more extreme stress levels.

DISCUSSION

- Menstruation was associated with a significant increase in vaginal Shannon diversity, likely due to a temporary reduction in *Lactobacillus* dominance. This supports our prior findings, and those of others, that menstruation disrupts microbial stability, suggesting that hormonal or physiological changes may drive shifts in vaginal composition.
- Moderate stress was associated with significantly higher average vaginal Shannon diversity compared to normal stress, suggesting a potential link between psychological stress and vaginal microbial composition. This pattern was not observed in higher stress categories, likely due to limited sample size.
- Progestin-only birth control methods were associated with lower vaginal Shannon diversity. Participants using local progestin methods had the lowest average diversity, indicating a stronger link between localized hormone delivery and vaginal microbial composition.
- Ongoing analyses include species-level analyses, mycobiome sequence data, and the interaction between the vaginal and gut microbiomes.

REFERENCES

[1] Cho, I. & Blaser, M. J. The human microbiome: At the interface of health and disease. Nat. Rev. Genet. 13, 260–270. https://doi. org/10.1038/nrg3182 (2012).
[2] Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C., Knight, R., & Gordon, J. I. (2007). The human microbiome project: Exploring the microbial part of ourselves in a changing world. Nature, 449(7164), 804–810. https://doi.org/10.1038/nature06244
[3] Cenit, M. C., Sanz, Y., & Codoñer-Franch, P. (2017). Influence of gut microbiota on neuropsychiatric disorders. World Journal of Gastroenterology, 23(30), 5486–5498. https://doi.org/10.3748/wjg.v23.i30.5486
[4] DeGruttola, A. K., Low, D., Mizoguchi, A., & Mizoguchi, E. (2016). Current understanding of dysbiosis in disease in human and animal models. Inflammatory Bowel Diseases, 22(5), 1137–1150. https://doi.org/10.1097/MIB.0000000000000750
[5] de Vos, W. M., Tilg, H., Van Hul, M., & Cani, P. D. (2022). Gut microbiome and health: Mechanistic insights. Gut, 71(5), 1020–1032. https://doi.org/10.1136/gutjnl-2021-326789
[6] Dinan, T. G., & Cryan, J. F. (2017). The Microbiome-Gut-Brain Axis in Health and Disease. Gastroenterology clinics of North America, 46(1), 77–89. https://doi.org/10.1016/j.gtc.2016.09.007
[7] Hou, K., Wu, Z.-X., Chen, X.-Y., Wang, J.-Q., Zhang, D., Xiao, C., Zhu, D., Koya, J. B., Wei, L., Li, J., & Chen, Z.-S. (2022). Microbiota in health and diseases. Signal Transduction and Targeted Therapy, 7(1), 135. https://doi.org/10.1038/s41392-022-00974-4
[8] Tetel, M. J., de Vries, G. J., Melcangi, R. C., Panzica, G., & O'Mahony, S. M. (2018). Steroids, stress and the gut microbiome-brain axis. Journal of neuroendocrinology, 30(2), 10.1111/jne.12548. https://doi.org/10.1111/jne.12548
[9] France, M., Alizadeh, M., Brown, S., Ma, B., & Ravel, J. (2022). Towards a deeper understanding of the vaginal microbiota. Nature microbiology, 7(3), 367–378. https://doi.org/10.1038/s41564-022-01083-2
[10] Graham, M. E., Herbert, W. G., Song, S. D., Raman, H. N., Zhu, J. E., Gonzalez, P. E., Walther-António, M. R. S., & Tetel, M. J. (2021). Gut and vaginal microbiomes on steroids: implications for women's health. Trends in endocrinology and metabolism: TEM, 32(8), 554–565. https://doi.org/10.1016/j.tem.2021.04.014
[11] Walther-António, M. R., Chen, J., Multinu, F., Hokenstad, A., Distad, T. J., Cheek, E. H., Keeney, G. L., Creedon, D. J., Nelson, H., Mariani, A., & Chia, N. (2016). Potential contribution of the uterine microbiome in the development of endometrial cancer. Genome medicine, 8(1), 122. https://doi.org/10.1186/s13073-016-0368-y
[12] Song, S. D., Acharya, K. D., Zhu, J. E., Deveney, C. M., Walther-Antonio, M. R. S., Tetel, M. J., & Chia, N. (2020). Daily Vaginal Microbiota Fluctuations Associated with Natural Hormonal Cycle, Contraceptives, Diet, and Exercise. mSphere, 5(4), e00593-20. https://doi.org/10.1128/mSphere.00593-20

ACKNOWLEDGEMENTS

We thank Charlie Gao, Samantha Finn, and the rest of the Wellesley College LTS staff for developing and maintaining the app used for data collection in this study. We would also like to thank Wellesley College and the Mayo Clinic for funding this project.