BIOCULTURAL CONSIDERATIONS OF SEX, GENDER, AND EMBODIMENT

BY

MEREDITH WILSON

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Anthropology
in the Graduate College of the
University of Illinois Urbana-Champaign, 2023

Urbana, Illinois

Doctoral Committee:

Professor Kathryn Clancy, Co-Chair
Professor Jenny Davis, Co-Chair
Professor Ripan Malhi
Professor Toby Beauchamp

ABSTRACT

This dissertation challenges the typical analytical framework of biological anthropology, particularly in the context of continued binarizing of sex and gender despite ample biological evidence to contest these categories, in broadly asking *how is the particular identity and experience of gender embodied?* The dissertation is split into two interconnected parts. The first will include a literature review and textual analysis of biological anthropology's current discourse around gender and sex (chapter 1). I identify 3 main ways by which biological anthropology research is re/producing sex gender binaries and cis- and heteronormativity. Next, Chapter 2 provides a discussion on how systems of harm, like white supremacy and patriarchy, are recreated not just in our current research discourse but have been historically maintained and reproduced even as dominant scientific paradigms shift. In this case, I examine how both deterministic and plasticity-based research on sex and gender maintain violent systems.

Part 2 consists of quantitative analyses exploring variation of two common biomarkers used in studies of embodiment, C-reactive protein (CRP) and cortisol in a Polish and Polish American sample. For CRP I analyzed potential menstrual cycle effects and compared different samples phenotypes. I found that the Polish and Polish American samples had distinct menstrual cycle CRP phenotypes. The Polish sample did not show any cycle effects. In the Polish American sample, post menses had a negative effect on CRP (estimate -.17, t-value -5.2), and there were increased CRP concentrations during the early follicular phase (median .406, $p<.05$), specifically the first three days of menstruation (median .466, $p<.01$). For cortisol, I examined the possible within sample variation and cortisol's potential relationship with estrogen and progesterone. I found an average cortisol cycle phenotype which varies through the menstrual cycle. However, this obscures within sample variation. I found 3 distinct cortisol phenotypes

($p<.05$). Progesterone cycle shape was correlated with cortisol cycle shape ($r=.64$, $p<.05$) and the cortisol group with the most consistent (e.g., invariable through the cycle) pattern had higher PdG exposure compared to the other groups ($p<.01$).

These analyses were conducted with the purpose of better understanding and incorporating biological variation so that these biomarkers can be used towards a more inclusive research design. Additionally, Chapters 3 and 4 use statistical analysis that do not try to find quantitative difference between groups decided *a priori* or to define a universal norm but instead examine within and between population variation to show that even bodies we assume are homogenous are incredibly diverse and varied. With these analyses, this dissertation on gender and biological embodiment aims to actively combat the racist, patriarchal, heteronormative, and cisnormative harm inherent in traditional scientific methodology.

ACKNOWLEDGMENTS

First and foremost, thank you so much to my advisors, Dr. Kate Clancy and Dr. Jenny Davis. Kate, thank you so much for the in depth and careful feedback on my writing and for listening to me complain constantly about it (my writing, not your feedback!). Our meetings, conversations and work together have greatly shaped my work as a scholar, and I know that I will take what I've learned from you with me for wherever I may land next. Jenny, thank you so much for being an advocate for me in times when I was not able to be, for providing me with guidance and for having some incredibly inspiring and generative conversations with me about my research. This dissertation could not be possible without the both of you.

I would very much like to thank all my whole support system both near and far. My parents, Mike and Sandy, and siblings, Heather, Shannon, and Nathan, for listening to me and giving me advice when I needed it most. I'd like to particularly commend my siblings for always opening and mostly always responding to my late-night writing snaps. I'd also like to thank my virtual bookclub, Katie Cassel, Amanda McDonnall, Alix Guyot, Leslie Herrmann, Hannah Parker Carver and Sarah Stauffer. Without you all, I never would have made it through the pandemic, let alone finished a dissertation. My Dickinson crew, Liz Benz, Melynda Dalzon, JK Ekas, Tori Eberle, Kate Nelson, and Ioni Kokodis, thanks for putting up with my nonsense. Snake Sex you definitely deserve a shout out too. Tessa Cicak, Daniel Ehlrich, Dan Plekhov, Elic Wiesel, and Kaity Haj, thanks for giving me stats support, grad school advice, and collective complaining whenever any of us needed it. Finally, for all of my past and current friends that I have made here at Illinois, Dr. An-di Yim, Dr. Katie Lee, Natasha Mazumdar, Dr. Allie Zachwieja, Cameron Merrill, Dr. Karthik Yarlagadda, Maria Cox, Dr. Valerie Sgheiza, Sana

Saboowala, Grace Shaw, Rachel Rusen, Navashni Naidoo, Yifan Wang, Dan Matis, Josh Diaz, Vivian Cheng, and Abby Houkes, I owe you all so much for your support.

This research would not be possible without the generous funding from the following: NSF (Clancy #1317140); NSF BCS-1732117; NSF BCS-1650839; NSF GRFP DGE-1144245,Wenner-Gren foundation for Anthropological Research (084918 Rogers, 089812 Lee), Lewis and Clark Fund for Exploration and Field Research (2015), UIUC graduate college (Rogers travel funding 2015; Lee travel funding 2017), UIUC Department of Anthropology Summer Research Funding, Sigma Xi Grants-in-Aid of Research (Rogers Grant ID is G2016100191355504 for Polish American recruiting).

I would also like to thank all of my collaborators for your thoughtful feedback and time spent engaging with my work. Especially the members of Clancy Lab past and present, Dr. Katie Lee, Dr. Mary Rogers-LaVanne, Dr. Michelle Rodrigues, Maria Cox, Dr. Valerie Sgheiza, Bryana Rivera, Urooba Ahmed Fatima, and Dr. Elahe Ganji. I would also like to thank Karolina Mi/lkowska, Piotr Hutka, Kamila Parzonka, Szczepan Jakubowski, Katarzyna Szulc, Aleksandra Wojtarowicz, Aleksandra Starnawska, Monika Szlachta, Pan Doktor Leszek Pieni(a,)(*z)ek , Pani Po/lozna Emilia Bulanda, Zarin Sultana, Rachel Mitchell, Agata Orkisz, Anna Pawi(n')ska, Szczepan Jakubowski, Monika Kukla, Klaudia Dziewit, Kristina Allen, Ansley Jones, Kaitlin Kanthak, Hayley Ban, Sara Gay, Ohm Shukla, Juliana Georges, Priya Bhatt, Rachel Mitchell, Zarin Sultana, Vilimira Asenova, Fatima Godfrey, Kevin Zavala, and Denise Herrera, who all contributed to the quantitative data in some way, shape or form.

Finally, I'd also very much like to thank my participants, who's voices unfortunately were not able to make it into this dissertation, though the things I learned about gender (my own and more broadly speaking) through speaking with you all influenced these analyses in more

ways than I can count. What I learned about myself and from you all was nothing short of inspirational and I will hold every conversation and shared bit of ourselves closely.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION AND CIS AND HETERONORMATIVITY IN BIOLOGICAL ANTHROPOLOGY 1

CHAPTER 2: EMBODIMENT, PLASTICITY AND THE RE/PRODUCTION OF GENDER, SEX AND RACE IN HUMAN BIOLOGY 27

CHAPTER 3: CYCLE EFFECTS ARE NOT UNIVERSAL: A CASE STUDY OF C-REACTIVE PROTEIN CONCENTRATIONS IN RURAL POLISH AND POLISH AMERICAN SAMPLES 51

CHAPTER 4: CORTISOL PHENOTYPE VARIES WITHIN A HOMOGENOUS POPULATION OF MENSTRUATING INDIVIDUALS 77

CHAPTER 5: CONCLUSION 100

REFERENCES 105

APPENDIX A: IRB APPROVAL LETTER FOR CHAPTERS 3 AND 4 129

APPENDIX B: CHAPTER 1 TEXTUAL ANALYSIS RESULTS 130

APPENDIX C: CHAPTER 3 SUPPLEMENTAL MATERIALS 131

APPENDIX D: CHAPTER 3 R code 151

APPENDIX E: CHAPTER 4 SUPPLEMENTAL MATERIALS 152

APPENDIX F: CHAPTER 4 R code 162

CHAPTER 1: INTRODUCTION AND CIS AND HETERONORMATIVITY IN BIOLOGICAL ANTHROPOLOGY

Biological anthropologists have a responsibility to attend to social categories, like gender, that contribute to harmful hierarchies of oppression. Instead, we have a history of using our research to naturalize and reify this and other categories like race and sex. Even now, more aware of this past, we re/produce these categories and make them seem natural with the questions that we ask and the methods that we use. This dissertation challenges the typical analytical framework of biological anthropology, particularly in the context of continued binarizing of sex and gender despite ample biological evidence to contest these categories, in asking *how is the particular identity and experience of gender embodied?* I am a biocultural anthropologist by training, whose research interests have always lain in understanding how our bodies and environments are intertwined: my work asks, how does our sociocultural environment influence our biology, or, how do we embody our experiences? My interest in gender and biological embodiment are combined in this dissertation's overarching theme or driving question: how are the identity and experience of gender embodied?

In order to address this question with care, I will need to first begin with smaller questions. The question of gender and biological embodiment has the potential to incorporate many different projects, questions and methodologies. My dissertation on gender and biological embodiment aims to actively combat the racist, patriarchal, heteronormative, and cisnormative harm inherent in traditional scientific methodology. Because of this, the work that I do spans numerous disciplines and methodologies that at first glance may seem disconnected but are necessary to explore to better conduct my research. The dissertation is split into two

interconnected parts. The first will include a literature review and textual analysis of biological anthropology's current discourse around gender and sex (this chapter) and conclude with a discussion on how these systems of harm are recreated not just in our research discourse but have been historically maintained and reproduced even as dominant scientific paradigms shift (Chapter 2). Part 2 consists of quantitative analyses exploring variation of two common biomarkers used in studies of embodiment, C-reactive protein and cortisol. These analyses were conducted with the purpose of better understanding and incorporating biological variation so that these biomarkers can be used towards a more inclusive research design.

Additionally, while chapters 3 and 4 are written in a way that followed the strict scientific research paper format and used language common to quantitative analysis, for publishing purposes, I made subversive choices to position these chapters as pseudo case studies for how to conduct human biology research in ways that doesn't reproduce systems of harm. First, I omitted the words evolution, adaptation, reproduction, female, woman, sex, and gender from these chapters. Additionally, I clearly defined and described the variation I was interested in exploring, i.e., variation from the hypothalamic-pituitary-ovarian axis and the menstrual cycle. I did not engage with or make any assumptions about *a priori* sorting of individuals within my populations except for population difference in chapter 3 (see chapter 3 for more information). I utilized statistical methods that are not about "proving" difference of distributions between groups or showing a group is more different from the norm than the other. Nor did I try to identify a universal norm but instead examine within and between population variation to show that even bodies we assume are homogenous are incredibly diverse and varied. Finally, I also maintained gender inclusive language throughout the chapters. While subtle and done in a way

that may not be obvious at first glance, I have attempted to not re/produce cis and heteronormativity in these ways.

Sex, gender and race in Western Science

To begin, I will briefly discuss the concepts central to my dissertation, specifically, the ways in which gender, sex and race are re/produced more broadly through science and popular understandings of science (see Chapter 2 for a more expanded discussion). Next, I will give a detailed analysis of the current biological anthropology discourse as it relates to the reproduction of cis- and hetero-normativity. Science is not way of knowing free from bias but rather another method of biopower used to categorize and control bodies within and without the state (Gill-Peterson, 2018; Schuller, 2018, Schuller and Gill-Peterson, 2020). Science has both historical and current (conscious or otherwise) participation in and re/creation of racist, hetero- and cis-normative, and patriarchal systems. Often, human biology research is designed and interpreted in such a way that re/produces sex as binary and as a purely biological, rather than socially constructed phenomenon. For instance, the National Institute of Health has called for an increased emphasis on the importance of including sex as a variable in clinical, medical, and biological research, bringing awareness to the absence of female subjects in scientific studies (Arnegard et al., 2020; National Institute of Health, 2015). However, this call requires researchers to "(1) to factor sex as a biological variable (SABV) into research designs, analyses, and reporting or (2) to provide strong justification for single-sex investigations" (p.858, Arnegard et al., 2020). This call does not define what it means by sex as a biological variable, but the second point, that justification must be made for single-sex investigations and by later referring to "both sexes" implies it is understood as binary (National Institute of Health, 2015).

Defining sex as a binary variable (female versus male) without consideration of gender (or often conflating gender and sex), continues to reify these categories, closing off the possibility of developing curiosity about or better standards for understanding the full range of variation in these phenomena.

The impacts of this discourse far-reaching. LGBTQIA+ individuals are at a higher risk for physical violence (National Center for Transgender Equality, 2015; Richey et al., 2019), poorer treatment within and access to healthcare (Clark et al., 2018; Goldberg et al., 2019; McCann et al., 2019), and mass incarceration, surveillance, and police brutality (National Center for Transgender Equality, 2015; Lamble, 2014; Edelman, 2014). Additionally, queer populations are at greater risk of mental health conditions. LGBTQIA+ adults are up to two to four times more likely to develop a mental health condition compared to cisgender and heterosexual adults (Medley et al., 2016; Wanta et al., 2019). These examples and statistics are much worse for queer people of color, as they navigate violent gender and sex systems that have been reinforced to further a white supremacist society (James et al., 2016; Ronan, 2021).

Much has been written about the history of western gender and sex norms and the science behind them by feminist and queer scholars (e.g. Bederman, 1995; Hyde et al., 2019; Fausto-Sterling, 1985; Fausto-Sterling, 2012; Fausto-Sterling, 2019; Jordan-Young, 2010; Fisher, 2011; Shattuck-Heidorn and Richardson, 2019; Schuller, 2019). For instance, Fausto-Sterling (1985), in *Myths of Gender*, shows that many of the assumed differences between men and women (and the research that supports them), like difference of intelligence, are unfounded. Additionally, gender and sex are also inherently tied to racial hierarchy. That is, heteronormativity and cisnormativity are racist, meaning that both have been created through and in order to uphold white supremacy (e.g. Somerville, 1997; Schuller, 2019; Spillers, 1987; Wynter, 2003;

Bederman, 1995; Beauchamp, 2019; Gills-Peterson, 2018). For example, Somerville (1997) argues that sex and sexualities were naturalized through scientific studies in tandem with (and utilizing the methods of) racist science. This process of defining (and assigning and denying and rejecting) gender in terms of whiteness is upheld and bolstered by western scientific thought, as I will show more concretely in Chapter 2. First, however, I will demonstrate how sex and gender, cisnormativity and heteronormativity are re/produced in current biological anthropology studies.

Cis and heteronormativity in current biological anthropology discourse

Biological anthropology still very much engages with and re/produces the conceptualization of discrete categories of sex, as well as cis and heteronormativity, at the introductory classroom level but also through published research. Biological anthropology research is grounded in evolutionary theory, which tends to emphasize reproductive success and the assumption of gender and sex division of behavior/labor. For example, the assumption that men hunt and women gather, which is being challenged (Haas et al., 2020) is still invoked in anthropology studies (e.g., Hill et al., 2020; Loponte and Mazza, 2021; Stibbard-Hawkes et al., 2020). These binary assumptions are introduced at an introductory level in biological anthropology. In the most recent edition of Biological Anthropology (fourth edition) the passage on testosterone identifies it as the "male hormone" and equates its function to only that of dominance and reproductive behavior. This is after positioning progesterone as a "female hormone," the function of which is only to prime a uterus for pregnancy (Stanford et al., 2019, p.506-507). This entry is based in cisnormative and heteronormative assumptions that men have testosterone and thus men have dominance behaviors, and woman have progesterone and are always in a holding pattern for pregnancy (the progesterone entry also discussed PMS in contrast

to the testosterone section which includes research on parental behavior, so progesterone (and women) is also associated with pathology). In truth, these hormones are neither male nor female and both have many functions beyond dominance and reproduction (Dubois and Shattuck-Heidorn, 2021). However, these other functions are not discussed outside of the section on reproductive behavior and strategies (for a detailed look at the shortcomings of testosterone estrogen research see Jordan-Young, 2012; Jordan-Young and Karkazis, 2019). The section goes on to discuss male risk taking and male cooperative hunting, further equating men and being male with risk, aggression, and action often all in the name of securing status, increasing their mate selection, and ultimately having more offspring.

Cisnormativity and heteronormativity are two related terms that refer to when binarized norms of cisgender and heterosexuality are assumed, enforced, re/produced, and naturalized, making it appear that both cisgender and heterosexual are the given states of being. In the discipline of biological anthropology, instances of cisnormativity and heteronormativity are common, often woven into researcher's questions, methods, and interpretations without much awareness of where these assumptions are coming from. This is particularly the case in studies interested in evolution and adaptation, as differential reproductive success and increased reproductive fitness are the main scientific determinants of adaptive traits. Many studies reproduce cis and heteronormativity, though it may not always be intentional. To show how this happens, I looked at biological anthropology journals, *the American Journal of Physical Anthropology* and *the American Journal of Human Biology*, over the course of two and a half (January 2020-July 2022) years and identify original research articles that are re/producing cis and heteronormativity in their research. These journals were selected because they are the

journals of the main association of biological anthropologists and the main association of human biologists and biocultural anthropologists in the United States.

This is not an exhaustive or systematic review and is bound by the last two and a half years so that research is current. As such I did not follow a "preferred reporting items for systematic reviews and meta-analyses", or PRISMA, format (Rethlefsen et al., 2021; Takkouche and Norman, 2011). Instead, I conducted a qualitative textual analysis of current biological anthropology research. I identified articles that were re/producing cis- and hetero-normativity in ways both subtle and overt. For example, articles that engaged with identifying/reifying gender and sex difference, pairing sex and gender in ways that are reductive, using sex to extrapolate gendered behaviors, or that emphasized reproduction and heteronormative pairings. I started my search by reading through the titles of each issue and selecting articles based on title that appeared to be conflating sex and gender, reproduced or tested sex and gender differences, reproduction, or where sex or gender might be connected to behavior. I then read the abstracts for each to better identify examples of gender essentialism, places where gender or sex appeared to be conflated or where gender was assumed from sex, places where intimate relationships were all positioned as heteronormative, and more. Some examples of this language are: "sexual dimorphism; sex differences; sex estimation; reproduction; violence" Finally, I more closely read a selection of 8 articles to use as examples in this chapter. The analysis was confined to *Homo sapiens sapiens* but included both archaeological and living human studies.

I identified over 116 articles that fit the above criteria, with the most common instances being conflating and binarizing gender and sex, often through quantification of morphological difference. Furthermore, almost none of the articles define or specify what they mean by gender or sex or how they determined one or the other in their sample. The main exception to this was in

archaeology, as they tend to outline their methods for determining sex. The archaeological studies mostly use some variation of Buikstra and Ubelaker (1994) to assess various pelvic and cranial traits to sort remains into female, male, likely female, likely male, or undetermined. Despite these multiple categories, such studies tend to conduct analyses on binary data categories, maintaining a binarized conception of sex within the field. Like this, I identified a number of common themes in how cis and heteronormativity are re/produced, which I will expand on below. However, due to the numerous articles identified, I will only be highlighting a few for each key theme. In order to see the entire list, along with article information, abstracts and basic data, see Appendix B.

Quantifying difference

The first major category of analysis were those studies specifically intended to identify sex differences or quantify sexual dimorphism in adults, children, and archaeological remains. Many of these were archaeological or forensics studies and looked at various morphological features. For example, teeth, including enamel, enamel peptide, odontometircs, and dental tissue size, are a popular feature to "better" determine the sex of both children and adults (e.g. Aris et al., 2020; Fernée et al., 2021; Gowland et al., 2021; Viciano et al., 2021). Additionally, many of these articles attempted to find new ways to quantify difference between pelvic morphology, cranial morphology and to a lesser extent long bone morphology of men and women (e.g. Del Bove et al., 2020; Marino et al., 2021). The main goal of these articles was to be able to better identify sex in the osteological record or to identify sexual dimorphism in living populations for forensics cases. However, none of the articles were able to binarize remains with 100% accuracy and instead developed methods that were 75-90% effective at categorizing remains into a binary

category of male or female, often removing “undetermined” or “intermediate” remains from analysis.

One article that exemplifies how cis and heteronormativity are reproduced is an analysis from Uy, Hawks, & VanSickle (2020) that looks at sexual dimorphism between the pelvis and gut volume in humans. The authors found that gut volume was associated with pelvic shape and with body size in males but not in females and suggest that this is due an “uncoupling” of the pelvic shape from the gut because females need to accommodate a uterus and thus pelvic shape and size in females is less related to supporting organs as much as it is to reproduction. They argue, “at any given GV [gut volume], we observe that females have wider outlets. This observation, coupled by the other regression analyses here showing a nonsignificant relationship between the pelvis and GV in females, perhaps signify that the role of the bony pelvis in support of the gut is minimized compared to its role in providing an adequately-sized birth canal in females” (p.137). This article stood out because of intense association of being female with reproduction, whereas being male was not tied to reproduction. By equating female pelvic form function with reproduction, at the expense of the more “male” function of support (literally holding up the gut), this article reproduces the narrative that females must reproduce, associates maleness with support/supporting, and implies all sex differences are because of females need to be able to reproduce.

While most of the studies did not try to explain how these methods can be applied beyond sorting individuals into male or female, Gowland et al., (2021), described how their novel method of analyzing peptide concentrations of tooth enamel can be used to identify the sex of infants, juveniles and children. They explain, “bioarchaeologists are now better equipped to explore questions related to sex-dependent cultural treatment of infants and juveniles, including

questions related to identity, weaning, infanticide, childcare, and puberty" (p.868, Gowland et al., 2021). This kind of application suggests we should assume sex-dependent differences exist and are meaningful. Furthermore it re/produces cisgender norms and causes researchers to assume that biological sex not only equals and has always equaled gender but that gender is and was binary and archaeologists can know subsequent cultural meanings of gender.

There were also a number of studies comparing skeletal, fat, and muscle mass between male and female athlete and non-athletes (e.g. Abe et al., 2021; Abe et al., 2020a; Abe et al., 2020b). These studies were all concerned with identifying differences between not just athletes and non-athletes, but also between the sexes. One of the studies, looking at whether resistance training has an effect on facial muscles, found that, while there were not differences between the high training and low training athletes, there were differences in size of the frontalis muscle, with women having larger frontalis muscles, and over all differences in size of the rest of the facial muscles, with men generally having larger face muscles (Abe et al., 2020b). The authors did not give much justification for why this sort of analysis was necessary, nor did they contextualize the findings in the discussion, thus perpetuating and over emphasizing sex difference. Further, it is unclear how authors defined and identified sex difference or gender difference. That is, data and results refer to participants as men and women while including the binary variable sex in analysis and there isn't any indication if this was a self-identified category, or a researcher identified categorization. For example, "therefore, sex differences in facial muscles are very unique, and it is unclear the reasons why some facial muscles are larger in women compared to men" (p.6). This study, which attempt to reifying and quantify binary sex difference, switches back and forth between male and men and female and women in ways that certainly erases variation present and works to further binarize and essentialize the categories of man and woman.

Violence, victimhood, and other gendered behaviors

Sex identification methods are often used in bioarchaeology to collect demographic information but to also draw conclusions about the lived experiences of presumed males and females in the archaeological record. Drawing on violence and conflict behaviors that are historically ascribe to males, several the articles looked at patterns of violence in the archaeological record. One example is a study of the Nasca Highlands of Peru circa 900-1400 CE (McCool et al., 2021). This study examined frequency and type of trauma differences between males and females and found more antemortem but not perimortem trauma in males. While the study is careful to use the word male throughout their articles, the authors ascribe the aggressors, raiders, and those committing the violent acts as males, while the victims are females and children, though evidence for this is scant. For example,

> "Noncombatants such as females (especially adolescents and young adults), children, and older adults were routinely the victims of lethal violence. Indeed, rates of perimortem trauma are higher among traditional noncombatants compared to those who typically make up the combatant profile. The majority of lethal encounters resulted in perimortem traumas to the posterior of the skull, suggesting victims were either ambushed from behind, were attempting to flee an assailant, or were executed" (p.626).

The authors of this study only ever describe female remains as "noncombatants" who were "were routinely the victims of lethal violence". This conceptualization of maleness and men being as being naturally more aggressive and violent and the historical perpetrators of violence has been problematized and critiqued, with calls for anthropologists to be more aware of when and how our research is re/producing these narratives (Gutmann, Nelson, & Fuentes, 2021). However, this kind of framing is still being perpetuated in recent and well-known disciplinary publications of biological anthropology.

Yet another archaeological study uses sex determination to describe gendered division of labor in a Pre-Roman population, Verona (Laffranchi et al., 2020). This article looks at muscle attachment sites in the upper and lower limbs and compares them between males and females. They found a significant difference between an attachment site for the right bicep and for the left gluteus medius and minimus muscles. Laffranchi et al., (2020) use this as an indication that there was a gendered division of labor in this community, "a result that may be related to the performance by men and women of distinct activities overall related to farming" (p.582). Notably, they did not find any other differences between general bone shape or other bony landmarks, in fact they found more similarities between individuals than differences. However, it is the differences that are highlighted and fit into existing literature and the similarities that need greater explanation, as if males and females being similar is surprising. These types of study designs, which look for differences based on sex differentiation through pelvic and cranial analysis (which is never 100% accurate, for example, in their own analysis they were not able to conclusively determine sex for 3 individuals but still sorted those 3 into female or male based on what they thought these individuals most looked like), always make me wonder what kind of story the data and results would tell if analysis did not start from a binary gender and sex difference. Additionally, the immediate assumption that a larger muscle attachment on the right humerus equals heavy lifting and thus manual labor is steeped in current assumptions about the division of labor by sex and gender that are not investigated but instead explained as givens.

Yet another way to quantify difference, this time in living humans, is through the 2d:4d ratio, a size ratio between the second and fourth fingers, usually on the non-dominant hand (e.g. Bagepally et al., 2020; Ertuğrul & Özener, 2020; Kalichman et al., 2020; Kirchengast et al., 2020; Lombardo & Otieno, 2021; Misiak et al., 2020). These studies use the 2d:4d ratio as a

proxy for *in utero* environment because this ratio is thought by some to be related to how much testosterone a person was exposed to as a baby. There is a more "feminine" type of the 2d:4d ratio, where the ratio is larger, and a more "masculine" type, where the ratio is smaller. This *in utero* exposure to testosterone (through 2d:4d proxy) has in turn been associated with everything from competition, more "masculine" personality traits, and longevity (Misiak et al., 2020; Kalichman et al., 2020; Bagepally et al., 2020). However, the 2D:4D digit ratio is a measurement that has been shown to be inaccurate and unfounded (e.g., Leslie, 2019).

One of the articles I want to highlight is by Bagepally et al., (2020). In this article, the authors measured the 2d:4d ratio on both hands and asked participants to complete a "big 5" personality and displaced aggression questionnaire in India. They then tested for any associations between the digit ratios of both hands and found several associations with digit ratio and personality traits. Namely they found a "link between 2d:4d and domains of Big Five personality factors among Indian men and inverse relationship between 2d:4d and more 'female' hands in the domains of disorganization, carelessness, and revenge planning even in men, emphasizing the effect of prenatal testosterone exposure on human personality" (p.1, Bagepally et al., 2020). In their discussion the authors reiterate the scientific narrative that *in utero* testosterone exposure leads to a more "masculinized" brain that is then associated with "male" behaviors, such as aggression, while "female" is associated with disorganization and carelessness. This narrative has already been disproven and *in utero* testosterone effects are inconsistent and context dependent (Jordan-Young, 2012; Jordan-Young and Karkazis, 2019; Leslie, 2019). By conducting a study like this the authors are embracing a stereotypical, binary, cisgender understanding of what it means to be man and male, mainly that high levels of testosterone lead to aggressive and "masculine" associated behaviors later in life. They are not only coupling male

and men together in that being a male means that being a man later in life is going to lead to specific types of personalities and behaviors, but also perpetuating an incredibly patriarchal and binary understanding of what that maleness and manhood looks like.

Masculine versus feminine features and behaviors and reproduction

Common in the studies of living humans is an attention to "feminine" versus "masculine" features, particularly as they relate to reproduction and attractiveness. One study that I want to highlight is by Żelaźniewicz et al., (2020). In this article their aims were to investigate associations between leptin levels and the facial attractiveness of young women from Poland. The authors work off "evolutionary theories [that] propose that facial attractiveness is a cue to an individual's biological condition, allowing [others] to assess a potential mate's fitness," and argue that "another trait, influencing an individual's attractiveness, is facial adiposity (or perceived weight in the face), possibly as it is an accurate indicator of BMI" (p.250-251). They determined facial attractiveness of women by online survey that recruited only self-identified men. They then tested whether participants' leptin levels (a proxy for body fat) were associated with the rating men gave them. They found that leptin was negatively associated with facial attractiveness, as was BMI, in other words, fat was associated with being unattractive. This study is harmful on several different levels. The assumption that only men can assess facial attractiveness of women AND that facial attractiveness is consistent across populations AND associated with reproductive potential AND that fat is universally considered unattractive is so incredibly reliant on heterosexual, cisnormative, and racist assumptions that without them, this logic would never be accepted. The association of fat with unattractiveness and unhealthiness is

rooted in white supremacy, with much of the fat phobic narrative emerging during the Enlightenment used to justify racial difference and hierarchy (Strings, 2019).

Another study concerned with identifying and quantifying sexual dimorphism, with explicit assumptions around attraction and race, looks at the sexual dimorphism of the faces of modern Buryats from Southern Siberia using geometric morphometrics (Rostovtseva et al., 2021). Rostovtseva et al., (2021) found several different facial features that were sexually dimorphic, and which matched with previous studies on Asian populations. They additionally found a unique facial feature, the upper face width to height ratio, that was sexually dimorphic in this population with women having higher ratios than men, which is the opposite of other reference populations. They suggest this is indicative of different genetic and ecological backgrounds. In the discussion they also go on to associate rounder and more "Caucasoid" (p.9) eyes with women and generally compare these and other features with results from other Asian populations and Caucasian populations. Not only is this article explicitly re/producing cisnormativity by quantifying sex difference and creating highly binarized understandings of "typical" female and male faces, but it is doing so in a way that reinforces white features are more attractive and more "typical" of an ideal gender type. Gender and sex difference has been and continues to be defined, produced, and reproduced in association with and through racial differences and hierarchies. Surprisingly, the authors report that these sex differences that they define account for only 8% of the variation of facial morphology in this population. Despite this, these differences are the main component of their analysis and discussion, demonstrating how dedicated these authors are to re/producing gender and sex difference and ascribing to cis and heteronormative binaries.

Many of the explanatory narratives used to bolster these differences in attraction and facial type are all about increasing reproduction and thus reproductive success. While reproduction is an important component of evolutionary theory, there is so much more to our lived experiences than existing for sex or having sex or sexual desires solely for the purpose of producing offspring or passing on heritable genetic material. The narrative around reproduction privileges heterosexual pairings as natural and attributing a certain suite of traits to females and women that increase their ability to bear children and make them more attractive to men while an additional set to males and men all for the purpose of getting more mates.

Key takeaways and conclusions

Cis and heteronormative assumptions are deeply entrenched in the study of biological anthropology, they are present in texts books and classrooms which re/produce these normativities at the foundational level in ways that allow them to be naturalized and then carried out in future research. After conducting my review of the most recent literature published in AJPA and AJHB, I have been able to identify a number of ways that cis and heteronormativity are commonly re/produced in biological anthropology. The largest group were those studies where the work is about first splitting sex into two categories, and then quantifying difference. By highlighting/quantifying sex difference, researchers are actively engaging with a sex binary that is then used as a proxy for gender. This is especially the case with archaeological studies, which cannot assess gender in the same ways that studies on living human might be able to (if those studies assess gender at all and aren't using sex as a proxy). These types of studies were some of the most common, despite decades of research already conducted on quantifying and identifying gender and sex difference.

Additionally, for living populations people are referred to as man and woman and for nonliving populations it is male and female, even though the basis for that distinction is on biological sex (assumed or identified by the researcher through various means, though it isn't made clear in all cases). In the archaeological studies, researchers are always careful to refer to people as male or female, while still discussing gendered behaviors and cultural features. However, in the studies examining living populations, researchers seemed to use the terms male and female most often when discussing data collection and results, but use the term man and woman in the introduction and discussion to refer to their participants Furthermore, the majority of the articles highlighted and listed in Appendix B end with broader impacts, like how can these studies be generalized and what can we now say about human evolution writ large given these results. Many of the archaeological and living human studies are conducted on groups of about 20-80 individuals and making sweeping generalizations about difference, adding to the re/production of cis and heteronormativity by making the differences between gender and sex seem natural, generalizable, and innate.

Finally, the anxiety around reproduction and reproductive fitness that I reported are very much reminiscent of similar narratives of reproductive success and fitness that were voiced throughout the eugenics movement, specifically positive eugenics, which is the type of eugenics concerned with increasing the birth rate of people with "desirable traits" (i.e., white, middle class, protestant). The eugenics movement of the early to mid-20th century was rooted in white supremacy and positive eugenics specifically included social programs and education aimed at increasing the reproduction of white populations (Kline, 2005; Ordover, 2003). Not only are the narratives around reproduction and heteronormativity that I outlined above entangled with narratives of racial hierarchy, but so are the narratives around attractiveness and beauty (Herzig,

2016; Jha, 2016; Strings, 2019), violence and "masculine" behavior (Gutmann, Nelson, & Fuentes, 2021; Bederman, 2008), all of which are predicated on colonial, imperialist, white-supremacist, capitalist, patriarchal systems (hooks, 2013). As I discuss in depth in Chapter 2, even the seemingly simple differentiation and re/production of gender and sex in human biology studies are necessarily coproduced together to maintain these oppressive systems.

This review of the two most recent volumes of AJPA and AJHB is not exhaustive. Deciding at the start to concentrate on articles that included gender and sex, reproduction, development or life history, and violence or conflict in their titles influenced my conclusions. There are likely ways that cis and heteronormativity are re/produced in biological anthropology that are not so explicitly related to gender and sex and reproduction that this brief overview missed. With all of this said, however, I do think it is important to note that identifying and researching gender and sex differences is not inherently bad, especially when purposeful and attentive to the ways that cis and heteronormativity create/reinforce differences in lived experiences. There are still health disparities and issues that could be erased or ignored if sex and gender where simply not accounted for within a study. However, these differences should be critically engaged with and the *a priori* categorization of participants into binary sex, and subsequently binary gender, needs to be revised. Much of the research listed in the Appendix is not purposefully trying to create inequalities, erase variation, or uphold harmful cis and heteronormative systems but are instead trying to highlight previously missed variation. The research that they are conducting however is developed in and through a cis and heteronormative system and thus those normativities and assumptions are worked into the research design in ways that do not name them. In the following chapters of this dissertation, I make the case for and

provide examples of using difference types of analyses which do not rely on *a priori* categorization, which has the potential to combat assumptions of binary sex and gender.

Chapter 2: Embodiment, plasticity and the re/production of gender, sex and race in human biology

While the introduction has demonstrated some of the ways in which our current biological anthropology discourse re/produces cis and heteronormativity, Chapter 2 will continue the discussion started here and will expand on it. Chapter 2 will demonstrate how biological anthropology not only recreates these systems of harm through uncritically re/producing cis and heteronormativity, but also through the frameworks and concepts we use to study human variation. One of the possible reasons for this re/production is due to human biologists assuming that by switching their frameworks (for example from determinism and thus racist science to plasticity and thus, they hope, anti-racist science) they have done enough to combat systems of oppression. While deterministic frameworks are often the most criticized in biology for harmful racist and sexist understandings of race and gender, plasticity, biosocial/biocultural and environment X gene interaction frameworks are not completely without fault. However, Western science as a discipline/process/construct is used to reify and naturalize oppressive categories and hierarchies, regardless of framework.

In chapter 2, I historically situate human biology research on embodiment, plasticity, gender and sex to show that even with large shifts in scientific understanding, in this case from determinism to plasticity, science is still a tool to create and maintain racist, patriarchal, cis and hetero normative systems. I begin by defining and problematizing embodiment and plasticity and review how and why these concepts have been taken up in human biology research. Next, I

engage the works of feminist, trans and queer scholars who have examined the connection between embodiment, plasticity and the creation of Western binarized sex and gender. Further, I present how the re/production of a sex and gender binary is entwined with the justification of racial hierarchies through plasticity and embodiment. While deterministic frameworks are often the most criticized in biology for harmful racist and sexist understandings of race and gender, plasticity, biosocial/biocultural and gene X environment interaction frameworks are not completely without fault. I conclude with recommendations and possible pathways forward for embodiment and plasticity research in human biology, suggesting that human biology research should engage with feminist science and technology critiques to be mindful of the way in which our concepts might be re/producing harm.

Part 2: Within population variation of common biomarkers of embodiment

Given the current state of biological anthropology discourse, how can we go about conducting research that explores human variation while not unknowingly re/producing cis and heteronormativity? Chapters 3 and 4 offer imperfect examples on how to study human biological variation that is both legible to traditional human biologists while not actively or uncritically re/producing binary gender and sex. The goals of these quantitative chapters are twofold. First these analyses were initially conceived to better explore within population and individual variation of common biomarkers used in studies of embodiment, C-reactive protein and cortisol. This was to better incorporate biomarker data of gender diverse bodies without having to remove or exclude individuals who might not fit a normative pattern. Second, I sought to describe and explore human variation in ways that do not re/produce cis and heteronormativity.

In order to accomplish these goals, I explored the variation present in two common biomarkers, cortisol and C-reactive protein, in a population of people who experience menstrual cycles. There is evidence that sociocultural experiences and environment can be biologically embodied through various mechanisms such as the hypothalamic pituitary adrenal (HPA) axis and inflammatory responses to stressors (ex: Chida and Steptoe 2009; Johnson et al., 2013; Zannas et al., 2015). Both cortisol (Allison et al., 2019; Desantis et al., 2015; García et al., 2017; Legatzke & Gettler, 2020; Lewis et al., 2010; Thayer & Kuzawa, 2014) and C-reactive protein (Measelle et al., 2019; Dubois, 2012; Goosby et al., 2015; Inoue et al., 2016) are common measures of these mechanisms and are used in numerous biocultural studies. However, these biomarkers do not function in their own vacuums, disconnected from other systems in the body, and there is evidence in both human and animal studies that they are interacting with other biological systems, especially the HPA and hypothalamic pituitary gonadal (HPG) axis (Carey et al., 1995; Handa et al., 1994; Heck & Handa, 2019; Kirschbaum et al., 1996; Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005; Viau, 2002). The HPA/HPG interaction might affect an individual's cortisol or C-reactive protein expression, especially if that individual is menstruating (Clancy et al., 2013). Few studies identifying and describing HPA axis and inflammatory response variation across entire menstrual cycles have been conducted; many existing studies offer infrequent sampling methodologies (cortisol ex: Kirschbaum et al., 1999; Montero-López et al., 2018; Villada et al., 2017; CRP ex: (Jilma et al., 1997; Wander et al., 2008; Wunder et al., 2006).

This biological portion of the dissertation is just the first exploratory step that will help to guide future decisions about which biomarkers to collect to study embodiment, and will fill in gaps about cortisol and CRP across the menstrual cycle currently present in the literature. First,

biomarkers like cortisol and C-reactive protein are likely being affected by both external and internal factors. External effects are those like the sociocultural environment in which a person lives and a person's lived experiences which impact concentrations of cortisol and CRP. Internal effects are the variables which are internal to a person's body, meaning they are the individual biological systems with which biomarkers might be interacting with, like the interaction between the hypothalamic pituitary adrenal axis and the hypothalamic pituitary gonadal axis.

Second, it is vital to acknowledge that different bodies experience and respond to external effects differently. Previous research on sex and gender suggests that there is a connection between our sex and gender lived experiences and our biological outcomes (ex: Fausto-Sterling et al., 2012; Gettler et al., 2013; Gettler et al., 2011; Joel et al. 2015; Kuzawa et al., 2010; van Anders et al., 2012), however each person has a different body and a unique way of being. Variation in biological embodiment due to the hypothalamic-pituitary-ovarian axis or menstrual cycle variation is important to describe and identify so studies of gender and biological embodiment can be as inclusive as possible. By better understanding, describing, and accounting for the internal effects, we will be able to compare the biomarkers of diverse bodies with more nuance, and better identify when variation is more attributable to lived experiences or internal variation.

Finally, while chapters 3 and 4 are written in a way that follows the strict scientific research paper format and uses much of the language common to quantitative analysis there are important omissions. In neither of the articles do I use the following words or concepts: evolution, adaptation, reproduction, female, woman, or sex. Instead, I very specifically use and define the sources of variation I am actually exploring, the HPO axis and the menstrual cycle and maintain gender inclusive language throughout the articles. Additionally, I do not engage with or

make any assumptions about *a priori* sorting of individuals within my populations and utilize statistical methods that are not about "proving" difference of distributions between groups or showing a group is more different from the norm than the other. Instead, I explore within population differences to show how there is ample variation in C-reactive protein and cortisol across the menstrual cycle, and that there is likely no "normal" menstrual cycle pattern of these biomarkers. While subtle and done in a way that may not be obvious at first glance, I have attempted to not re/produce cis and heteronormativity in these ways.

Chapter 3: Cycle effects are not universal: a case study of C-reactive protein concentrations in rural Polish and Polish American samples.

This chapter examines why studies of C-reactive protein (CRP) concentrations across the menstrual cycle have been inconsistent, reporting either no menstrual cycle effects or variable timing of effects. I explore menstrual cycle CRP variation in two geographically diverse samples of Polish and Polish American individuals. First, I identify when the menstrual cycle is influencing CRP concentrations. Second, I examine whether CRP phenotypes are population specific. Analyses were conducted on 76 Polish and 22 Polish American daily first morning void urine samples starting on the first day of menstruation until the start of their next period. Urinary C-reactive protein, estrone-3-glucuronide, and pregnanediol-3-glucuronide were assayed. Cycles were aligned by ovulation and cycle lengths and hormone concentrations were scaled using geometric morphometric methods. We constructed sample specific linear mixed models to examine cycle effects and compared median CRP concentrations across cycle phases using Kruskal-Wallace and Dunn tests. The Polish and Polish American samples had distinct menstrual cycle CRP phenotypes. The Polish sample did not show any cycle effects. In the Polish

American sample, not menstruating had a negative effect on CRP (estimate -.17, t-value -5.2), and there were increased CRP concentrations during the early follicular (median .406, $p<.05$), specifically the first three days of menstruation (median .466, $p<.01$). Our paper suggests that menstrual cycle effects are not universal across populations. CRP concentrations do not change across the menstrual cycle in the Polish sample. However, in the Polish American sample, CRP is highest during the early follicular, specifically the first three days of menstruation.

Chapter 4: Cortisol phenotype varies within a homogenous population of menstruating individuals

In chapter 4, I explore that assumption that ovarian hormone effects on cortisol through the menstrual cycle are similar across spontaneously cycling, ovulating and generally healthy individuals. This chapter seeks to test whether menstrual cycle effects on cortisol are universal within a homogenous population, and to explore a potential source of menstrual cycle cortisol variation, hypothalamic-pituitary-ovarian axis function. Analyses were conducted on 76 Polish daily first morning void urine samples starting on the first day of menstruation until the start of their next period. Urinary cortisol, estrone-3-glucuronide, and pregnanediol-3-glucuronide were assayed. Cycles were aligned by ovulation and cycle lengths and hormone concentrations were scaled using geometric morphometric methods. I conducted a principal components analysis and cluster analysis to identify cortisol shape patterns in the sample and to determine if individuals sort into statistically significant phenotypes. Two block partial least squares were used to test if cortisol cycle shape was correlated with estrogen or progesterone cycle shape. Pairwise t tests with FDR correction were used to compare estrogen and progesterone area under the curve across cortisol phenotype groups. Overall average cortisol cycle shape appears to follow a cyclic pattern similar to ovarian hormone patterning, but this average obscures significant group

differences. I found 3 distinct cortisol phenotypes ($p<.05$). Progesterone cycle shape was correlated with cortisol cycle shape ($r=.64$, $p<.05$) and the consistent cortisol group had higher progesterone exposure compared to the other groups (AUC PdG =, $p<.01$). My study suggests that ovarian hormone effects on cortisol patterning through the menstrual cycle are not universal and may account for much of the disagreement in the literature. Individuals' cycles appear to differentially impact cortisol curves across a cycle, with some showing very little cyclicity and others showing stronger cyclicity.

Chapter 5: Conclusions

The overarching question of how gender is embodied necessarily involves bringing together a number of different, seemingly disparate and disconnected theoretical perspectives and methods of both sociocultural and biological disciplines. Though as many feminist and queer scholars have pointed out these two are not and have never been separate; science does not happen in a cultural vacuum and our sociocultural spheres do not operate separate from or regardless of our physical bodies (ex: Haraway, 1988; Harding, 1986; Oreskes, 2019; Pigg and Adams, 2005). While this dissertation on the surface is keeping the sociocultural and the biological separate for now, it has critically and carefully engaged with the theories and methods of each so that future researchers can access feminist, queer, trans theoretical frameworks and quantitative methodologies. By demonstrating that current biological anthropology discourse is still actively re/producing gender and sex, I hope to bring awareness to the subtle ways that cis and heteronormativity are entrenched in our discipline and research. Additionally, by showing that human biology research, regardless of the popular framework of the day, has historically been invested in this re/production in order to maintain oppressive systems of harm, like race, I

invite human biologist to more critically engage with their own research. Describing in great detail the biological variation of cortisol and C-reactive protein, including considering both external (such as sociocultural environment) and internal (such has HPO and HPA interactions) effects, will help future researchers better incorporate biological variation from internal effects (i.e. accounting for menstrual cycle variation of cortisol and C-reactive protein in a population that has a diverse range of bodies) and tease apart variation from external effects, like my primary interest, gender and gendered lived experiences. Additionally, these analyses provide an example of how human biologists can conducted quantitative research in a way that tries to not uncritically re/produce gender and sex.

CHAPTER 2: EMBODIMENT, PLASTICITY AND THE RE/PRODUCTION OF GENDER, SEX AND RACE IN HUMAN BIOLOGY

Introduction

Biological determinism is the framework that posits that an organism's biological structures (like, hormones, genotypes, neurological organization, etc.) determines everything about phenotype, including behavior and social position. Even seemingly more benign manifestations of determinism, such as the Genome Wide Association Studies of the last 15 years, still attempt to map complex, culturally specific, behaviors to specific alleles (e.g. de Vries et al., 2020; Kong, et al., 2017). Biological determinism has rightfully been critiqued as a framework that produces racist and sexist science because it assumes organisms have essential traits, that these traits cannot be changed, and there are stark demarcations between groups with and without particular traits (e.g. Graves, 2015; Lewontin, 1980; Miller and Costello, 2001). In contrast and often positioned as the remedy to determinism, is plasticity. Plasticity refers to the ability of an organism to express or change their phenotype to respond to an environmental change, with different phenotypes in different environmental circumstances (Mascie-Taylor and Bogin, 2011; Nettle and Bateson, 2015; Sultan, 2021; West-Eberhard, 2021) and is a central assumption of embodiment in human biology research. That is, without an organism's ability to respond *to* environment, there would be no embodiment *of* environment. However, plasticity, like determinism, is not cut off from the sociocultural and political world within which we conduct research.

Many anthropologists have written about the shortcomings and pitfalls of plasticity as a solution to or progression from determinism, especially in the realm of epigenetics (ex: Lock,

2013; Meloni, 2016; Niewohner, 2011; Warin, Kowal, and Meloni, 2016), however plasticity is still often situated as an "after" to determinism, the natural step forward in scientific progress, and little attention is paid to how plasticity has historically been used to re/produce[1] not only racial and socioeconomic hierarchies, but also gender and sex. Even with large shifts in scientific understanding, in this case from determinism to plasticity, human biology must be aware of its part in the re/production of these systems and that science itself is a component of these systems. This paper explores how embodiment research, through its use of plasticity, has historically been used as a tool to create and maintain colonial, imperialist, white-supremacist, capitalist, patriarchal systems (hooks, 2013), particularly through the biological study of sex and gender.

Through critically engaging with plasticity, we can further challenge our understanding of it as an antithesis to determinism and better conceptualize and study embodiment. This is particularly applicable to the study of gender and sex. Despite ample biological evidence to contest these categories (ex: Fausto-Sterling 1993; Fausto-Sterling, 2008; Jordan-Young, 2011; Shattock-Heidorn and Richardson, 2019; Joel et al., 2012; Joel et al., 2015), gender and sex are often conflated with each other, taken as given, immutable categories (for example, binarized in statistical analyses), and determined sex at birth is assumed to determine current gender or sex (through questions such as "are you male or female"). Because of the potential harm human biology work can have, we must be exceedingly mindful of how our research might be taken up to reinforce rigid binaries of sex and gender and in turn, hierarchal categorizations of race.

To better interrogate why embodiment and plasticity as concepts fall short of being the cure all to racist and deterministic human biology research that many view them as being, I will

[1] The terms re/produce and re/production are used throughout this article to highlight how gender and sex are both reproduced within and through a historical and cultural context and actively produced, tweaked and changed to seem both natural and ahistorical.

begin first with a discussion of what embodiment and plasticity mean and how they are used and problematized in human biology research. Next, I situate plasticity historically and I engage more specifically with feminist, trans and queer scholarship to better understand why simply adopting a plasticity framework is not enough to separate our research from reproducing harm. I do this by examining how plasticity has been historically used in the re/production of gender and sex, with an emphasis on how these categories are intertwined with the categories of race in Western sciences. Finally, I conclude with a discussion of current human biology research that is utilizing feminist, queer and trans perspectives and suggest that human biology research should engage with science and technology critiques to be mindful of the ways in which our definitions and frameworks might be re/producing harm.

Embodiment

Embodiment and embodying refer to the process of a person or group incorporating and internalizing the physical and sociocultural world around them (Csordas, 1990; Kimmel, 2008). This process is not unidirectional, meaning that embodying does not act on only on a passive object but also that a subject embodies and in turn recreates and changes that which they are embodying (Csordas, 1990; Saboowala et al., this issue). Embodiment is also not solitary nor is it only between a person or group and the outside world but is an interpersonal and social process (Csordas, 1990; Kimmel, 2008). There is much more beyond biological mechanism that can be considered modes of embodiment, including, dance, vocalization, movements, behaviors, and clothing (e.g. Jones, 2002; Reed, 1998; Rees, 2017; Weidman, 2014; Zimman and Hall, 2009).

The definition most commonly used in human biology, medical, clinical and public health disciplines comes from Krieger (2005) and emphasizes potential biological mechanisms for embodiment. In her paper, Krieger defines embodiment as:

> "A concept referring to how we literally incorporate, biologically, the material and social world in which we live, from in utero to death; a corollary is that no aspect of our biology can be understood in the absence of knowledge of history and individual and societal ways of living… ''embodiment'' for epidemiology is best understood: (a) As a construct, process, and reality, contingent upon bodily existence; (b) As a multilevel phenomenon, integrating soma, psyche, and society, within historical and ecological context, and hence an antonym to disembodied genes, minds, and behaviours; (c) As a clue to life histories, hidden and revealed; and (d) As a reminder of entangled consequences of diverse forms of social inequality" (p.352).

While Krieger's definition pays necessary attention to the multidirectional, historically and ecologically situated entangled processes of embodiment, it removes the possibility for embodiment beyond biological mechanism. As such, much of the subsequent literature of embodiment in human biology is concerned only with biological outcomes through biological mechanisms and has operationalized this definition in ways that imply a unidirectional cause and effect (Saboowala et al., this issue). This move is likely a response to the absence of the body in medical and sociocultural theories of embodiment, which saw biological anthropologists attempting to bring the "body" back to embodiment (Lock, 2013).

While the move to consider the body of the embodiment is important, the current swing towards only considering biological mechanism, biological outcomes, or health outcomes is an over correction. The assumptions that embodiment can only happen through biological means and results in differential health outcomes, negative health outcomes are primarily experienced by marginalized communities and individuals, and that a person's life can be fully known by teasing apart specific biomarkers should be critically engaged with. Our current mode of researching and understanding embodiment has the potential to cause additional harms by upholding the oppressive

systems which we study (Saboowala et al., this issue). Numerous biological and biocultural anthropologists have begun to problematize this unidirectional and biologically oriented view. For example, Hokes and McDade (2014), discuss the framework of biosocial inheritance, which is "the process through which social adversity or advantage is transmitted across generations through mechanisms both biological and social" (p.194), as a form of embodiment. Biosocial inheritance is useful for reentangling the biological with the social, however, the main outcome of interest is health, and the study of the biological mechanisms which lead to differential health outcomes (Hoke and McDade, 2014).

This single-minded focus on biological and health outcomes has two potential consequences. The first is that it connects embodiment to mainly negative health outcomes through marginalization and social inequalities, seen in point "d" of Krieger's definition. This connection between negative health outcomes and marginalized lived experiences, while an important addition to highlight the necessity of considering sociocultural inequalities when interrogating health outcomes, has an unspoken, though likely unintended, effect. Namely, in only naming social inequalities as a source of embodiment and differential health outcomes, it masks normative experiences (that is, white, male, cisgender and heterosexual) as invisible and makes them the default. Clancy and Davis (2019) have pointed out through their interventions for the use and definition of WEIRD populations in human biology research, that WEIRD really means white, and that human biology positions research comparisons specifically between white and non-white populations, furthering distance between groups and constructing white as the normative, invisible experience and everyone else the nonnormative experience. By only naming social inequalities and by embodiment and plasticity researchers often only studying the health of non-white or marginalized groups in comparison or contrast to the health of white groups (explicit or otherwise),

our current framework of embodiment produces the same binary. For example, within this journal, articles which include the words “embody” or “embodiment” are all about race, racism, and racial inequalities. There is little attention paid to positive embodiment or embodiment that is associated with positive lived experiences further associating negative health disparities with the embodiment of race (Torres and Torres Colon, 2020) and pathologizing whole groups of people as impaired (Meloni et al., 2022).

Second, this definition implies that an individual’s lived experiences and life history, as Kreiger writes both “hidden and revealed,” can be read or known through their biology. An overemphasis on health reinforces this view. That is, an individual’s (or group’s) phenotype is a direct outcome of their environment and lived experience and through these phenotypes researchers can know their experiences. A biological phenotype makes real the otherwise unknown lived experience. Fassin (2009) terms this framing of life being reduced to biology as “biolegitimacy” (p.49-51). That is, through suffering bodies (e.g. people who use drugs and through their suffering gain legitimacy/importance for social programs, Larocque and Foth, 2021) or identified biological characteristics a group is recognized by the state (Fassin, 2009). A major consequence of this is that if biological evidence cannot be found for a lived experience, then this lived experience is assumed to be not meaningful or that this experience must not exist in the way researchers thought important. Just because there is no biological evidence for something doesn’t mean that it is not meaningful or important to the individuals or groups that human biologists are researching. These results could have material consequences in terms of access to and types of funding, biomedical interventions, and social programs that end up doing more harm through

biopolitical[2] and state surveillance (Warin et al., 2020). For example, Murray (2018), working with Indigenous children in British Columbia, argues that a popular epigenetic survey the Early Development Instrument is based on the eugenics era classification of "vulnerable Aboriginal children" (p.225). Through the use of this instrument, there is more justification for state intervention and control of Indigenous groups, like through the forced relinquishment of children (Murray, 2018). Additionally, the phrasing "hidden and revealed" implies a certain level of knowability of a person's past, regardless of whether that person or persons may want those things to be known. While this understanding of embodiment is currently being framed to identify assumed-to-be beneficial intervention programs (and through these increased state control), it has terrifying potential to be utilized in more overtly violent ways through bio surveillance.

A more expansive way of conceptualizing embodiment is the multidimensional, dynamic, and varied process of incorporating and impacting the material and social world through many different means (Saboowala et al., this issue). This conceptualization of embodiment is similar to Torres and Torres Colon (2020) who argue, "since all experience materializes as real, embodiment occurs with every instance of sensed collective bodily distinction" (p.183). That is, embodiment can include all forms of bodily experience. How we can embody these experiences is only confined by what a person or organism can be/do/experience/become/perceive/imagine (which can and does change over time). This kind of conceptualizing of embodiment is vast and expansive. Considering embodiment in this way does not put any judgment on an individual or group for any kind of

[2]Foucault's concept of biopolitics shows where the State, concerned with population control and surveillance, seeks to describe, define, categorize, and mark specific bodies as normative (and thus residing within the State sanctioned structure) or nonnormative. These defining categories are produced, naturalized, and reinforced within scientific discourse and, through their naturalization, work to establish differences that seem inherent and unchanging between other categories, especially racial difference (Foucault, 1976).

embodying, does not privilege mechanism or health over all else, and does not assume that a person's life and being can be uncovered through a biological sample. Biological mechanism is only one potential mode through which we, as beings who are constantly becoming, experience, internalize, interpret, and produce and reproduce ourselves and the world around us. This mode of theorizing embodiment, which emphasizes multidirectional and expansive potential pathways, helps to combat some of the unintended interpretations of Krieger's definition (2005). Like Krieger (2005), it still relies on the assumption of plasticity. Plasticity and the process of plastic responses to environment are not inherently negative, and many argue that plasticity has the potential to be the connecting line between the sociocultural and biological realms (Hicks and Leonard, 2014; Krieger, 2011; Meloni, 2015). However, plasticity, as the next section will demonstrate, needs to be considered critically and as part of a larger socio-historical context.

Plasticity

Krieger's and my own definitions, though never explicitly saying so, depend greatly on the assumption of plasticity. Plasticity, broadly speaking, refers to the ability of an organism to express or change their phenotype to respond to an environmental change, with different phenotypes in different environmental circumstances (Mascie-Taylor and Bogin, 2011; Nettle and Bateson, 2015; Sultan, 2021; West-Eberhard, 2021). The assumption of plasticity is ubiquitous in much of the literature published on human biology. For example, a quick search of "plasticity" in the *American Journal of Biological Anthropology* yielded 1,135 articles and chapters, 561 articles in the *American Journal of Human Biology*, and 137 articles in *Human Biology*. Studies of embodiment through biological mechanisms, while they may not explicitly name plasticity, work

on the underlying assumption that organisms respond to their environments and incorporate that response; it is their bodies responding to, internalizing, and embodying their environment.

The popularity of plasticity of a concept has ebbed and flowed in human biology research since the mid 1800s (more on this in the next section). Hicks and Leonard (2014) outline a brief history of human biology research since the publishing of Darwin's *On the Origin of Species*, and argue that plasticity (in this case specifically developmental plasticity, or the plasticity of an organism during development both in utero and as the organism grows), is a useful concept to study the process of inequality while not removing evolution and adaptation from our work. West-Eberhard (2003) argues that plasticity is itself an adaptive trait impacted by evolutionary forces and can lead to evolution through genetic accommodation, or the process where novel traits expressed during development are shaped by selection. Hicks and Leonard (2014) rightly caution against and point out that human biology research runs the risk of teleological thinking but that by adopting plasticity as a grounding framework and utilizing sociocultural and mixed methodologies, human biologists can avoid this trap.

However, plasticity in practice has not been the answer to teleological, racist, or genetic determinist science that many human biologists hope or want it to be. Many human biologists working in and examining the field of epigenetics, a field that is at the forefront of plasticity research, have noted the shortcomings of plasticity as a potential way forward. While the current iteration of plasticity, particularly through epigenetic research, is positioned as a corrective to determinism and solution for human biologists in the postgenomic world (Meloni, 2015; Meloni 2016), it instead has a risk of switching the determinism from a genetic source to an environmental or historical source (Meloni, 2017; Meloni et al., 2022; Warin et al., 2020). Additionally, Lock (2013) cautions about wholly accepting epigenetic research at face value, as doing so will likely

lead to a tendency to reduce studies of human experience and health outcomes to easily measurable variables, thus leaving oppressive social and political systems unexamined. Researchers also point out that epigenetic research, like much of molecular biology, may reduce historical and intergenerational processes to singular moments in time, shifting attention to individual and one-off interventions instead of addressing the oppressive systems that are the root cause of health and wellbeing disparities (Lock, 2013; Niewohner, 2011; Saboowala et al., this issue). Furthermore, the plasticity of epigenetics has often been positioned as both mechanism and cure when it comes to health disparities from inequalities through individual intervention (Meloni and Testa, 2014; Meloni, 2016; Saboowala et al., this issue). For example, the assumption that individuals can make their own personal intervention in cancer prevention by increasing their green tea polyphenol epigallocatechin-3-gallate (EGCG) intake (for review on epigenetic research on EGCG see Li, et al., 2022). However, as discussed above, plasticity (and embodiment) is itself likely to be used as a form of biopolitical surveillance and control by means of state or government-controlled intervention programs (Meloni, 2016; Meloni, 2019; Warin et al., 2020).

Despite these timely critiques within the realm of epigenetics, plasticity is often still positioned as a response to either past sociocultural research on embodiment that neglected the body (Hoke and McDade, 2014; Lock, 2013) or to the harmful, deterministic narratives around gender, sex, and race that can pervade the sciences (Gills-Peterson, 2018; Pitts-Taylor, 2016; Richardson, 2017; Schuller, 2018; Schuller and Gills-Peterson, 2020; Weasel, 2016). Many of us who use the concept of plasticity, either explicitly or implicitly through the study of embodiment, have not considered its historical use in the study of human biology. Instead, we frame it as a response or a way of connecting the biological and social (Hicks and Leonard, 2014). Many feminist scholars have called for a more critical understanding of embodiment and plasticity, one

that historically situates these concepts not as a brand new and an ahistorical way to combat racism and sexism in the sciences but as frameworks which have been historically used to create and uphold racism and sexism (Pitts-Taylor, 2016; Richardson, 2017; Weasel, 2016). Interrogating this history of how plasticity has been used to reproduce gender and sex could help to elucidate why its current iteration falls into the same traps as determinist frameworks and is not the cure all we hope it to be.

The plasticity of gender and sex historically situated

Feminist, queer, and trans scholars have made incredibly important interventions into how gender and sex are defined and conceptualized and have shown both categories to be unstable and mutable over time (see table 1.1 for expanded definitions and discussion). This critical scholarship has shown that gender, sex, and race are not natural, inherent, or monolithic things, but are instead naturalized, constructed, and reinforced to appear concrete and normal (e.g. Cipolla et al., 2017; Fuentes, 2019; Gill-Peterson, 2018; Gupta and Rubin, 2020; Schuller, 2018; Snorton and Haritaworn, 2014; Somerville, 2000; Stryker and Aizura, 2013; Tallbear, 2019). Gender and sex especially have historically been tied together to define what an acceptable, normal (that is, white, cisgender, heterosexual) body looks and acts like (Gill-Peterson, 2018; Schuller, 2018; Spillers, 1987; Wynter, 2003). These scholars have also made pertinent interventions into the study of human biology, interrogating how it is conceptualized, how scientific work is understood and positioned within and outside of the academy, how it is conducted, and what kinds of scientific inquiry produces/reproduces and naturalizes categories of sex, gender, and race. This section will show how human biology research, whether utilizing a deterministic or plasticity model, has historically been used as a tool to re/produce and bring legitimacy to these constantly changing and unstable categories.

Scientific definitions of a male versus a female body and who is designated male/man, female/woman, and other, have been coproduced with creating racial difference. Sylvia Wynter (2003) traces the colonial project of the creation of Man in Western culture as a white, male, middle class, and heterosexual. Wynter discusses how in modernity, Man has come to be defined through biological means, and that through biological sciences, Man becomes the only representative of humanity. Wynter's analysis of the creation of Man and Western culture's attempts to define what it means to be human/man demonstrates how sex, gender, race, and also class are defined by and created through biological and scientific discourse. Those who are not Man but instead defined as Other, thus are not human and are denied the categories of Man (such as gender). For example, Spillers (1987) argues that black women and men are "ungendered" (specifically ungendered from gender which is defined as white and patriarchal) so that they become flesh, a commodity for white, capitalist consumption (p.68).

This process of defining (and assigning and denying and rejecting) gender in terms of whiteness is upheld and bolstered by Western scientific thought. In her book *Biopolitics of Feeling*, Schuller (2018) builds upon Wynter (2003) and Spillers (1987) and focuses in specifically on embodiment and plasticity frameworks used by science to uphold and recreate sex, gender and race in the 19th century. Schuller (2018) discusses in depth how distinct, binary sexes and gender stereotypes were created through scientific studies in order to reconcile the racist, hierarchal views of the 19th century United States, placing white male bodies as the peak of evolution. Through her discussion of the biopolitics of feeling and sentimentality, Schuller (2018) shows that white men and women are distinguished not just by their genitals but also by their capacity for feeling. White people in 19th century America were considered superior because of their "impressibility" or their ability to be impressed upon and molded by their civilized cultures, that is they are more malleable

(literally can be pressed on and formed by culture), responsive and plastic to cultural environments and biologically embody civilization more thoroughly (p.7). In contrast to white impressibility, people of color were not able to evolve past a "primitive state" because they lacked the capacity to be impressed upon by civilizing forces (p.8). This social construction presented a problem however, in that being full of sentiment, sentimentality and impressionability could possibly cause a species to be weak or over influenced. So, how does white supremacy reconcile this? By further delineating sex and gender. That is, white women came to embody the "sentimental" sex, while white men came to embody the rational, impressing sex. The language of impressionability and plasticity used by the scientists in the 19th century that Schuller (2018) describes would not look too out of place in the more current biosocial/biocultural and environment x gene interaction frameworks of embodiment that have become more popular. For example, Meloni et al., (2022), in their review of epigenetic and Developmental Origins of Health and Disease studies of race/ethnicity and health, found that 58% of their sample reported "a 'multifactorial combination' or 'complex interplay' between genetic and environmental exposures," and another 23% referenced the environment as a direct source of phenotypic difference (n=49) (p.12).

Gill-Peterson's (2018) book, *Histories of the Transgender Child*, situates another subject within a biological, medicalized, racialized history of embodiment and plasticity, the transgender child during the mid-20th century. The transgender child, Gill-Peterson (2018) argues, is currently spoken about as an invention of the 21st century with no history at all. As Gill-Peterson (2018) argues this is not the case. What's more, the medicalized and scientific narratives around trans children are set within assumptions of racial difference, especially racialized difference in plasticity. For example, Gill-Peterson (2018) states,

> "far from being a progressive vector of malleability or change, the racial plasticity of sex and gender was a decidedly disenfranchising object of governance from the

> perspective of trans children. At its institutional best, it granted access to a rigid medical model premised on binary normalization. At its institutional worst, it allowed gatekeeping clinicians to reject black and trans of color children as *not plastic enough* for the category of transsexuality, dismissing their self-knowledge of gender as delusion or homosexuality" (p.4).

Gill-Peterson, like Schuller, identifies plasticity as a specifically white characteristic, one through which a person's self-knowledge of gender and sex (and the concomitant qualities of the binarized sexes and genders, like feeling) are considered valid only for those who also possess whiteness. Gill-Peterson (2018) further traces how "in the late nineteenth- and early twentieth-century life sciences, sex underwent two key transformations: sex became synonymous with a concept of biological plasticity that made it an alterable morphology, and, through experiments by largely eugenic scientists, it was racialized as a phenotype" (p.35). Sex became plastic through the studies of animal experiments, endocrinology, embryology, and later childhood development. However only white bodies were able to access that plastic response and embody the "binary normalization" of male and female, while black and trans of color children were "not plastic enough''(p.4). Sex, being plastic, could be impressed upon, manipulated, and guided by scientists in childhood in order to achieve what the clinicians of the era considered to be the optimal body; white, able, and binarized.

Schuller and Gill-Peterson (2020) continue their discussion of plasticity, in their special issue of Social Text, *The Biopolitics of Plasticity*. They explicitly name plasticity as "a central axis of biopolitical governance" (p.1). That is, plasticity as a concept uses the potential malleability of a body and enforces state power onto the body in order to "engineer an individual and population" (p.2). Thus, plasticity is used to further delineate populations. In this case, plasticity is read as whiteness, while Blackness is denied plasticity and the ability to embody and self-transform. Historically, plasticity has been used to ascribe Man (Wynter, 2003) with the ability to be formed

by his experiences and environment, and the greater the ability to be formed and embody, the more personhood and thus more human an individual is. This type of forming and reforming, however, is only possessed by individual white bodies, and bodies of color are instead denied this humanity. This plasticity on the individual scale is used to differentiate between racial categories. During the eugenics movement, the concept of plasticity was applied instead to the population level, with entire populations being able to be manipulated and changed through eugenics programs. However, as individual plasticity was still a characteristic of whiteness, the ability to influence a population's movement progressively forward through time towards an idealized form was only available to a white population, while Black populations remained defined by their formlessness (Schuller and Gill-Peterson, 2020).

Table 2.1: Key definitions

Gender
Gender is difficult to define and numerous scholars and disciplines have attempted to better understand and operationalize the concept. Trans and queer scholars (Halberstam, 2014; Stryker 2008) define gender as a marker of social difference that results in social organization. Gender is a historical category that changes from place to place, through time, and as a category it depends on a lot of different things coming together to make it "real". With that, gender is perceived to be organic or ingrained and is invisible and immutable, especially to the normative gender(s). Importantly, these scholars include gender as a form of oppression in their definitions. Gender is used to sort bodies into binary (in the United States) categories that are subject to various forms of social control. Gender, though not necessarily connected to the physical form, is assigned into one of two types of genders based on genital sex identified at birth. Gender has been further defined and broken down into multiple different levels and dimensions in the social sciences. Gender and how gender is experienced is social, interpersonal, structural and individual and can change over time (Connell, 2012; Fausto-Sterling, 2012; Fausto-Sterling, 2019; Hyde et al., 2019; Shattuck-Heidorn and Richardson, 2019; Tate et al., 2014). Gender can be a set of social norms about what it means to be a certain gender. Gender can be individual in that a person has a gender and a set of internalized beliefs which they use to interpret, interact with, and participate in social norms about gender. Gender can be experienced and re/produced through structural, institutional, and societal norms and systems. Gender is a learned, cultural, political, porous, and changing category/process of categorization/organization that can mean different things at different times in different locations. In the patriarchal, racist, and colonial context of the United States, a person's gender is understood through their sex which was identified/categorized at or before birth. That is, our society relies heavily on the binarization and subsequent identification of sex by genitals in order to forcibly sort bodies into a specific gender category.
Sex
The concept of sex itself is a construction and not a biological truth or universal (Fausto-Sterling, 1993; Fisher, 2011; Joel et al., 2012; Pigg and Adams, 2005; Richardson, 2017; Somerville, 1994). Most

definitions of sex specify karyotype, however, genital phenotype is used to assign a human body either male or female. While a person's phenotype is related to their genotype, it can vary greatly from person to person. For example, Fausto-Sterling (1993) theorized sex as not being binary, but instead as having as many as five different sexes, depending on different arrangements of phenotypic traits. Joel et al., (2012), considered as many as 9 possible sexes, depending on a person's gonadal, genital, and genetic makeup. Many critical scholars have talked in depth about how sex, like all other social categories, is constructed and given meaning that is then naturalized or considered innate (e.g. Fausto-Sterling, 1993; Fisher, 2011; Joel et al., 2012; Pigg and Adams, 2005; Richardson, 2017; Somerville, 1994). When human biology studies start with sex without defining what they mean by this category and how they are categorizing participants, they are at risk of perpetuating the assumption that sex is rigid, binary, natural and unchanging. Sex is often difficult to define, and the definition and traits of particular sexes are changing and overlapping. Despite this, scientists often assume that every reader knows exactly what they are talking about when using a male and female category, without considering how this assumption is incorrect and works to try to stabilize sex as a rigid category. The category of sex is a culturally meaningful category that is itself gendered. While sex greatly influences how a person is socialized in the US, gender also influences our understanding of sex (Fausto-Sterling, 1993; Jordan-Young, 2011). Sex does not determine gender, but sex impacts where and how bodies are attempted to be categorized in this society. In this way, gender and sex, while describing two different categories that can and should be uncoupled, are linked together.

Table 2.1 (cont.).

The Past and Present of Human Biology

What this history demonstrates is that human biology research and Western science as a discipline is founded on white, patriarchal, colonial ideals and that no matter the framework du jour, we as human biologists are still reckoning with and in many cases, reproducing the structural inequalities that we study. Given this critique of the concept of plasticity, how might human biology, biological anthropology, and biocultural studies of human embodiment especially, engage plasticity? Gill-Peterson and Schuller's work, summarized above, show how plasticity has historically been used as a biopolitical tool to uphold state power and is not the ahistorical foil to the racist and sexist determinism framework of yesteryear. While Schuller and Gill-Peterson (2020) are more so addressing other critical scholars, they state that, "despite a critical pessimism about its amenability to any dissent from its deeply entrenched biopolitical function, [scholars] also make a case for the productivity of plasticity. While the historical baggage attendant to thinking with plasticity is immense — and perhaps nowhere more so than in its racialized forms — the concept is for the same reason essential to thinking corporeal change across a range of

scales" (p.11). Schuller and Gill-Peterson (2020) are not calling for the throwing away of the concept and framework of plasticity, but instead, considering its formulations and instances both outside of science discourse and within it can call attention to the ways in which race, sex and gender are (co)creating and reinforcing each other. Their argument instead is that plasticity should not be uncritically embraced and championed as a tool to dismantle racist structures and that, "understanding plasticity to be a tool of biopolitical power can help us identify moments when Left frameworks themselves are invested in valorizing bodily capacity and potential and thereby fall into the logics of racialization and debilitation rather than subverting them" (p.13).

This last point, that Left frameworks, but also, I would argue, human biologists, should "identify moments…invested in valorizing bodily capacity and potential," is vital for our work. Schuller and Gill-Peterson (2020), through their analysis are inviting not just science studies scholars to think critically about plasticity, but also human biologists and other scientists invested in the narrative of plasticity. The narrative of plasticity especially would be better addressed by reflecting on the language that we as human biologists use to introduce, describe, and interpret our data. Are we inherently placing a value judgement on the malleability of the body? Is this malleability being situated within a broader framework of natural selection and how? Importantly, what bodies are now included in a plasticity narrative when many biological anthropology studies draw from and reproduce research conducted by white scientists? Which bodies are positioned as healthy and which as unhealthy?

These questions are particularly important when thinking about which bodies are plastic in the current discourse of embodiment. Many human biology studies on embodiment are either conducted on non-white or marginalized populations, are investigating biological or health outcomes that are perceived as negative or are investigating these negative outcomes in

marginalized populations (Saboowala et al., this issue) often in contrast to white, cis, heteronormative and WEIRD groups who are studied or positioned as the control (Clancy and Davis, 2019). In our current discourse, it is often bodies of color and bodies which are marginalized that are ascribed plastic responses, the opposite of the historical scientific discourse that Schuller and Gills-Peterson (2020) describe. These plastic responses often are tied to negative, non-normal (i.e., white), or unhealthy outcomes through perceived negative biological responses (e.g., increased baseline cortisol). Plasticity in our modern research is positioned and used in ways that appear to be in opposition to plasticity at the turn of the 19th century – that is, plastic responses are the realm of white bodies versus bodies of color, plasticity results in positive versus negative responses, and plasticity is the framework of racial difference versus the framework of antiracist science. However this framework is still being used in a binarizing and differentiating way. What implicit narratives are being constructed when only bodies of color are susceptible to negative health outcomes? What does it mean that plasticity is only considered within negative contexts, what judgement on those who embody and have plastic responses does this imply? How is plasticity still being used, if not in more subtle ways, to create and maintain gendered and racial difference?

Embodiment, plasticity, gender and sex are all interconnected and used in human biology discourse in tandem with the re/production of racial difference. When considering embodiment, plasticity, gender and sex as topics of study, we cannot think of them as a well demarcated, wholly separate, unbiased, ahistorical categories outside of racism. We as human biologists need to attend to these frameworks' and categories' re/production of racial, cis, and hetero normative assumptions and their use to maintain and reinforce racial difference and hierarchy. Gill-Peterson (2018) wrote, "sex and gender were reconceived as plastic phenotypes during the twentieth

century, which makes *all human embodiment*, including cisgender forms, a racial formation" (p.27, emphasis my own). In any projects that are directly interested in embodiment and, especially the embodiment of gender, Gill-Peterson's (2018) argument that all embodiment is a racial formation should be a starting point for how we conceive of embodiment and plasticity. Gill-Peterson's argument follows, as many have pointed out that the positivist and "objective" position of Western Science is a tool used to legitimize and make "natural" a white and white supremacist reality (Harding, 1995; Fleming, 2018; Melville et al., 2022; Wynter, 2003). By being aware of where our scientific inquiry fits within the historical scientific discourse, we can then be more attentive to the language we use, the research design we implement, and the interpretation of our results.

Potential futures

Anthropologists working within both the biological and sociocultural disciplinary realms have begun to put forward new ways of theorizing embodiment, plasticity, and evolutionary processes that aim to not binarize social versus biological, sociocultural variables versus biological outcome, plasticity versus determinism (Ingold and Palsson, 2013; Niewohner and Lock, 2018). Ingold and Palsson (2013) suggest understanding humans and our study of ourselves as "biosocial becomings," which emphasizes the "processual, developmental, and relational" biological and social processes that are entangled together in the becoming of life (Inglold and Palsson, 2013, p.9 and p.20). Niewohner and Lock (2018), introduce the concept of "situated biologies" with the intent of emphasizing not only the biological and differential health outcomes most often of interest to human biologists but to also incorporate the richness of ethnographic research which emphasizes how co-constructed humans' biologies, experiences, cultures, and histories are. They argue that situated biologies help to bring attention to how the boundaries between material,

culture, environment, and body are constantly disintegrating and being rebuilt. Importantly, they state that incorporating sociocultural theory and ethnographic methodologies into our studies of human biology are necessary for interrogating human lived experience. Clancy and Davis (2019), along with emphasizing ethnographic and qualitative methodologies, also make the point that human biologists will likely need to move away from prioritizing statistical significance, replicability, and rigid categorizations in order to better encompass all forms of human variation.

Many biological anthropologists are also incorporating humanistic and queer theoretical perspectives into their work to propose alternate ways of doing human biology that also bring attention to the ways in which human biology re/produce categories of oppression through our research. Feminist, queer, and trans scholarship is important to incorporate to show how the re/production of a sex and gender binary is entwined with the justification of racial hierarchies through plasticity and embodiment. Smith (2021) argues that scientific knowledge should be brought under the purview of this thinking. That is, scientific knowledge production should be grounded in and analyzed through theoretical frameworks beyond evolutionary theory and white, capitalistic, patriarchal cis and heteronormative ways of knowing. Recent work by numerous biological anthropologists have begun to problematize the binaries of gender and sex and utilize queer theory to think beyond the normative (e.g. Astorino, 2019; Dubois, Puckett and Langer, 2022; Dubois and Shattuck-Heidorn, 2021; Dubois et al., 2021; Meredith and Schmitt, 2019; Smith and Archer, 2019). For example, Dubois and Shattuck-Heidorn (2021) critically engage with the ways in which the emphasis on biological normalcy as the level of analysis, with common statistical analyses that binarize, normalize, and categorize gender/sex, continues to reproduce these categories. What is critical moving forward is to make sure that these researchers and perspectives are taught not only at a graduate or independent study level, but as foundational to

our discipline. What would future biological anthropology and human biology research look like if introduction to biological anthropology textbooks had chapters that included critical historical perspectives on evolution, adaptation, and plasticity and sociocultural theory and methods as part of the study of human variation?

In the meantime, while we work towards teaching and incorporating these perspectives as foundational for our discipline of biological anthropology and work to dismantle systems of oppression more broadly, there are some ways that we can mitigate our reproduction of these systems of oppression. Gender and sex are complicated and unstable as categories and can mean very different things to different people and disciplines (see Table 2.1). While both are complex and in flux, these categories have very real effects on how people live, experience, move through the world, and understand themselves and their positions. For human biologists interested in embodiment, combining the extremely varied lived experiences related to gender and sex would only erase this variation. Instead, researchers should be explicit about what they mean by, how they are defining, and how they are determining the sex and gender of participants (see Dubois and Shattuck-Heidorn, 2021, for an example of a gender and sex inclusive questionnaire). Researchers need to be sure not to conflate gender and sex (for example, using self-reported gender as a proxy for sex) and not to limit gender and sex to only man and woman or male or female.

Along with this, we can also interrogate the study design and methods that we use. First, human biology researchers should conduct their work from a place of mixed methodology (Clancy and Davis, 2019; Neiwohner and Lock, 2018), which incorporates ethnographic and qualitative data not just for creating surveys and population specific categories, but as an equal unit of analysis to examine human variation. If unable to incorporate mixed methods and qualitative data, human biologists can try to ask questions and choose methods that do not reproduce scientific narratives

of difference but instead attempt to blur the lines between categories and refuse normal/average/mean identification. For example, considering how sex and gender are defined from the start of a project and why these categories might even be necessary for the research project are all simple actions researchers can take to not further re/produce sex and gender. Furthermore, researchers can explore statistical methods that do not rely on *a priori* group identification which then produce normative groupings or situate one group over the other (for example, "group A has greater inflammation than group B and thus is more stressed"). My own work on the embodiment of gender is committed to exploring alternative forms of statistical analysis such as generalized linear mixed models (Bates et al., 2015; Bolker et al., 2009; Bolker and others, 2022), geometric morphometrics (Ehrlich et al., 2022; Dryden and Mardia, 2016; Rohlf, 1999), and latent class analysis (McCutcheon, 1987; Nylund-Gibson and Choi, 2018) as ways of moving beyond group A versus group B hypothesis testing. All of these suggestions are relatively easy to implement at the research design phase and will help elucidate more nuance when studying embodiment.

Broader impacts and conclusions

Maintaining and reproducing racist, colonial, capitalist, patriarchal, cis and hetero normative systems is harmful to anyone who isn't white, male, middle-upper class, cisgender and heterosexual. For example, LGBTQIA individuals have a higher risk of experiencing physical violence, challenges accessing healthcare and poorer treatment, mass incarceration, surveillance, and police brutality. People of color are even more likely to face these problems (James et al., 2016). In 2021 and 2022 we witnessed a serious increase in anti-trans legislation in the United States and acts of physical violence against trans and non-binary women of color (Ronan, 2021). At the time of this writing, there are currently 387 anti- LGBTQ bills being proposed, many of

which have or would ban and criminalize trans healthcare and dressing in drag and drag show performances (ACLU, 2023). Many of these bills are justifying these bans under the guise of "biology," arguing that they are maintaining a natural order protecting the healthy mental and physical development of children (ex: Arkansas SB43; Mississippi HB1125; Tennessee SB1; Tennessee SB 3). These trans and drag bans are a case study of how both embodiment and plasticity and determinism are being used in ways to uphold a hetero and cis normative system by claiming they are protecting children from possible exposure to persons that could influence their own mental, emotional, and physical development. Though plasticity has been taken up as a foil to determinism by human biologists, sociopolitical usage shows how both concepts can be used to maintain racist, colonial, capitalist, patriarchal, cis and hetero normative systems. By claiming these bills will protect the "immutable characteristics…determined by anatomy and genetics" of children from becoming influenced by "prurient" external influence, proponents for these bills tap into the biological language of both determinism and plasticity, lending themselves legitimacy (ex: Tennessee SB1; Tennessee SB 3).

Especially because the current harm being enacted through the language of human biology, plasticity and determinism, biological anthropologists and human biologists have a responsibility to not reproduce but to actively dismantle harmful systemic hierarchies of oppression based on categories like gender, sex, and race. Embodiment and plasticity are positioned as a solution to racist and sexist deterministic science but as many scholars have discussed, embodiment and plasticity often fall short of true change, switching the determinism from genes to environment and continuing to uphold categories of oppression within and outside of our disciplinary realms. This re/production of categorization can be seen in our historical and current study of gender and sex, which have far reaching consequences. For example, in political

discourse around gender and sex both plasticity and determinism are used in tandem to enact violence against LGBTQIA groups. Through examining the historical context of plasticity in human biology, especially as it is used to re/produce gender and sex, we can see that like gender and sex, the frameworks that we use are unstable and changing, often in service to maintaining the status quo. Simply changing the frameworks that we use is not enough as science itself is a structure enmeshed in the broader sociocultural and political systems at work. Swinging too far into the biological realm, seeing the sociocultural as only another variable to be studied, and viewing embodiment only through the terms and mechanisms of plasticity will always fall short of creating meaningful change through our scientific endeavors. Through interrogating our use of plasticity in embodiment research, with special attention paid towards the re/production of gender, sex and race, I hope to bring our own scientific inquiry under the purview of critical scholars and theorists. By being aware of the historical context of our own frameworks and of the assumptions about what *a priori* categories (like gender, sex, and race) mean, we can begin to imagine new ways of questioning, studying and interpreting embodiment in human biology.

CHAPTER 3: CYCLE EFFECTS ARE NOT UNIVERSAL: A CASE STUDY OF C-REACTIVE PROTEIN CONCENTRATIONS IN RURAL POLISH AND POLISH AMERICAN SAMPLES

Introduction

C-reactive protein (CRP) is an acute phase protein and inflammatory biomarker that has the potential to be impacted by physiology of menstrual cycle variation, such as from cyclical changes of ovarian hormones (Clancy et al., 2013). This can introduce noise into the measure of concentration of this protein, which can be a problem when it is only measured a handful of times, as is common in many biocultural studies (e.g., Inoue et al., 2016; Konishi et al., 2014; Kranjac et al., 2022; Shattuck‐Heidorn et al., 2021). Some studies examined variation in CRP across an entire menstrual cycle, usually with less than daily sampling methods (e.g., Jilma et al., 1997; Wander et al., 2008; Wunder et al., 2006). These studies have variable findings, which could be a result of sampling, population, or other sources of variation. Therefore, the question remains as to whether CRP varies through the menstrual cycle, and whether this variation results from sampling methods or characteristics of a population (e.g., geographic location, subsistence behavior). This study seeks to address this question, exploring in detail CRP variation across an entire menstrual cycle in two samples of menstruating individuals from Poland and the United States.

CRP is a measure of general inflammation in the body, is synthesized in the liver, and is highly responsive and sensitive to trauma, infection and external stressors (Pepys & Hirschfield, 2003). CRP is a measure of current stressors or states, reaching peak values within 48 hours of the stressor or infection and has a plasma half-life of 19 hours, thus quickly falling to pre-stressor

levels after the stressor has been resolved (Pepys and Hirschfield, 2003). CRP is often used to identify and understand embodiment of sociocultural stressors, such as interpersonal stress, socioeconomic status, racial discrimination, childhood adversity, and employment stress (e.g., Danese et al., 2009; Johnson et al., 2013; Kiecolt-Glaser et al., 2011; McDade et al., 2006; Nazmi & Victora, 2007; Owen et al., 2003). For instance, perceived discrimination and self-reported discrimination are associated with increased CRP concentrations (Flores et al., 2008; Guthrie et al., 2002; Lewis et al., 2010; Pascoe & Richman, 2009). Additionally, CRP has been associated with gendered lived experiences. For example, increased CRP was associated with transition-specific stressors among transgender men. In particular, those participants who reported higher levels of stress related to "passing" as someone who is assigned male at birth had higher CRP concentrations (Dubois, 2012).

CRP does not follow a seasonal cycle (Pepys and Hirschfield, 2003), nor is there a pronounced diurnal cycle, except for a slight increase at awakening (Izawa et al., 2013). However, CRP could be affected by both external and internal factors. External factors include a person's environment, like their socioeconomic status, where they live, and their lived experiences. These external variables are often the variables of interest to biocultural anthropologists. Internal factors are the variables which are internal to a person's body, meaning they are the individual biological systems with which biomarkers might be interacting. One possible source of internal factors comes from the hypothalamic-pituitary-ovarian axis, which regulates cyclical menstrual changes of ovarian hormones like 17-beta estrogen and progesterone (Mikael et al., 2019).

Additionally, CRP is a very sensitive marker of tissue damage and inflammation and, as such, tissue remodeling during the menstrual cycle could affect CRP (Clancy et al., 2013). For

instance, CRP could be elevated at the start of menses: right before menstruation there is a decrease in progesterone levels (an anti-inflammatory hormone) and an increase in pro-inflammatory markers, leading to tissue breakdown and bleeding (Azlan, et al., 2020; Evans and Salamonsen, 2012). Previous research on regularly cycling, generally healthy participants have had mixed results, with some studies finding increased CRP in the luteal phase compared to follicular phase (Jilma et al., 1997) or no differences across the menstrual cycle (Wunder et al., 2006). Other studies found increased CRP in the follicular phase (Blum et al., 2005; Gaskins et al., 2012; Vashishta et al., 2017; Wander et al., 2008). Additionally, when examining ovarian hormones, estrogen levels has been negatively associated with CRP concentration (Blume et al., 2005; Clancy et al., 2016; Clancy et al., 2013; Gaskins et al., 2012; Wander et al., 2008), however, there are also studies that find no associations between estrogen and CRP (Wunder, et al., 2006). Progesterone has also been tied to CRP, though in the opposite direction as estrogen, with increased progesterone in some cases associated with increased CRP (e.g., Wander et al., 2008; Jilma et al., 1997). It is important to note that these studies focused on endogenous hormones, whereas studies examining exogenous hormones, in the form of birth control pills or hormone replacement therapy, find reversed associations, with exogenous estrogens associated with increased CRP (Walsh et al., 2000; Dreon et al., 2002; Kluft et al., 2002; Kovacs et al., 2005) and exogenous progesterone associated with decreased CRP (Skouby et al., 2002; Gol et al., 2006).

In this study, we analyzed daily first morning void urinary CRP concentrations across one full menstrual cycle in two separate populations: from Poland (n=76) and from the United States (n=22). We have attempted to account for several physiological and methodological confounders. To address the possibility that CRP is elevated due to tissue remodeling at menses,

we need to consider whether prior to menses, during the duration of menses, or the most physiologically active part of menses is where we might see this effect. Taking the above points together, we identify the first three days of menses as most relevant to study as this is a day or so after an increase in pro-inflammatory biomarkers (Evans and Salamonsen, 2012), thus when CRP is likely reaching its peak (Pepys and Hirschfield, 2003) and when tissue remodeling is occurring (Clancy et al., 2013). Another frequent issue is that variable menstrual cycle lengths pose challenges for exploring variation at the very start or end of cycles, such as at menses, since most collection protocols are from first day of first menses to first day of next menses. To address this, we use aligning and scaling methods to investigate relationships present at these times in the cycle.

We propose two main hypotheses. First, we hypothesize that menstruation is an inflammatory state that drives an increase in CRP in menstruating people. We predict that the first three days of the menstrual cycle will have higher CRP than other days of the cycle, however CRP across the remainder of the cycle will be non-cyclical. Second, we hypothesize that how affected CRP is by menstrual cycle factors in an individual is population specific. Exploring variation in CRP concentration across menstrual cycle could help researchers design optimal collection timing and will likely impact methodological considerations for future research. It is vital that we better explore cyclical variation that could be impacting results in menstruating individuals, in a way that recognizes that even when we can document them, these cycle effects are not universal across populations or people.

Materials and Methods

Data collection and population

Data were collected over 2014-2017 at the Mogielica Human Ecology Study Site in the Beskid Wyspowy region of southern Poland (Jasienska and Ellison, 2004) and the University of Illinois, Urbana-Champaign. 98 individuals who were ovulating, not pregnant (determined with by presence/absence of HGCB) and completed collection across one entire menstrual cycle were included in this analysis. Of those 98, 76 were Polish participants whose data were collected at the Mogielica Human Ecology Study Site and 22 were Polish American participants whose data were collected primarily in the Chicago and Urbana-Champaign areas of Illinois. Participants were healthy, regularly menstruating, non-smoking, not breastfeeding, not on any form of hormonal birth control, and ranged in age from 18-46. Participants collected daily first morning urine samples starting on the first day of menstruation and kept collecting until the start of their next period. Samples were stored in participants' freezers until samples could be dropped with researchers.

Both populations are generally homogenous and are of white European ancestry, with the main difference being geographic location. The Polish population consists of a transitioning subsistence agricultural environment and women have moderate levels of physical activity (Colleran, 2014; Jasienska et al., 2006; Lee et al., 2019). In contrast, the Polish American sample is mainly urban and suburban, does not engage with subsistence farming and has a more sedentary lifestyle with bouts of intentional exercise (Lee et al., 2020). The Polish population is an ethnically and religiously homogenous group (around 98% practicing Catholics) from a small, insular, region in Southern Poland (Colleran and Mace, 2015). The Polish American population was recruited from across Illinois and Polish ancestry was an eligibility criterion. As such, the

Polish American sample population is likely more ethnically diverse than the Polish sample. Analyses were first conducted on the sample as a whole (combining the Polish and Polish American populations) and results are similar to the Polish results (supplemental). However, we decided to sort participants into population group to explore potential effects of incredibly different lived experience, i.e. growing up in rural Southern Poland versus growing up in the United States. Research protocols were approved by the University of Illinois at Urbana-Champaign Institutional Review Board (UIUC IRB #13856), and all participants provided informed consent.

Laboratory analysis

Urine samples were stored in -20 freezers at the University of Illinois, Urbana-Champaign until samples could be assayed. Samples were aliquoted into 1 ml tubes for assays to avoid numerous freeze/thaw cycles and specific gravity was measured to adjust for hydration levels (Miller et al., 2004). Urinary estrone-3-glucuronide (E1G, a metabolite of estradiol), pregnanediol-3-glucuronide (PDG, a metabolite of progesterone), and CRP were assayed using the Quansys BioSciences ELISA multiplex system at the Clancy Lab at the University of Illinois, Urbana-Champaign. Lab work followed protocol described in Salvante et al. (2012) and assay specific dilution values were determined in conversations with Quansys. Biomarker data were cleaned and compiled using R code developed and written by Lee, Rogers, and Wilson. Only samples with CVs <30% are included in analyses, however, samples with CVs greater than 20% were rerun when able. See supplemental for inter- and intra-assay CV information.

Aligning and scaling menstrual cycle data

Hormone concentrations were aligned across the menstrual cycle by mid-cycle drop date as described in Lipson and Ellison (1996). To incorporate all cycle day data from all participants, we scaled cycle day (Ehrlich et al., 2022). Unscaled data has a variable number of days in each cycle (range 24-32), and we cannot assume that one person's day 10 of 24 is the same or homologous to another person's day 10 of 32. Cycle days were scaled under a geometric morphometric framework outline in Ehrlich et al. (2022), which does not rely on 24-hour days. Rather, this method works by identifying three "fixed landmarks," that can be reliably identified across individuals and truly considered homologous. These landmarks are first day of menses (day -1), day of ovulation (day 0), and first day of next menses (day 1). The remaining days are allowed to "slide" along this scale converting to values between -1 and 1. Missing data was inputted using the k=3 nearest-neighbors and all menstrual cycles were scaled to 28 days, which is the average cycle day length of the total sample (n=98). CRP was quantified for all days across the menstrual cycle for most (n=68) individuals. Many cycles (n=41) were missing only 1-4 days of CRP concentrations, and few (n=8) were missing 5+ days of CRP concentrations. The majority of cycles were missing the first or second day of collection (64%), however our method of imputation of k=3 nearest-neighbors is appropriate for our data (Beretta and Santaniello, 2016). Using k nearest neighbors is appropriate for inferential statistics but the structure of the dataset may change with increasing k. A PCA shows us the structure of our data: similar spreads of individuals despite data missingness tells us that for our specific analysis, k=3 does not alter the structure of the data (see supplemental for more information on the PCA analysis and missingness). This type of scaling ensures that observations are homologous and allows us to include total cycle data instead of truncating some individuals' cycles. Phases derived from the scaled cycle days are defined as early follicular (days <-.7), follicular (days >-.7 to -.2),

periovulatory (days -.2- .25), luteal (days .25 to .52) and late luteal (days >.52); the values for each day correspond to early follicular, follicular, periovulatory, luteal and late luteal definitions of days before -10, days -10 to -3, days -3 to 3, days 3 to 6, and days after 6 respectively (Lipson and Ellison, 1996; Barrett et al., 2013; Clancy et al., 2013). For more information on hormone and cycle day scaling see Ehrlich et al., 2022.

In addition to x axis scaling, we also scaled CRP (48.1ng/ml-1 091.5 ng/ml), E1G (3.9ng/ml-847.5ng/ml), and PdG (363.8ng/ml-244 832.4ng/ml) values to account for the incredibly wide range in hormone concentration. We apply min/max scaling; min/max scaling is similar in concept to z-scores or log transformations, to make massive ranges in a dataset more comparable. However, it is different in that it removes almost all variation due to these disparate ranges. This type of scaling is more sensitive to possible patterns across the menstrual cycle that might have otherwise been hidden by using raw or z score values. This method allows us to control for inter-individual differences in amplitude (concentration), while preserving relative differences in amplitude for each individual. We could then identify patterns across a menstrual cycle that would otherwise be covered by the noise of massive concentration ranges. All analyses were conducted on both the unscaled CRP values and the scaled CRP values, however only scaled will be discussed in depth. The unscaled results can be found in the supplemental. Only scaled E1G and PdG values were used as predictive variables in models, as the ranges of both were too large for unscaled values to be included in models.

Menstrual cycle effects

To test our first and second hypotheses, we developed linear mixed (effects) models (LMM) in order to better understand how menstrual cycle effects (the first three days of menses,

day of cycle, E1G, and PdG) and individual effects might be associated with CRP in each of the studied populations. The first three days of menses were chosen instead of the full number of days an individual was actively bleeding because previous research suggests in the days leading up to menses there is a decrease in anti-inflammatory markers but an increase in proinflammatory biomarkers up until bleeding (Azlan, et al., 2020; Evans and Salamonsen, 2012). CRP generally reaches peak 48 hours after stimulus (Pepys & Hirschfield, 2003) thus, CRP may not be higher in the entire menstrual or follicular phase. Additionally, the first three days of the menstrual cycle are when menstrual repair mechanisms are most active (Clancy et al., 2013). For each sample, from the Polish and Polish American populations, there are two sets of models, one set using unscaled CRP data and one set using scaled CRP data. In these models, CRP is the dependent variable, the first three days of menstruation is a main effect, and the individual is a random effect. We then added day of cycle, E1G and PdG to the models to examine possible full cycle or ovarian hormone effects. Possible cofounders, such as age, body fat percentage and waist to hip ratio are next included in the models. The model sets were constructed adding variables in a step-wise manner.

Conditional Akaike's Information Criteria was used to determine model of best fit: cAIC as opposed to traditional AIC is considered more appropriate for mixed model choice (Saefken et al., 2021; Saefkin et al., 2014; Greven and Kneib, 2010). In contrast to linear regression, which makes many assumptions about data normality and distribution, LMMs are more flexible and account for random individual effects that might be associated with CRP concentrations, making them better suited to these data, which are not normally distributed (Bates et al., 2015; Bolker et al., 2009; Bolker and others, 2022). Finally, confidence intervals and partial R^2 were computed to better interpret the results of the best fitting LMM (Edwards et al., 2008; Jaeger et al., 2016,

Nakagawa and Schielzeth, 2013). P-values are not computed for this type of mixed modeling and are thus not included in results (Bates et al., 2015; Bolker and others, 2022).

Cycle phase and menstruation differences

In addition to the LMMs, to test both of our hypotheses, we also assessed whether or not there are any phase differences of unscaled and scaled CRP concentrations for both the Polish and Polish American samples. We defined phases as early follicular (<-10 days), follicular (-10 to -3), periovulatory (-3 to 3), luteal (3-6) and late luteal (>6) (Lipson and Ellison, 1996; Barrett et al., 2013; Clancy et al., 2013). Data were found to be non-normally distributed, so phase difference was examined by first conducting a Kruskal-Wallace test, which identifies if there are any differences in distribution between all phases but does not tell us which individual phases are different from others. If there was a significant Kruskall-Wallace test, a post-hoc Dunn Test with Bonferroni correction was then performed to identify which groups are different from one another. In order to assess whether the very beginning of the menstrual cycle (defined as the first three days of menses or bleeding) exhibits higher levels of inflammation compared to the rest of the menstrual cycle, we conducted additional Kruskal-Wallace tests for both the Polish and Polish American samples. Alpha was set at .05 for Kruskal-Wallace tests and .05/2 for post-hoc analyses.

Results

Descriptive statistics

The average age of the Polish population was 34 years, with ages ranging from 24-46 years (Table 1). The average age of the Polish American population was considerably younger at 24, with a larger range of 18-42 years (Table 3.1). There are very large ranges and standard

deviations for all of measured hormones in both populations. The Polish population had slightly higher concentrations of C-reactive protein, with an average of 229.2 (ng/ml), compared with the Polish American population, with an average of 205.6 (ng/ml).

Table 3.1: Descriptive statistics for Polish and Polish American samples.

	Polish participants (n=76)		Polish American participants (n=22)	
	Average (sd)	Range	Average (sd)	Range
Unscaled C-reactive protein	229.2 ng/ml (169.0)	48.1-1091.5 ng/ml	205.6 ng/ml (36.9)	97.8- 426.4 ng/ml
Unscaled E1G	105.8 ng/ml (102.2)	4.0- 776.4 ng/ml	95.8 ng/ml (104.0)	10.6-847.5 ng/ml
Unscaled progesterone	13873.0 pg/ml (20,836.5)	412.2-244,832.4 pg/ml	15, 126.0 pg/ml (24,624.1)	363.8-176,371.1 pg/ml
Cycle length	28 days (3.0)	20-38 days	29 days (4.8)	19-42 days
Age	34 years (8)	19-46 years	24 years (8.1)	18-42 years
Body fat %	28.9 (7.7)	8.7-44.5	28.3 (7.0)	16.6-45.5
Waist to hip ratio	.77 (.07)	.67-.95	.75 (.07)	.64-.99

Visually assessing menses and menstrual cycle effects

To first assess the impact of the menstrual cycle and menses on CRP, we graphed both unscaled and scaled CRP data. When cycle day is unscaled CRP does appear to have some

variation through the menstrual cycle (Figure 3.1a). However, the significant variation at the start and end of the cycle could be attributed to cycle effects, to low sample sizes at each end, and/or to different cycle lengths. We scaled cycle day to help solve these problems; when doing so, CRP appears relatively invariant through the menstrual cycle in the Polish population, indicating that linear modeling is appropriate, and that menses or cycle day may not be associated with CRP concentrations (Figures 3.1b and 3.1c).

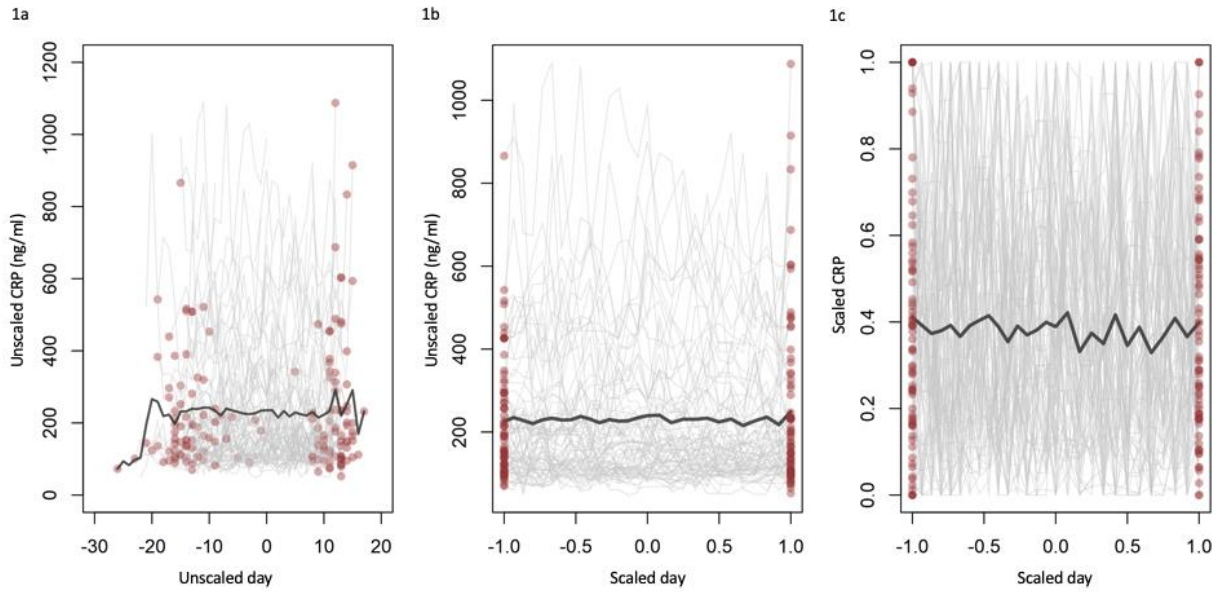


Figure 3.1: Figures showing comparison of unscaled day and unscaled CRP concentrations (1a), scaled day and unscaled CRP concentrations (1b), and scaled day and scaled CRP concentrations(1c). Individuals are Polish and aligned by ovulation (day 0). Red dots represent the first day of bleeding at the start and end of each cycle.

Next, we compared how CRP changes across the menstrual cycle with the different scaling methods for the Polish American population. In this sample, both unscaled day/CRP and scaled day/unscaled CRP suggest no cycle day effect (Figures 3.2a and 3.2b). It is only when both day and CRP are scaled that we see a different pattern in the Polish American sample (Figure 3.2c). CRP is higher at the beginning of the menstrual cycle, with an additional spike and

decrease in the periovulatory period, and a third increase at the very end of the menstrual cycle, suggesting both menses and cycle day effects.

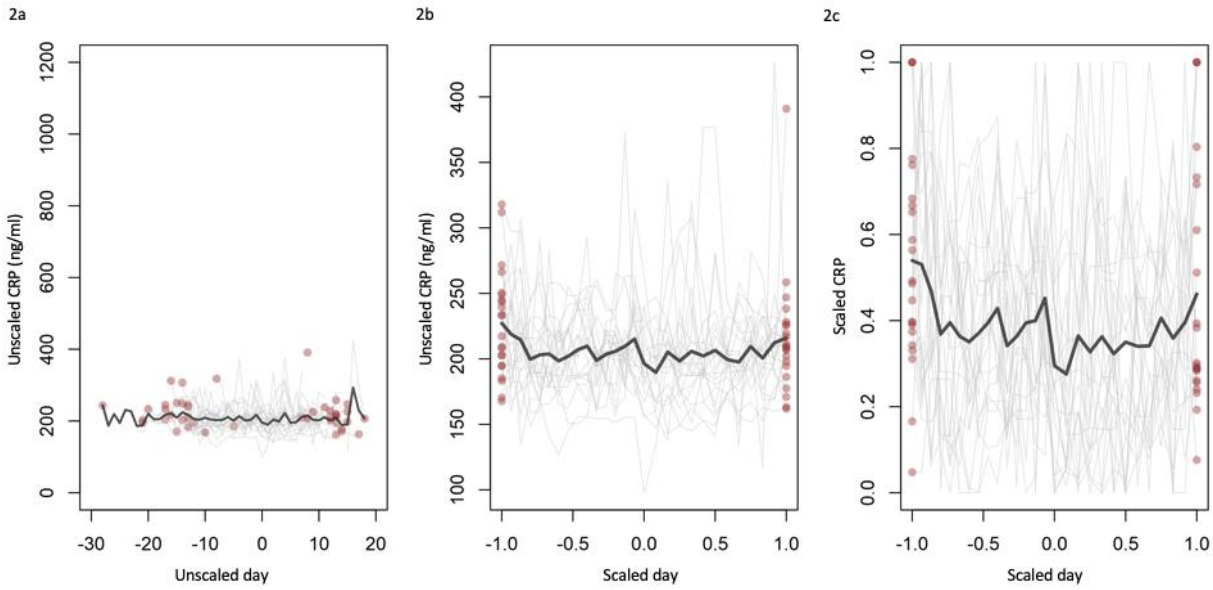


Figure 3.2: Figures showing comparison of unscaled day and unscaled CRP concentrations (2a), scaled day and unscaled CRP concentrations (2b), and scaled day and scaled CRP concentrations(2c). Individuals are Polish American and aligned by ovulation (day 0). Red dots represent the first day of bleeding at the start and end of each cycle.

Assessing first 3 days of menstrual cycle effects and population difference

The models of best fit for both the Polish and Polish American samples were model 4 for the scaled analyses, which in included first three days of menses, cycle day, scaled E1G, and scaled PdG (Table 3.2). Our first hypothesis, that CRP will be higher during menstruation is only partially supported. The Polish scaled model (Table 3.3) explains almost no CRP variation in the population (r^2beta=.01) and shows a negligible, negative effect of after the first three days of menstruation on CRP concentrations (estimate -.02, t value -1.1), meaning there is little to no evidence that menses and tissue remodeling is affecting CRP concentrations. This contrasts with the Polish American sample model (Table 3.4), which explains slightly more variation

(r^2beta=.07) and shows a larger negative effect of not menstruating on CRP concentrations (estimate -.17, t value -5.2).

Table 3.2: cAIC results for the Polish and Polish American scaled CRP models. *model 4 chosen by cAIC for both samples. *CRP - C-reactive protein; E1G - estrone-3-glucuronide, PdG - pregnanediol-3-glucuronide*

		cAIC for Polish Sample	cAIC for Polish American Sample
Model 0	scaled CRP ~ 1+ (1|id)	139.2	-74.9
Model 1	scaled CRP ~ menses + (1|id)	140.8	-98.5
Model 2	scaled CRP ~ menses + cycle day + (1|id)	142.1	-96.5
Model 3	scaled CRP ~ menses + cycle day + scaled E1G + (1|id)	129.0	-112.0
*Model 4	scaled CRP ~ menses + cycle day + scaled E1G + scaled PdG + (1|id)	128.5	-113.5
Model 5	scaled CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + (1|id)	128.7	-113.4
Model 6	scaled CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + body fat % (1|id)	128.9	-113.2
Model 7	scaled CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + body fat % + waist to hip ratio + (1|id)	129.1	-113.1

Table 3.3: LMM results for the Polish scaled CRP model 4. *E1G - estrone-3-glucuronide, PdG - pregnanediol-3-glucuronide*

	Estimate	Std. error	t value	95% CI	r^2beta
Intercept	.39	.02	15.8	.34 - .44	.01 (full model)
After menses	-.02	.02	-1.1	-.06 – .02	.001
Scaled day	-.001	.01	-.10	-.03 – .02	.000
Scaled E1G	.08	.02	3.8	.04 – .13	.009
Scaled PdG	-.03	.02	-1.4	-.08 – .01	.001

The Polish and Polish American LMM results support our second hypothesis that menstrual cycle CRP phenotypes are influenced by population-specific variables in an individual. Not only is the effect of menstruating more pronounced in the Polish American

population (Table 3.4), but E1G concentrations have a larger positive effect on CRP concentrations (estimate .15, t value 4.3) when compared to the Polish sample (estimate .08, t value 3.8). Unscaled results can be found in the supplemental and complement the scaled results (Supplementary Table C.2).

Table 3.4: LMM results for the Polish American scaled CRP model 4. *E1G - estrone-3-glucuronide, PdG - pregnanediol-3-glucuronide*

	Estimate	Std. error	t value	95% CI	r^2beta
Intercept	.52	.04	13.0	.44 – .59	.07 (full model)
After menses	-.17	.03	-5.2	-.24 – -.11	.04
Scaled day	.01	.02	.66	-.03 – .05	.001
Scaled E1G	.15	.04	4.3	.08 – .22	.03
Scaled PdG	-.07	.04	-1.7	-.14 – .01	.005

Testing cycle phase differences between populations

We further tested whether menses or menstrual phase had any effect on CRP concentrations by comparing the medians and distributions of CRP across different cycle phases, as well as a comparison of early menses versus the rest of the cycle. The Polish sample had no significant phase differences for the scaled analyses (Figure 3.3 and Table 3.5). Additionally, there were no significant differences between the first 3 days of menstruation to the rest of the cycle in the Polish sample (Figure 3.4 and Table 3.5). For the Polish sample, there is no evidence that the beginning of menstruation is a time of elevated CRP concentrations or that there are any cycle phase CRP concentration differences. These results complement the linear mixed model results above but do not support our first hypothesis that menstruating is an inflammatory event.

Table 3.5: Medians of cycle phases and the first three days of menses for the Polish population for the scaled data. No significant differences were found.

	Median scaled CRP concentrations
Early follicular	.343
Follicular	.341
Periovulatory	.330
Luteal	.342
Late luteal	.335
First three days of menses	.365
Rest of the cycle	.335

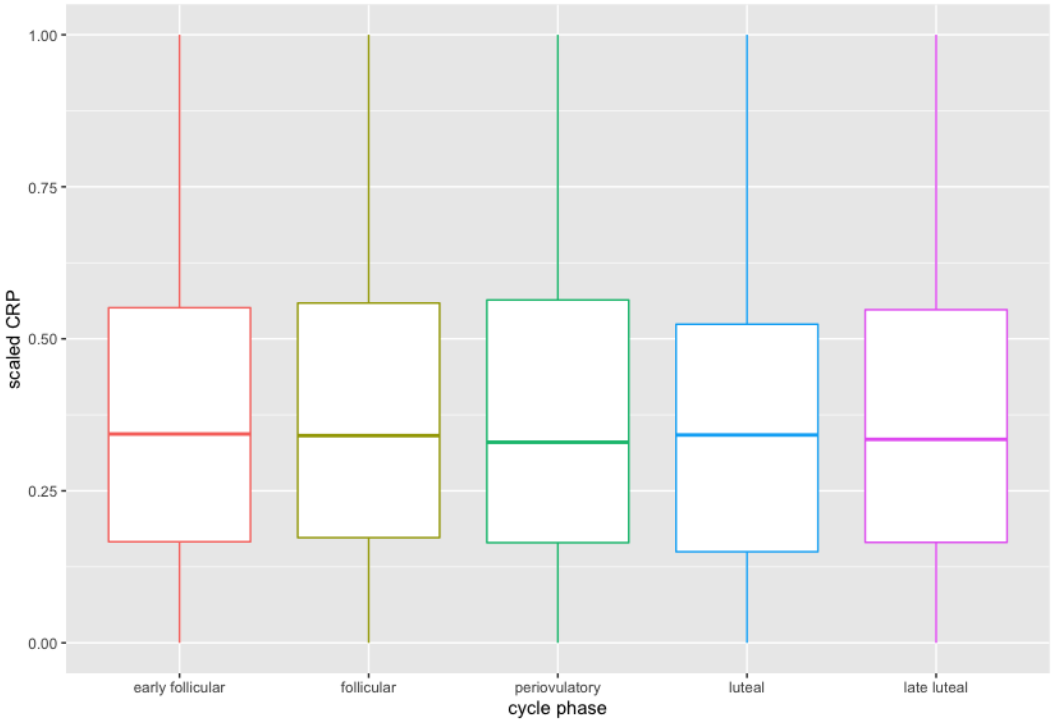


Figure 3.3: Box plots of Polish scaled CRP by cycle phase. No significant differences were found.

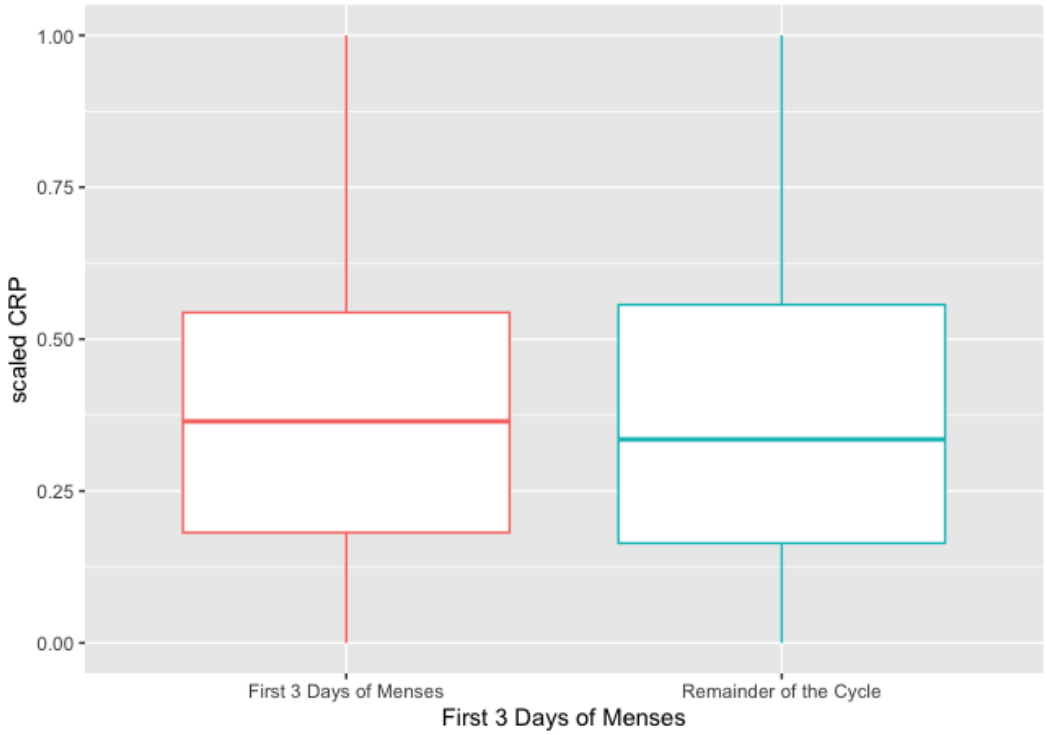


Figure 3.4: Boxplot of Polish scaled CRP between the first three days of menses and the remainder of the cycle. No significant differences were found.

Like the linear mixed model results, the Polish American sample again demonstrated a different pattern compared to the Polish sample, supporting our second hypothesis that there would be population specific menstrual cycle CRP phenotypes. Early follicular phase CRP was higher than periovulatory and luteal phase CRP (Figure 3.5 and Table 3.6). Additionally, we found CRP concentrations from the first three days of menstruation were significantly higher than CRP from the rest of the cycle in the Polish American sample (Figure 3.6 and Table 3.6). These results support our first hypothesis that the first three days of the menstrual cycle will have higher CRP than at other points in the cycle. Unscaled results can be found in the supplemental and complement the scaled results.

Table 3.6: The median scaled CRP values for the Polish American population. **denotes significant group difference by Dunn Test (p<.05) and ^ significant difference by Kruskal-Wallace (p<.01).*

	Median scaled CRP concentrations
Early follicular	.407*
Follicular	.355
Periovulatory	.328*
Luteal	.277*
Late luteal	.364
First three days of menses	.466^
Rest of the cycle	.340^

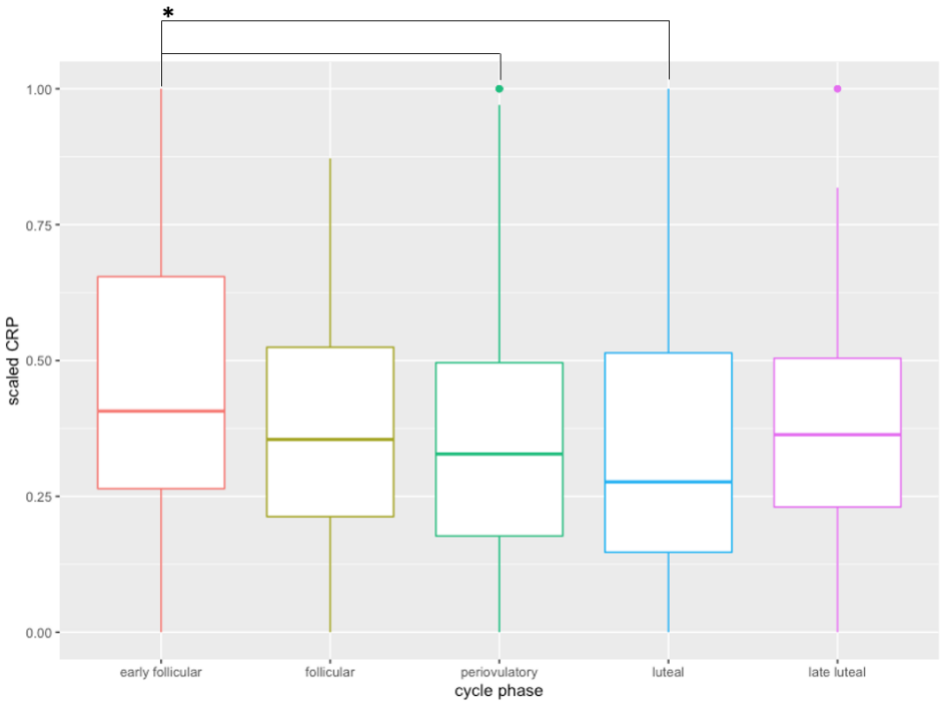


Figure 3.5: Box plots of Polish American scaled CRP by cycle phase. A Kruskal-Wallace test showed significant differences between groups (p<.05) and post hoc Dunn Test showed that the early follicular was significantly higher than the periovulatory and the luteal phase (p<.05). * denotes significant (p<.05) group differences.

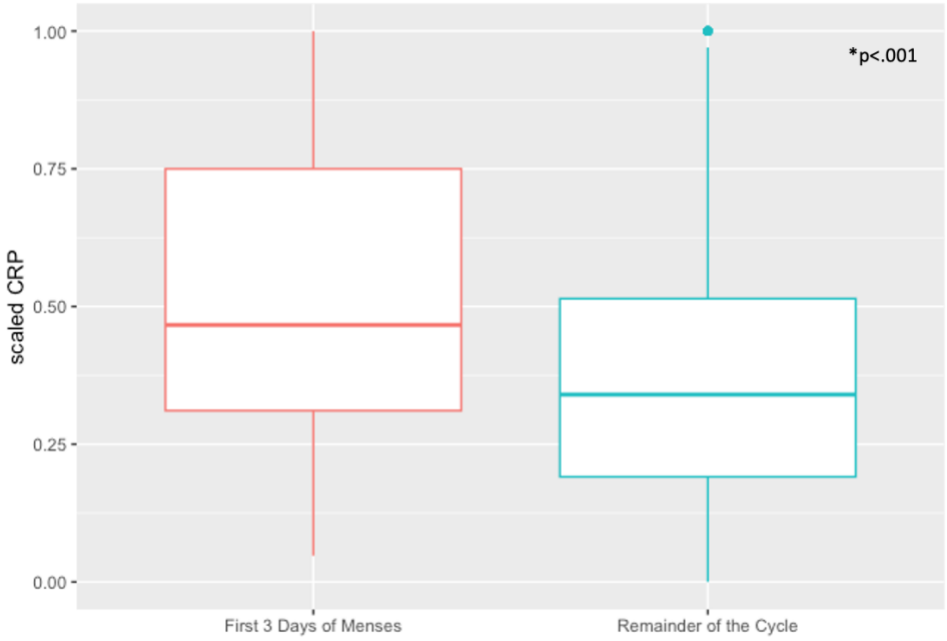


Figure 3.6: Box plots of Polish American scaled CRP comparing the first three days of menses (median = .466) with the rest of the cycle (median = .340). A Kruskal-Wallace test showed significant group difference (p<.001).

Discussion

We sought to better explore and understand CRP variation across the menstrual cycle in two different samples of Polish and Polish American individuals. As mentioned above, CRP could be affected by both external and internal factors. Two possible internal effects derive from ovarian hormones and the tissue remodeling inherent in menstrual bleeding. By examining cycle day effects and cycle phase difference by proxy, we examine both possible internal sources of variation. We tested hypotheses regarding cycle day effects and cycle phase difference in both samples: first, we hypothesized that the start of menses would increase CRP concentrations but that CRP across the rest of the cycle would be non-cyclical, and second, that population-specific variables influence how affected CRP is by menstrual cycle factors in an individual.

Supporting our first hypothesis, the Polish American sample have elevated CRP concentrations at the very beginning of menses, and while there is slight additional cycle

patterning beyond the beginning of menses in the scaled sample, these cycle day effects and phase difference are not statistically significant. Our first hypothesis is only partially supported with the Polish sample, where there were negligible menses and cycle day effects, no significant cycle phase differences, and no significant difference between the beginning of menses versus the rest of the cycle. These pattern differences between the two samples supports our second hypothesis that population has a mediating role in determining cyclicity of CRP.

Visual assessment of the unscaled and scaled CRP for the Polish sample supports the interpretation that menstruating and cycle day effects are not strong or meaningful (Figures 1b and 1c). Both CRP average lines are generally horizontal, and become even more so with the scaled CRP data that allows us to see shape changes without their being overshadowed by amplitude changes. When a linear relationship is near or essentially horizontal, we can hypothesize that the y axis effect is near zero, suggesting that timing of menstrual cycle is not impactful on individuals' CRP concentrations in our Polish sample.

This is in contrast to our Polish American sample. While cycle day still explained a very small amount of variation, included in this model was the first 1 to 3 days of menses, which explained a greater fraction of variation and had a larger estimate. The scaled cycle phase analysis showed that the first three days of the menstrual cycle had significantly higher concentrations of CRP than the rest of the cycle, suggesting that CRP concentrations tend to be higher at this point in the cycle among participants in this sample. This interpretation is supported by our visual assessment of the min/max CRP across the menstrual cycle (Figure 2c), which shows a recognizable increase in CRP at the very beginning of the menstrual cycle. The visual assessment also reveals a possible slight increase right before, and drop right after ovulation, however this cycle day effect might be too small or too short for the linear mixed

model to pick up on or for there to be significant phase differences. The Polish American sample shows a distinct CRP menstrual pattern that likely implies time of sample collection across menstrual cycle should be considered depending on population.

These results support previous findings that suggest that the very beginning of the menstrual phase is likely an inflammatory event that can be picked up by systemic inflammation biomarkers (Azlan, et al., 2020; Clancy et al., 2013; Evans and Salamonsen, 2012). Additionally, they show the usefulness of complete daily menstrual data, as just comparing broad cycle phases was not enough to see this short-term effect. In our scaled Polish American cycle phase analysis, we found that the early follicular phase was significantly higher than the periovulatory and luteal phase, likely driven by the increased CRP concentrations of the first three days of menstruation. These results demonstrate that there are population specific differences in how CRP looks across the menstrual cycle, and that the very beginning of menstruation exhibits higher levels of inflammation in the Polish American sample but not in the Polish sample.

Previous research of cycle differences of CRP has been mixed, with results showing higher follicular CRP (Blum et al., 2005; Gaskins et al., 2012; Vashishta et al., 2017; Wander et al., 2008), higher luteal CRP (Jilma et al., 1997), and no phase differences (Wunder et al., 2006). One possible reason for these discrepancy in results could be population specific differences, and there is clear evidence of average CRP concentration differences between populations (see Shattuck-Heidorn et al., 2020 for summary table). Our paper supports the possibility of there being distinct population differences in how or whether components of the menstrual cycle (e.g., estrogen or progesterone concentrations, endometrial remodeling) impact CRP concentrations.

There are several possible sources of variation that could account for the different relationships found in these two samples. One might be age, as CRP is known to be age

dependent, with CRP increasing as we age (Hutchinson et al., 2000; Pepys and Hirschfield 2003). Additionally, in tests of CRP response and sensitivity, a lower CRP baseline resulted in an increased CRP response to vaccination (McDade et al., 2015), suggesting individuals with lower baseline CRP might have a more sensitive inflammatory response and thus might be more sensitive to changes across the menstrual cycle. The average age of the Polish sample is 34, whereas the average age of the Polish American sample is 24 years, and the Polish sample has a higher average CRP concentration than the Polish American sample. However, when combining the samples and splitting into a young and older group, cycle effects disappeared and both samples resembled the Polish results (see Supplemental section 3), suggesting age is not driving the sample-dependent cyclical patterning observed here. Another possible source is body composition, as higher abdominal adiposity and body fat is associated with higher CRP concentrations (Brooks et al., 2010; Pannacciulli et al., 2001; Tchernof et al., 2002). However, we did not find any cycle day effects or cycle phase differences associated with measures of body fat, including a bioimpedance measure of body fat percentage as well as waist to hip ratio (see Supplemental section 4 and 5).

While population differences may account for some of the variability in menstrual cycle CRP concentration results, another possible reason for the discrepancies we see in the literature is sampling methodology. The studies cited do not have daily sampling across a full cycle, nor are they able to differentiate between early follicular, follicular, periovulatory, luteal and late luteal phase differences (including our own lab's earlier analyses of CRP, see Clancy et al., 2013). Our study includes daily CRP measures across one full menstrual cycle and employs a novel GM approach so that we can include all days of collection. This is in contrast to typical methods of truncating the days analyzed within a cycle in order to account for methodological

issues around variable cycle length and therefore very low sample sizes at either tail of the cycle. Scaling cycle day rather than not analyzing those days (or risking low powered analyses because of aforementioned low samples at those points in the cycle), we now see variation at the very beginning and the very end of the cycle. With scaling of day, we can also now be confident that one person's first three days of menses is homologous to the next person's as opposed to knowing that day 3 in a 12 day follicular phase may not be homologous to another person's day 3 in a 15 day follicular phase. Because of the cycle day scaling method that we used and because we have daily hormone data for an entire menstrual cycle in our sample, we were able to include early follicular (including menses), follicular, periovulatory, luteal and late luteal phases in our analysis with more confidence.

An additional benefit of the GM scaling method is how we scaled CRP using min/max scaling. With this scaling, we are able to uncover sources of variation obscured by large concentration differences. Previously, Ehrlich et al., (2022) demonstrated how a geometric morphometrics approach could help elucidate previously obscured menstrual cycle variation and patterning of ElG. The same geometric morphometric scaling and methods were used to analyze CRP in this paper. This benefit is particularly noticeable when considering the differences between the unscaled and scaled Polish American results (Figures 2b and 2c). Taken together, these results demonstrate how controlling for hormone concentration can provide a more sensitive tool to detect patterns between individuals that might otherwise be obscured by large concentration ranges. This is expected under the principles of geometric morphometrics, which considers size and shape to be two independent factors contributing to the overall form of an object (Dryden and Mardia, 2016; Rohlf, 1999). In other fields of biology, we see how size can be a confounding factor (e.g., allometry) (Jolicoeur, 1963; Jungers et al., 1995).

One last surprising finding, in both models for both samples, E1G had the second largest effect on CRP concentrations. This is in line with previous research that has demonstrated some effect or relationship between estradiol and CRP (Blume et al., 2005; Clancy et al., 2016; Clancy et al., 2013; Gaskins et al., 2012; Wander et al., 2008). However, the direction of the effect in our samples is the opposite of what was found in previous research. The direction of effect in our models is positive, meaning that as E1G increases CRP increases, whereas previous research has found a negative relationship (e.g., Blume et al., 2005; Clancy et al., 2016; Clancy et al., 2013; Gaskins et al., 2012; Wander et al., 2008). While PdG explained very little variation, the direction of effect was also in the opposite direction than in previous literature (e.g., Wander et al., 2008; Jilma et al., 1997). Our results, with E1G associations being positive and those with PdG being negative, more closely matches findings on exogenous hormones, as with studies of hormone replacement therapy and hormonal contraception (Dreon et al., 2002; Gol et al., 2006; Kluft et al., 2002; Kovacs et al., 2005; Skouby et al., 2002; Walsh et al., 2000). However, eligibility criteria meant no participants were taking exogenous hormones, all were spontaneously cycling, and all were premenopausal.

Our results could have broader disciplinary and methodological implications. By visualizing and analyzing CRP across the menstrual cycle in a number of different ways, in two separate samples, we have been able to describe CRP variation in greater detail. Our first hypothesis, that the very beginning of menstruation will have higher levels of CRP, was partially supported. These results suggest that collection during the first three days of menstruation may result in higher concentrations of CRP and may have a confounding effect on results. The results for both populations suggest that the mid to late follicular, periovulatory, luteal and late luteal might be comparable, though it is important to record when during the menstrual cycle data was

collected and adjust for possible population and/or project specific differences. Despite this, cycle day effects for both populations are low and this lack of strong cycle day effects on CRP could make CRP a more appropriate biomarker when infrequent sampling is necessary, compared to other stress biomarkers that vary more with menstrual cycle. This is in contrast to cortisol, which research suggests is consistently more sensitive to cycle day and menstrual cycle effects (e.g., Kirschbaum et al., 1996; Kirschbaum et al., 1999; Viau, 2002; Heck and Handa 2019).

Our second hypothesis, that our two different samples would have different CRP profiles across the menstrual cycle, was supported. These results are important to consider for future studies interested in external effects, like environmental, sociocultural and psychosocial variables, and suggest that, when the biomarker of interest is CRP, the need for daily menstrual cycle measurements or to control the timing of collection for each menstruating participant, depends on population. In our samples neither age nor body composition accounted for these pattern differences, however, there are many other potential sources of this variation. A potential future avenue of research would be to examine whether rural populations (the Polish sample) might have a different menstrual cycle CRP concentration phenotype than suburban populations (the Polish American sample).

While these results provide a detailed look at CRP variation across the menstrual cycle in two populations of Polish and Polish American participants, there are some limitations that should be considered. First, these results are likely not applicable to all populations, all contexts, or all geographic locations. Our population includes individuals of European descent, with the main difference being geographic location. As such these results may not be (and are likely not) applicable to all or more populations that are geographically different or have different lived

experiences with oppression and privilege. Additionally, while the min/max scaling methods we utilized can be used no matter the timing or how often researchers perform biomarker collection, an effective GM sliding scale of cycle day will need multiple data points throughout a menstrual cycle. Finally, the mediums of CRP analyzed, for example, urine versus serum versus salivary, are not always directly comparable. As such, care should be taken when applying these results and our interpretation to other types of CRP or study designs.

Conclusion

Our paper adds to the literature about cyclical variation of CRP across the menstrual cycle and identifies important methodological considerations by showing that a possible reason for the inconsistencies in results may be due to population specific differences and sampling methods which miss short term cycle effects (like the spike in CRP during the first three days of menstruation in the Polish American sample). Additionally, Ehrlich et al. (2022) have already demonstrated the utility of geometric morphometric approach to estrogen and progesterone hormones, namely E1G. This paper further establishes the applicability of these scaling methods on other common biomarkers used to study lived experience, like CRP. Not only does it ensure homology of cycle days in menstrual cycle studies and allows us to include all cycle days for participants, but it also utilizes min/max scaling which controls for inter-individual differences in amplitude (concentration), while preserving relative differences in amplitude for each individual. Future directions should consider where population variation might be coming from. For example, differences in rural versus urban, socioeconomic status, diet, perceived stress, life history events and environmental contexts, could all play a role in the different menstrual patterns that we see between these two populations.

CHAPTER 4: CORTISOL PHENOTYPE VARIES WITHIN A HOMOGENOUS POPULATION OF MENSTRUATING INDIVIDUALS

Introduction

Cortisol, and by proxy hypothalamic pituitary adrenal (HPA) axis function, is one of the more popular biomarkers used as a stand-in for environmental stressors, particularly psychosocial stressors, in biological anthropology studies (e.g. Allison et al., 2019; Desantis et al., 2015; Dubois et al., 2017; Garcia et al., 2017; Legatske and Gettler, 2020; Pollard, 1995; Thayer and Kuzawa, 2014). For studies interested in psychosocial effects via HPA axis function, it is important to account for sources of biological variation, like the menstrual cycle. While there is often an assumption that the menstrual cycle impacts cortisol concentrations, there has been disagreement about when and to what extent this is the case (e.g. Ahn et al., 2011; Gröschl et al., 2001; Kudielka & Kirschbaum, 2003; Wolfram et al., 2011). However, should an individual's responsiveness to ovarian hormones vary, it introduces additional variation into our understanding of cortisol cyclicity.

The HPA axis is an environmentally responsive feedback loop, through which the body can respond to a constitutive stressor. HPA axis function can be examined by measuring cortisol concentrations. The HPA axis is a highly evolutionarily conserved system found in reptiles, amphibians, fish, and mammals that generally shows increased activation when an animal is confronted with a stressor (Sapolsky, 2021; Thayer et al., 2018). The HPA axis itself is highly variable and can be assessed in a number of different ways. For example, we see that both acute stressors (short term that can range in severity) and increased chronic stress (long term stress that can range in severity) over time are associated with an overall increase of glucocorticoids

(Wingfield & Kitaysky, 2002). However, HPA axis reactivity to an acute stressor is affected by chronic stress, which is associated with HPA axis suppression (Matthews et al., 2001) and a blunted diurnal cortisol curve (Miller et al., 2007).

However, we do not know how acute and chronic effects on cortisol interact with cycle variability. Previous research has found that there is some cortisol variation between cycle phases. A recent meta-analysis found that cortisol is higher during the follicular phase, though these results are from studies which collected saliva, blood, or urine, and used variable cortisol measures (e.g., 12h, 24h, morning, afternoon, cortisol awakening response (CAR)) (Hamidovic et al., 2020). However, type of cortisol measure likely has a large impact on results. For example, in a sample of German individuals, researchers found that the increase of salivary cortisol response during awakening was increased during ovulation, but there were no differences between follicular and luteal phases (Wolfram et al., 2011). However, a different study of Korean individuals examining the area under the curve of salivary CAR found no differences across the menstrual cycle (Ahn et al., 2011). Yet another study of young (age 15-22) German participants found no difference between follicular and luteal phases for waking salivary cortisol (Gröschl et al., 2001). Bao et al., (2004) examined the 24 hour salivary diurnal cortisol curve in a sample of Chinese adults and found differences between menses and the periovulatory and late luteal phases in terms of width of morning cortisol peak.

On source of cortisol variation across the menstrual cycle is the hypothalamic pituitary ovarian (HPO) axis, specifically the ovarian hormones of estrogen and progesterone. The HPO and HPA axes are interconnected and influence each other (e.g. Carey et al., 1995; Handa et al., 1994; Heck and Handa 2019; Kirschbaum et al., 1996; Kudielka et al., 1999; Kudielka and Kirschbaum 2005; Viau, 2002). Studies have found a relationship between estrogen

concentrations and cortisol, showing that estrogen replacement therapy is associated with increased cortisol (Edwards & Mills, 2008; Hampson & Duff-Canning, 2016) and there is some evidence that the peri-ovulatory phase (when estrogen is highest) is associated with increased cortisol (Wolfram et al., 2011). Progesterone has also been positively associated with cortisol concentrations in people on hormonal birth control, but not in spontaneously cycling individuals (Wirth et al., 2007). However, progesterone and cortisol are positively associated with one another in cycling individuals when collected during the follicular phase (Herrera et al., 2016; Nepomnaschy et al., 2004) and through the luteal except for mid luteal (Nepomnaschy et al., 2004). Progesterone has also been associated with increased stress (Herrera et al., 2016) and follows a similar diurnal pattern as cortisol (Gröschl et al., 2003), though this pattern is blunted in postmenopausal people and during menstruation (Ahn et al., 2011).

As evidenced above, there are numerous studies on the impact of the menstrual cycle and various cortisol phenotypes (i.e., CAR, waking cortisol, cortisol reactivity, etc.), however, results are inconsistent and suggest lots of variation. There could be several reasons for these discrepancies. First, depending on what aspect of cortisol is being studied and the medium of sample collected, results might not be comparable. While correlations between salivary, serum, plasma, and urinary cortisol are high there is high inter individual variation (Aardal and Holm, 1995; Levine et al., 2007; Neary et al., 2002). Additionally, medium might be representing different things. For example, for measures of waking cortisol, salivary cortisol is a snapshot of current state and reflects diurnal activity whereas urinary cortisol is an average measure of cortisol exposure over the nighttime and since last urination (Jerjes et al., 2006; Sarkar et al., 2013). Additionally, the studies above did not include daily sample collection through the menstrual cycle. This lessens the ability to determine the reliability of the differences found and

the confidence that sample collection timing is consistent across all menstrual cycles (as cycles themselves are variable lengths). Researchers may also use different methods to determine timing of collection (e.g. recall of first day of last period, urinary luteinizing hormone test) and align cycles (e.g. aligning by ovulation, day of first period, reverse cycle day). Finally, these studies take an average or pool together their sample and compare menstrual phases or effects without first exploring whether menstrual cycle effects are consistent within sample.

Our paper aims to better explore how consistent menstrual cycle effects are, or are not, within a relatively homogenous sample. We perform a unique application of geometric morphometric methods on a sample of spontaneously cycling Polish individuals which includes daily urinary collections, providing a rich data set with the potential for greater insight into why there is a lack of consensus in the existing literature on the HPA axis and the menstrual cycle. To accomplish this, we ask the two main questions and propose the following hypotheses:

Question 1: What does cortisol look like across the menstrual cycle? Is there a cyclical pattern and are there within population phenotype differences?

Hypothesis 1_0: There will be no average sample cyclical pattern of cortisol across the menstrual cycle.
Hypothesis 1_a: There will be a sample average cyclical pattern of cortisol across the menstrual cycle.

Hypothesis 2_0: There will be no within sample variation of menstrual cycle cortisol patterning.
Hypothesis 2_a: There will be within sample variation of menstrual cycle cortisol patterning, with varying levels of "responsiveness" to the menstrual cycle.

Question 2: Are ovarian hormones a source of cortisol variation? Are these different cortisol phenotypes associated with ovarian hormone characteristics?

Hypothesis 3_0: E1G shape or exposure will not vary across cortisol phenotype groups.
Hypothesis 3_a: E1G shape and exposure will vary between cortisol phenotypes.

Hypothesis 4_0: PdG shape or exposure will not vary across cortisol phenotype groups.
Hypothesis 4_a: PdG shape and exposure will vary between cortisol phenotypes.

While we are not able to address every possible source of variation due to methods (for example, type of sample as we only have daily morning void urine), we can begin to address some of the other possible sources of cortisol variation across the menstrual cycle. First, our study has daily cortisol and ovarian hormone measures across one full menstrual cycle, better accounting for the issues of sampling, timing, and alignment. Second, instead of only examining overall sample average, we statistically examine whether there is menstrual cycle cortisol variation within sample by applying a unique geometric morphometric analysis that allows us to analyze and compare cortisol shapes across an entire menstrual cycle.

Materials and methods

Data collection and population

These data were previously collected over 2014-2017 for the Sto Lat Ecological Determinants of Health project at the Mogielica Human Ecology Study Site in the Beskid Wyspowy region of southern Poland (Jasienska and Ellison, 2004) includes 76 ovulatory menstrual cycles. Research protocols were approved by the University of Illinois at Urbana-Champaign Institutional Review Board (UIUC IRB #13856), and all participants provided informed consent. Participants were native Polish, healthy, regularly menstruating, non-smoking, not breastfeeding, not on any form of hormonal birth control, and ranged in age from 18-46. Participants collected morning void urine daily starting on the first day of menstruation and collecting until the start of their next period. Samples were stored in participants' freezers until researchers could pick up samples. The sample consists of a transitioning subsistence agricultural

environment and participants experience moderate levels of physical activity (Colleran, 2014; Jasienska et al., 2006; Lee et al., 2019). See Table 4.1 for sample characteristics.

Laboratory analysis

Urine samples were stored in -20 freezers at the University of Illinois, Urbana-Champaign until samples could be assayed. Samples were aliquoted into 1 ml tubes for assays to avoid numerous freeze/thaw cycles. Specific gravity was measured (refractometer PAL-3, Atago U.S.A., Inc., Bellevue, WA) to adjust for hydration levels (Miller et al., 2004). Urinary estrone-3-glucuronide (E1G, a metabolite of estrogen), Pregnanediol-3-glucuronide (PDG, a metabolite of progesterone), and cortisol were assayed using the Quansys BioSciences ELISA multiplex system at the Clancy Lab at the University of Illinois, Urbana-Champaign. Lab work followed the protocol described in Salvante et al. (2012) and assay specific dilution values were determined in conversations with Quansys. Biomarker data were cleaned and compiled using R code developed and written by Lee, Rogers, and Wilson (2020). Sample CVs were <30%, however all samples with CVs >20% were rerun when possible. See supplemental for inter and intra assay CV information.

Aligning and scaling menstrual cycle data (hypothesis 1)

Hormone concentrations were aligned across the menstrual cycle by mid-cycle drop date as described in Lipson and Ellison (1996). To incorporate all cycle day data from all participants, we scaled cycle day (Ehrlich et al., 2022). Unscaled data has a variable number of days in each cycle (range 24-32), and we cannot assume that one person's day 9 of 20 is the same or homologous to another person's day 9 of 38. Cycle days were scaled under a geometric

morphometric framework outline in Ehrlich et al. (2022), which does not rely on 24-hour days. Rather, this method works by identifying two "fixed landmarks," that can be reliably identified across individuals and truly considered homologous. These landmarks are first day of menses (day -1) and first day of next menses (day 1). The remaining days are allowed to "slide" along this scale converting to values between -1 and 1. All menstrual cycles were scaled to 28 days, which is the average cycle day length of our sample. This type of scaling ensures that observations are homologous and allows us to include total cycle data instead of truncating some individuals' cycles.

We also scaled cortisol (30ng/ml-2,183.6ng/ml), E1G (3.9ng/ml-847.5ng/ml), and PdG (363.8ng/ml-244,832.4ng/ml) values to account for the incredibly wide range in hormone concentrations. We apply min/max scaling (MMS); min/max scaling is used to make massive ranges in a dataset more comparable and is similar in concept to z-scores. However, it is different in that it removes almost all variation due to these disparate ranges. This type of scaling is more sensitive to possible patterns across the menstrual cycle that might have otherwise been hidden by using raw or z score values. This method allows us to control for inter-individual differences in amplitude (concentration), while preserving relative differences in amplitude for everyone. We can then identify average patterns across the menstrual cycle in our whole sample that would otherwise be covered by the noise of massive concentration ranges (hypothesis 1). Missing data was inputted using k=3 nearest-neighbors. Cortisol was quantified for all days across the menstrual cycle for most (n=41) individuals. Many (n=13) are missing only 1 day; N=7 are missing 2 day; N=6 are missing 3 days; N=3 are missing 4 and 5 days each (n=6 total); and N=6 are missing 8 or more days (max13). The majority of cycles were missing the first or second day of collection (64%), however our method of imputation of k=3 nearest-neighbors is appropriate

for our data (Beretta and Santaniello, 2016). Using k nearest neighbors is appropriate for inferential statistics but the structure of the dataset may change with increasing k. A PCA shows us the structure of our data: similar spreads of individuals despite data missingness tells us that for our specific analysis, k=3 does not alter the structure of the data (see supplemental for more information on the PCA analysis and missingness). For more information on hormone and cycle day scaling see Ehrlich et al., 2022.

Geometric morphometric analysis (hypothesis 2)

We evaluate cycle cortisol phenotype(s) through the following steps. First, we used Principal Component Analysis to explore the primary axes of shape variation (Kaiser, 1960). TO determine meaningful PCs, we used the minimum proportion of an input variable. In the context of this study, the hormone values of each day were considered as separate variables. Each of the 28 intervals can therefor contribute 1/28 = 3.6% which we use as our threshold to limit meaningful components. This value is the contribution of a single day to the overall cycle as defined by the Kaiser-Guttman rule (Kaiser, 1960). Next, we conducted Cluster Analysis on these PC scores to infer subgroups (Ward, 1963). The R package NBclust (Charrad et al., 2014) was used to evaluate significant groupings via the Jaccard coefficient. Average and individual phenotypes are plotted for each subgroup, and permutation testing was carried out using the Morpho package (Schlager, 2017) in R to evaluate inter-group distances. At 10,000 rounds of testing a p-value less than .05 indicates significantly different groups/group shapes. These steps allow us to first identify meaningful shape and pattern differences within the whole sample. It then allows us to determine if individuals sort into statistically significant phenotypes based on

the shape and pattern characteristics of each individual menstrual cycle cortisol curve (hypothesis 2). Alpha was set at .05 for all analyses.

The R package moRphomenses (Ehrlich, 2022) was developed to facilitate analyses and make the method accessible, and additional methodological details can be found there. The GM approach used here is broken down into several distinct steps, as described above: 1) alignment and scaling of cycles; 2) scaling of hormone concentration; 3) imputation of missing data; and 4) evaluation of cycle morphology. This last step includes using Principal Component Analysis to describe shape trends, Cluster Analysis to characterize and infer subgroups, and evaluated using permutation testing of inter-group distances, the Euclidean distance of PCA coordinates. For more information on only of these steps see Ehrlich, 2022.

Relationship between cortisol, E1G and PdG (hypotheses 3 and 4)

We assess whether there are any connections between cortisol, E1G and PdG two different ways. First, we assessed whether E1G shape is associated with cortisol shape. To do this, we first conducted the same GM analysis on E1G for each individual. Then, we evaluated whether there were any E1G shape trends across cortisol phenotypes by conducting a two-block partial least squares analysis (Rohlf, 2000). This type of analysis tests whether our different individual cortisol phenotypes are correlated to our individual E1G phenotype shapes. Additionally, we computed area under the curve (AUC) of unscaled E1G concentrations for each individual and tested whether average AUC of E1G of each cortisol phenotype group was different with a pairwise comparison using t-tests and FDR correction. This second analysis tests whether overall E1G exposure across the entire menstrual cycle is different between our cortisol phenotype groups (hypothesis 3). Next, we conduct the same gm scaling, two-block partial least

squares, AUC, and t tests with FDR correction analyses on PDG to assess whether PdG shape or PdG exposure are different between our cortisol phenotype groups (hypothesis 4). Alpha was set at .05 for all analyses.

Table 4.1: Descriptive statistics for Polish sample that includes averages, standard deviations, and min-max range of cortisol, E1G, PdG, cycle length, and age for each phenotype group.

	Group 1 (n=76)		Group 1 (n=76)		Group 1 (n=76)	
	Average (sd)	Range	Average (sd)	Range	Average (sd)	Range
Unscaled Cortisol	272.1 ng/ml (227.3)	30-2,183.6 ng/ml	264.5 ng/ml (165.7)	15.1-1,269.6 ng/ml	253.9 ng/ml	31.4-976.1 ng/ml
AUC Cortisol	.45 (.11)	.25-.64	.64 (.10)	.39-.89	.82	.62-1.1
Unscaled E1G	96.5 ng/ml (97.7)	4.0-1,042.8 ng/ml	95.0 ng/ml (85.6)	9.4-757.4 ng/ml	119.3 ng/ml (106.1)	15.3-682.3 ng/ml
AUC E1G	.56 (.19)	.22-1.05	.53 (.14)	.24-.86	.58 (.18)	.24-.87
Unscaled PdG	14,325.5 pg/ml (19,333.5)	412.3-151,185.3 pg/ml	11,382.1 pg/ml (14,399.1)	576-146,175.4 pg/ml	19,545.2 pg/ml (27, 049.3)	747.7-244,832.4 pg/ml
AUC PdG	.49 (.12)	.26-.77	.50 (.14)	.22-.81	.43 (.10)	.25-.66
Cycle length	28.2 days (2.75)	21-33 days	27.5 days (3.6)	20-38 days	27.2 days (2.2)	23-32 days
Age	34.1 years (8.2)	19-46.4 years	32.3 years (8.3)	19.1-45 years	36.2 years (6.6)	25-45 years

Results

Hypothesis 1: Average sample cyclical pattern of cortisol across the menstrual cycle

After scaling both cycle day and cortisol, there appears to be some menstrual cycle patterning of cortisol in the whole sample (Figure 4.1). There is a slight increase of cortisol during the very beginning of the follicular phase, a small spike right before ovulation, and an increase in the latter half of the luteal phase. If there was no average sample cyclical pattern of cortisol across the menstrual cycle then we would see a relatively straight line across the entire curve, however, we can see a noticeable pattern that partially resembles an average estrogen pattern, supporting our first hypothesis.

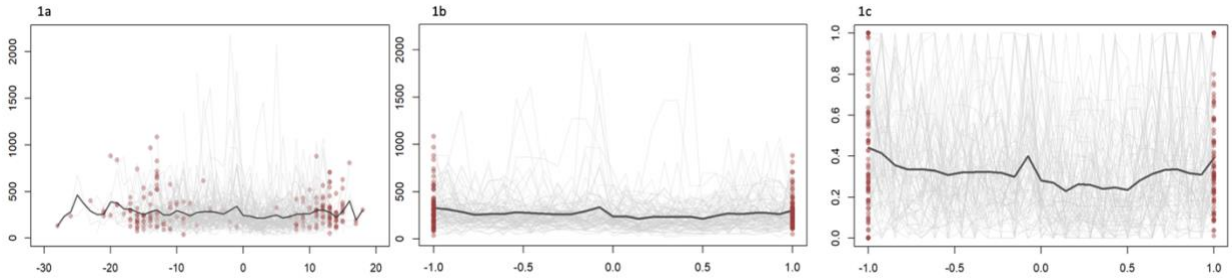


Figure 4.1: Figures showing comparison of unscaled day and unscaled cortisol concentrations (1a), scaled day and unscaled cortisol concentrations (1b), and scaled day and scaled cortisol concentrations(1c). Individuals are aligned by ovulation (day 0). Red dots represent the first day of bleeding at the start and end of each cycle.

Hypothesis 2: Within sample variation of menstrual cycle cortisol patterning

Our geometric morphometric analysis of cortisol results in three significantly different phenotype groups, supporting our second hypothesis (Figure 4.3: group 1, in red, n=29; group 2, in green, n=27; group 3, in blue, n=23). Individuals were grouped based primarily on two PCAs that explained 25% of the variation and show clear clustering when visualized on an XY coordinate (Figure 4.2a) (for more information on PCA3 and onward, see supplemental). PCA 1

is namely differentiated by higher versus lower MMS cortisol values (Figure 4.2b) and PCA 2 is characterized by low follicular, high luteal, and not periovulatory spike versus high follicular, low luteal, and a periovulatory spike of MMS cortisol values (Figure 4.2c).

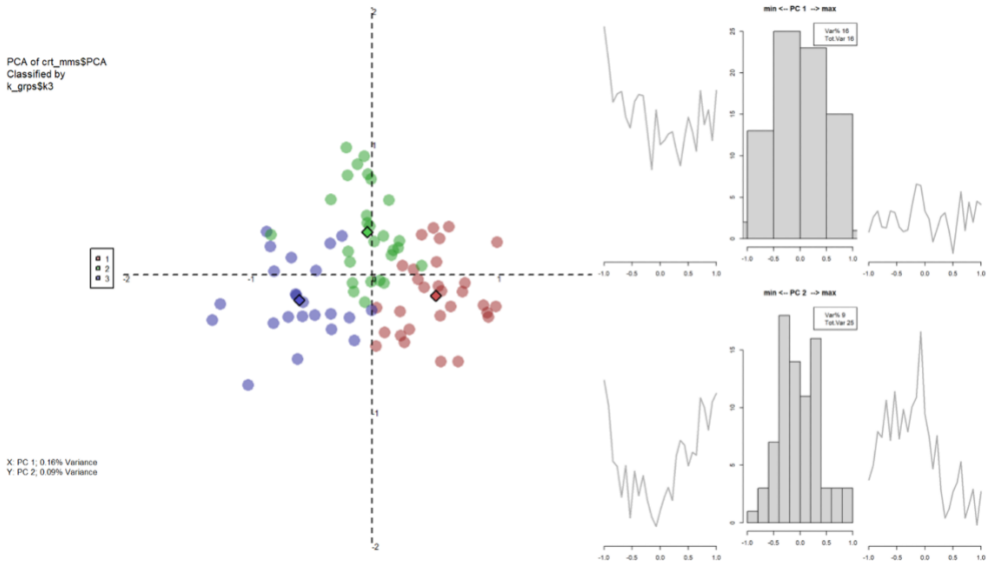


Figure 4.2: PCA plot of scaled cortisol showing how individuals cluster across our two main PCAs (2a). Bar graph showing distribution of individuals across PC 1 and the most extreme phenotypes of PC 1 (2b). Bar graph showing distribution of individuals across PC 2 and the most extreme phenotypes of PC 2 (2c).

Group 1 has larger, more variable spikes of cortisol across the cycle (Figure 4.3a). Group 2 most closely resembles the overall sample average and likely drove much of that curve (Figure 4.3b). Group 3 has a large spike at the very start of menses and an increase in the late luteal phase but is otherwise invariable through the cycle (Figure 4.3c). The main feature all three groups share is a slight spike right before ovulation. See supplemental for more detail on pairwise t test comparisons between cycle phases.

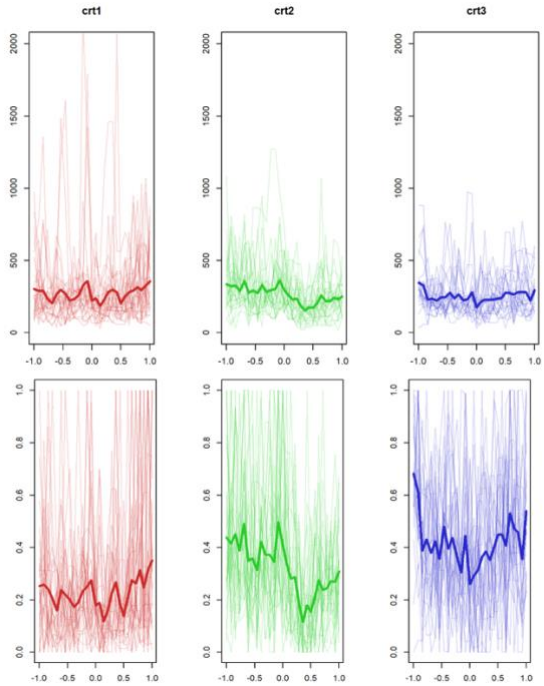


Figure 4.3: Unscaled (top) and scaled average (bottom) cortisol phenotypes of the three significant cortisol groupings.

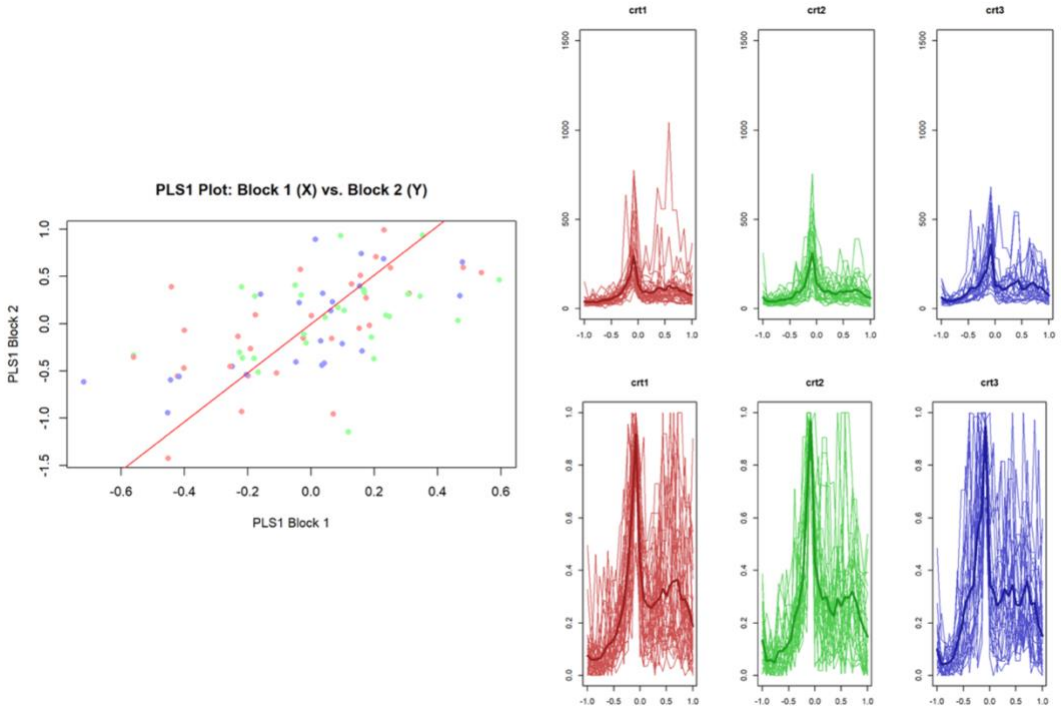


Figure 4.4: Figure 4a two-block partial least squares results comparing individual cortisol shape and individual E1G shape (r=.58, p>.05). Figure 4b shows average unscaled and scaled E1G shapes of each cortisol phenotype type.

Hypothesis 3: E1G shape and exposure across cortisol phenotype groups

There was no significant association of E1G shape (Figure 4.4) nor were there any significant differences of E1G AUC between the three cortisol phenotype groups (Figure 4.5) and thus we are not able to reject our null hypothesis, that E1G shape or exposure will not vary across cortisol phenotype groups. However, there was a near significant result where group three had higher E1G exposure compared to the variable and the responsive (p=.12 for both group 1 and group 2 comparisons).

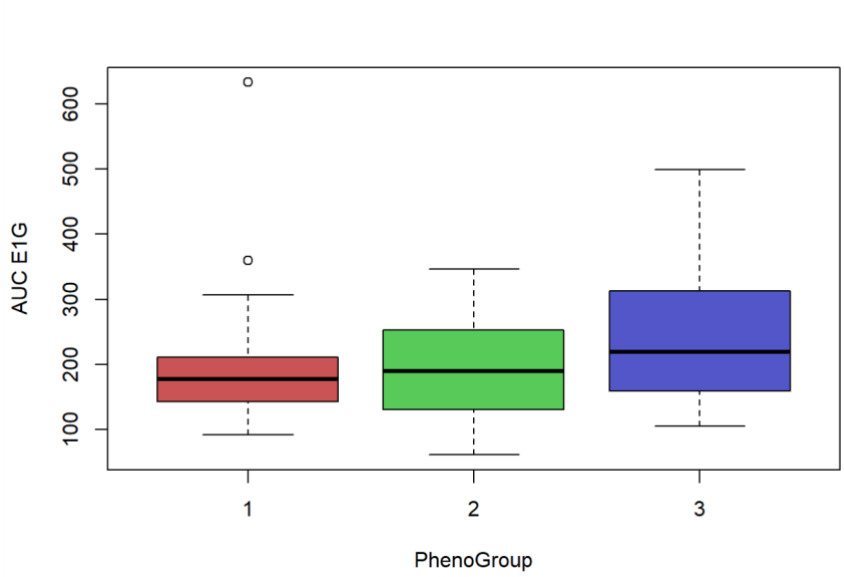


Figure 4.5: Box plot showing the E1G AUC values for group 1, 2, and 3. No significant differences, but group 3 has nearly significant increased E1G AUC (p=.12 for both group 1 and group 2 comparisons).

Hypothesis 4: PdG shape and exposure across cortisol phenotype groups

There is a significant correlation between individual PdG phenotypes and individual cortisol phenotypes (r=.64, p<.05, Figure 4.6a). Further, we see distinct PdG curves in each cortisol phenotype group (Figure 4.6b). Additionally, group three showed the highest AUC

values for unscaled PdG compared to groups one and two (p<.01, Figure 4.7). Also, group two had the lowest PdG, and was nearly significantly different from group three's PdG (p=.1, Figure 4.7). These results partially support our fourth hypothesis.

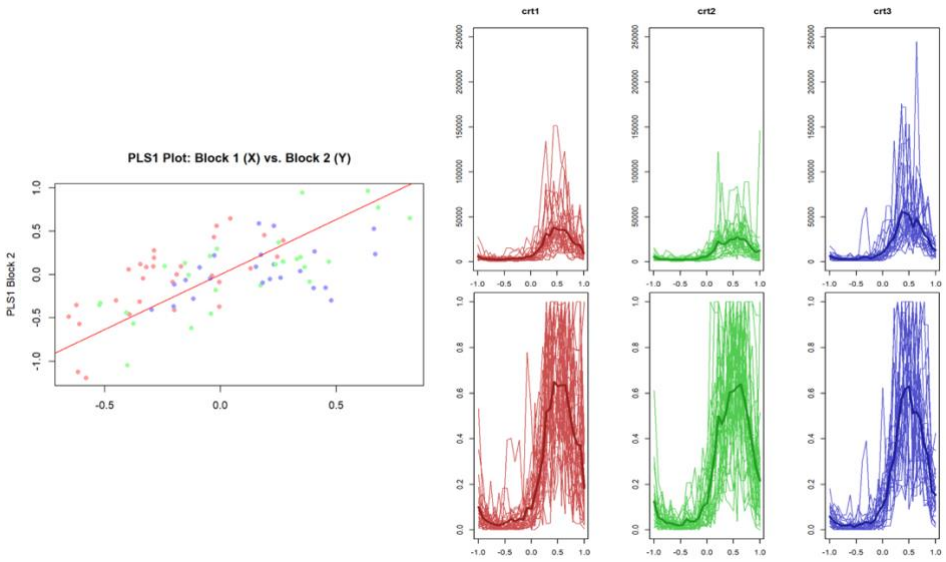


Figure 4.6: Figure 6a two-block partial least squares results comparing individual cortisol shape and individual PdG shape (r=.64, p<.05). Figure 6b shows average unscaled and scaled PdG shapes of each cortisol phenotype type.

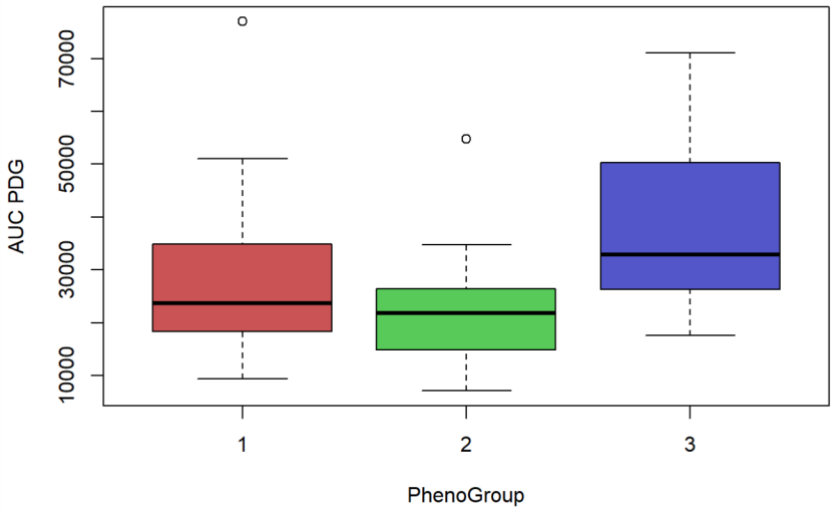


Figure 4.7: Box plot showing the PDG AUC values for group 1, 2, and 3. Group 3 has significantly increased PDG AUC (p<.01) compared to both group 1 and group 2 comparisons.

Visualizing cortisol, E1G and PDG together

Finally, when cortisol, E1G and PDG are graphed together, we can see that the responsive group has a convergence of cortisol, E1G and PdG just after ovulation, whereas the variable group is the least well matched, and the consistent group somewhere in between the two (Figure 4.8). In other words, this convergence represents that point at which E1G and cortisol are falling at the same time PDG is rising in responsive group.

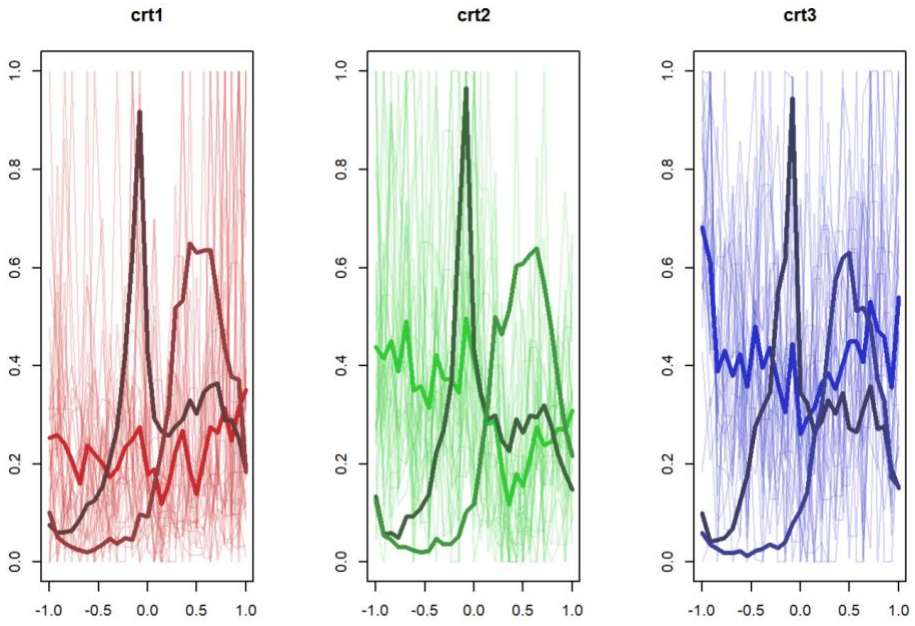


Figure 4.8: Average scaled curves of E1G, PdG and cortisol for each group. Group 1 (red) is the variable group, group 2 (green) is the responsive group, and group 3 (blue) is the consistent group.

Discussion

This study attempted to better explore cortisol variation across the menstrual cycle within a generally homogenous sample of spontaneously cycling Polish individuals. We conducted these analyses with the goals of accounting for several sampling issues and of testing the assumption that menstrual cycle effects are universal within a population. By utilizing a unique application

of geometric morphometrics and analyzing daily waking urinary samples across one full menstrual cycle, we provide evidence that menstrual cycle effects are unlikely to be universal. Our first hypothesis, that there is an average, overall cyclical sample shape to cortisol was supported, suggesting that there are menstrual cycle effects on cortisol. However, our second hypothesis, that there would be within sample variation of menstrual cycle cortisol patterning, was also supported and we found three statistically significant cortisol phenotype groups. Our third hypothesis, that E1G would be different across our cortisol phenotype groups was not supported with either of our analyses. However, our final hypothesis, that PdG would vary by cortisol phenotype was supported. We found that both individual cycle PdG shape was correlated with cortisol shape and overall PdG exposure was highest in the third group.

Overall sample average cortisol shape

Prior research on cortisol across the menstrual cycle has been very mixed. Some studies have found higher waking cortisol in the follicular phase (Hamidovic et al., 2020). Some studies have found no cycle phase differences (Ahn et al., 2011; Gröschl et al., 2001) and some have found only periovulatory cortisol to be higher with no other phase differences (Wolfram et al., 2011). The results for cortisol reactivity have been equally mixed (e.g. Andreano, Arjomandi, & Cahill, 2008; Duchesne and Pruessner, 2013; Kirschbaum et al., 1999; Maki et al., 2015; Montero-Lopez et al., 2018; Villada et al., 2017). The variable findings from past research could be due to either methodological issues, such as sampling frequency, and/or to menstrual cycle effects being highly variable between individuals. Our paper, with daily samples, novel aligning, and unique scaling techniques, helps to account for issues of sampling frequency and provides a much more fine-toothed exploration of cortisol across the menstrual cycle. With this, we can see that there is

an overall cyclical cortisol curve across the menstrual cycle. However, what this overall average obscured is even greater cyclical variation within our own sample.

Three distinct cortisol phenotypes

Within our own sample, we have identified three distinct menstrual cycle cortisol phenotypes. The first group appears to have the most cortisol variation, with large, inconsistent spikes across the entire menstrual cycle and a massive unscaled cortisol range. The second group has a distinct menstrual cycle pattern for both the unscaled and the scaled average shapes. This group follows a more typical menstrual pattern with a more noticeable mid cycle spike and shows clear elevation of cortisol during the follicular phase and a decrease in cortisol during the luteal phase. Though it looks like it has the highest MMS values and some variability in the MMS scaling, the third group has the most consistent cortisol concentrations across the menstrual cycle when we look at the unscaled data, except for the first few days of menses. The MMS scaling removes most variation due to amplitude so that we can more clearly see shape patterning. Thus, in groups with massive unscaled ranges, like the group 1, MMS values will appear lower, whereas groups with a smaller range, like group 3, will appear to have higher MMS values. This group also has the highest cortisol exposure of the three, though it is not significant (see supplemental). Interestingly, all groups were around the same size (n ranged from 23-29), suggesting that these groups are equally represented in this sample and likely representing meaningful phenotypes.

The variation in cortisol phenotypes that we found within our homogenous sample of spontaneously cycling Polish individuals is important to describe, as it could be one of the potential reasons for the large amount of variation we see in the literature about cortisol and

cycle day effects. Additionally, most of the studies on menstrual cycle variation of cortisol are only able to take a few days of samples across a cycle. Depending on sampling frequency and timing of collection, this could have a large impact on results, especially if a sample consists of many variable individuals who have large increases and decreases of cortisol across the entire cycle.

E1G and the cortisol phenotypes

To explore other potential reasons for why we see this variation in cortisol phenotype and responsiveness to the menstrual cycle we asked whether ovarian hormones, namely estrogen and progesterone, might be associated with these phenotypes. Prior research on cortisol and estrogen has generally found that exogenous estrogen is associated with an increase in cortisol (Edwards & Mills, 2008; Hampson & Duff-Canning, 2016). However, another study of post-menopausal people found no difference of 24 hour urinary cortisol concentrations between the estrogen replacement group and the no replacement therapy group (Prinz et al., 2001). Our results follow this, as we found no relationships between estrogen and cortisol.

A possible explanation for this finding is that estrogen, in contrast to cortisol, is less responsive. While there is evidence that estrogen concentrations are affected by ecological constraints (Ellison, 1990; Jasienska and Ellison, 1998; Vitzthum, 2009), developmental effects (Apter, Reinilä and Vihko, 1989), energetic stressors (De Souza and Williams, 2004; Williams et al., 2010), and psychosocial stressors (Allsworth et al., 2007), there is also evidence that estrogen is not related to or impacted by environmental and internal variables, like possible cortisol cyclicity. An alternate explanation could be that the effect of estrogen in our sample is too small to be seen in our sample size. While we did not see any significant correlation between estrogen

phenotypes and cortisol phenotypes, nor was estrogen exposure different across our cortisol groups, we do see a small spike in all three groups right before ovulation that corresponds with the estrogen spike. This qualitative assessment is supported by previous research (Wolfram et al., 2011).

PdG and the cortisol phenotypes

In contrast to our estrogen results, PdG shape was correlated with cortisol shape and overall PdG exposure was highest in group 3. This is in line with some previous research which has found a positive association between progesterone and cortisol (Herrera et al., 2016; Wirth et al., 2007), suggesting a relationship between the menstrual cycle cortisol curve and progesterone curve. However, our results are in the opposite expected direction, as group 3, which also had the lowest cortisol concentrations, had the highest concentrations of progesterone across the menstrual cycle. One explanation for these results is that progesterone, like cortisol, is an environmentally responsive hormone, meaning that it responds to sociocultural context, behavior, and life history events (e.g. Ellison et al., 1993; Ellison and Lager, 1986; Jasienska et al., 2017; Nunez-De La Mora, 2008; Warren, 1980). Other research has shown that progesterone is associated with decreased stress and anxiety in animal models (Frye and Walf, 2004; Picazo and Ferna, 1995), but with increased anxiety in human studies (e.g. Gonda et al., 2008; Nillni et al., 2012; Reynolds et al., 2018; van Veen et al., 2009). Additionally, the adrenals excrete progesterone in response to external stressors, like social stress tests (Childs et al., 2010; Gaffey and Wirth, 2014). Finally, progesterone concentrations can vary from cycle to cycle within the same person (Eisenlohr-Moul and Owens, 2020; Jasienska et al., 2017; Jasienska and Jasienski, 2008). Since progesterone is so variable across the menstrual cycle, within and between

populations, and also across lived experiences, the relationship between progesterone and cortisol may also be population or lived experience specific. Despite this variability, our results suggest that the progesterone curve might account for more of the cortisol phenotype variation we are seeing across the menstrual cycle than estrogen.

Limitations

While these analyses provide a detailed description of cortisol across the entire menstrual cycle in this sample, there are some limitations. The first, is that these results are likely not applicable across different study designs or populations. While we show evidence refuting the assumption that menstrual cycle effects are universal or ubiquitous, this implies that studies of menstrual cycle effects are likely population or sample specific and thus our three phenotypes might not be replicated in other populations. Second, our study was conducted using daily morning urine samples and this type of waking cortisol is likely not representing the same thing as salivary waking cortisol. Salivary cortisol is a snapshot of current state and reflects diurnal activity whereas urinary cortisol is an average measure of cortisol exposure over the nighttime and since last urination (Jerjes et al., 2006; Sarkar et al., 2013), these results are likely not applicable to salivary or serum samples. Furthermore, there are other ways of measuring HPA axis function beyond baseline waking cortisol, for example diurnal cortisol curves and cortisol reactivity, and the results are not directly applicable to other measures of HPA axis function. Additionally, while our min/max scaling can be applied no matter the number or frequency of biomarker samples, the cycle day slide scaling requires multiple data points (Ehrlich et al., 2022). Finally, an alternative explanation for our null results for estrogen could be that our sample is underpowered to identify any possible relationship. However, our three cortisol

phenotypes are equally represented in our sample of 76. The variable group has 29, the responsive group has 27, and the consistent group has 23 individuals, making these groupings relatively comparable. For both our two-block partial least squares (which tests total sample) and our pairwise t tests (which compares means between the three groups) we find null results, suggesting estrogen's effect may be too small find in this sample.

Implications

Despite some limitations, there are multiple implications of this study. First, our fine-grained analysis of cortisol across the menstrual cycle in a homogenous population demonstrates that menstrual cycle effects are not ubiquitous or universal in ovulating, spontaneously cycling, generally healthy individuals. This finding is likely accounting for much of the variation that we see in the literature about cortisol and menstrual cycle effects. Previous research by Erhlich et al., 2022 has found that even the "normal" estrogen curve is not the majority phenotype when examining within-population shape, and our results further challenge the equating of normal, average, and healthy with each other. Additionally, these findings challenge the assumed power the menstrual cycle has on other bodily systems, as not all groups had the same cycle pattern in their cortisol concentrations, and none corresponded to E1G variation. While the menstrual cycle is certainly interacting with other bodily systems, there is significant individual variation, particularly when studying environmentally responsive biomarkers like estrogen, progesterone, and cortisol or behavior (for a review of literature on behavior and estrogen see Jordan-Young, 2011).

Finally, our results have methodological implications for future work on cortisol in spontaneously cycling individuals. While it is difficult to obtain daily measures of baseline

cortisol, our results suggest that researchers should attempt to make multiple collections during each phase (including, if possible, the early follicular, follicular, periovulatory, luteal and late luteal) across an entire menstrual cycle if trying to determine menstrual cycle effects. Collecting few samples across the entire follicular or the entire luteal phases may not be enough to reveal cyclical patterns. Additionally, for more typical biocultural studies looking at sociocultural or psychosocial variables and cortisol, it is likely important to control for the timing of cortisol collection in spontaneously cycling individuals, as researchers will likely not know whether or how these individuals' cortisol concentrations might be interacting with their cycles.

Conclusion

In conclusion, we found evidence supporting an overall average cyclicity of cortisol across the menstrual cycle. However, this average obscured significant variation within our sample. When exploring cortisol phenotypes using GM methods, we found three distinct, unique cortisol curve patterns across the menstrual cycle in our sample. These findings challenge the typical assumption that spontaneously cycling individuals are impacted by the menstrual cycle in the same way and to the same extent. To better understand where this cortisol variation might be coming from, we further explored the possible relationship between the HPA and HPO axis. We did not find evidence to support E1G having a large effect on cortisol, but our results suggest that there is a correlation between cortisol shape and PdG shape and that a consistent cortisol shape may correspond to greater progesterone exposure across the menstrual cycle. Our paper also shows the utility of the GM scaling methods of both cycle day and hormone concentrations by creating a more complete image of hormones across the menstrual cycle and uncovering shape nuance that was otherwise obscured by large hormone ranges.

CHAPTER 5: CONCLUSION

The overarching question of how gender is embodied necessarily involves bringing together a number of different, seemingly disparate and disconnected theoretical perspectives and methods of both sociocultural and biological disciplines. Though as many feminist and queer scholars have pointed out these two are not and have never been separate; science does not happen in a cultural vacuum and our sociocultural spheres do not operate separate from or regardless of our physical bodies (ex: Haraway, 1988; Harding, 1986; Oreskes, 2019; Pigg and Adams, 2005). While this dissertation on the surface is keeping the sociocultural and the biological separate for now, it has critically and carefully engaged with the theories and methods of each so that future researchers can access feminist, queer, trans theoretical frameworks and quantitative methodologies.

By demonstrating the ways in which current biological anthropology discourse is still actively re/producing gender and sex in chapter 1, I hoped to bring awareness to the subtle ways that cis and heteronormativity are entrenched in our discipline and research. I conducted a qualitative textual analysis of the biological anthropology journals, *the American Journal of Physical Anthropology* and *the American Journal of Human Biology,* over the course of two and a half (January 2020-May 2022) years. I started my search by reading through the titles of each issue and selecting articles based on title that appeared to be conflating sex and gender, reproduced or tested sex and gender differences, reproduction, or where sex or gender might be connected to behavior. I then read the abstracts for each to better identify examples of gender essentialism, places where gender or sex appeared to be conflated or where gender was assumed from sex, places where intimate relationships were all positioned as heteronormative, and more.

Some examples of this language are: “sexual dimorphism; sex differences; sex estimation; reproduction; violence.” Finally, I more closely read a selection of 8 articles to use as examples in chapter 1. I identified 116 articles through my reading and 3 common themes: quantifying sex or gender difference; violence, victimhood, and other gendered behaviors; masculine versus feminine features and behaviors and reproduction. These themes are all tied together through a narrative of evolutionary theory and a subset specifically through reproduction. It’s important to note however, the research I engage within this chapter is not purposefully trying to create inequalities, erase variation, or uphold harmful cis and heteronormative systems but are instead trying to highlight previously missed variation. These assumptions are woven into our training and methods and as such they can be hard to see.

In chapter 2, I demonstrated that human biology research, regardless of the popular framework of the day, has historically been invested in this re/production of sex and gender in order to maintain oppressive systems of harm, like race. In this chapter, I defined and problematized embodiment and plasticity and reviewed how and why these concepts have been taken up in human biology research. Then, I examined the works of feminist, trans and queer scholars who have made the connection between embodiment, plasticity and the creation of Western binarized sex and gender. Related to this, I showed that the re/production of a sex and gender binary is entwined with the justification of racial hierarchies through plasticity and embodiment. I ended with recommendations and possible pathways forward for embodiment and plasticity research in human biology. Through this analysis, I invited human biologists to more critically engage with their own research.

The second half of my dissertation was more quantitative. In it, I described in detail the biological variation of cortisol and C-reactive protein. First, in chapter 3, I explored menstrual

cycle CRP variation in two geographically diverse samples of Polish and Polish American individuals. I found that CRP variation is population specific and may not change across the menstrual cycle depending on sample. Additionally, CRP may be elevated during the first few days of menstrual bleeding in other groups. Next, in chapter 4, I explored whether menstrual cycle effects on cortisol are consistent within a homogenous population, and a potential source of menstrual cycle cortisol variation, hypothalamic-pituitary-ovarian axis function. I found that individuals' cycles appear to differentially impact cortisol curves across a cycle, with some showing very little cyclicity and others showing stronger cyclicity. Furthermore, E1G is not related to cortisol variation, but there is evidence PdG is.

I hope this research will help future scholars better incorporate biological variation from internal effects (i.e. accounting for menstrual cycle variation of cortisol and C-reactive protein in a population that has a diverse range of bodies) and tease apart variation from external effects, like my primary interest, gender and gendered lived experiences. Additionally, it is vital to acknowledge that different bodies experience and respond to external effects differently. Previous research on sex and gender suggests that there is a connection between our sex and gender lived experiences and our biological outcomes (ex: Fausto-Sterling et al., 2012; Gettler et al., 2013; Gettler et al., 2011; Joel et al. 2015; Kuzawa et al., 2010; van Anders et al., 2012), however each person has a different body and a unique way of being. Variation in biological embodiment due to the hypothalamic-pituitary-ovarian axis or menstrual cycle variation is important to describe and identify so studies of gender and biological embodiment can be as inclusive as possible.

Finally, these analyses provide an example of how human biologists can conduct quantitative research in a way that tries to not uncritically re/produce gender and sex. While

chapters 3 and 4 were written in a way that followed the strict scientific research paper format and used language common to quantitative analysis there were important omissions. In neither of the chapters did I use the following words or concepts: evolution, adaptation, reproduction, female, woman, or sex. Instead, I very specifically used and defined the sources of variation I was actually exploring, the HPO axis and the menstrual cycle. Additionally, I maintained gender inclusive language throughout the articles. I also did not engage with or make any assumptions about *a priori* sorting of individuals within my populations and utilized statistical methods that are not about "proving" difference of distributions between groups or showing a group is more different from the norm than the other. Instead, I explored within population variation to show how there is ample difference in C-reactive protein and cortisol across the menstrual cycle, and that there is likely no "normal" menstrual cycle pattern of these biomarkers. While subtle and done in a way that may not be obvious at first glance, I have attempted to not re/produce cis and heteronormativity in these ways.

Future directions

This dissertation was intended to be the first steps of a longitudinal and biocultural project on gender. While the information and research I conducted could hopefully still be used in a capacity that challenges sex and gender binaries and systems of oppression, I will not be able to carry on this work. Potential applications of my research, particularly chapters 1 and 2 could be, could be hopefully used more broadly in classroom applications. I believe that learning early in training that our science is not unbiased and in fact, intentionally or not, can re/produce the harmful systems many of us wish to dismantle. Chapters 3 and 4 I hope will provide future researchers with examples of how to conduct human biology research that doesn't seek to

binarize, bin, or differentiate between people in ways that come from harmful assumptions. Additionally, I hope they provide insight into which biomarkers might be most appropriate for studies that will include diverse bodies, sexes, and genders and how to best interpret results when no biomarker is truly separate from any other variable, internal or otherwise.

This dissertation began with the question *how is the particular identity and experience of gender embodied?* In order to address this, I had to first situate my disciplinary training and methods within the larger historical and sociocultural context through which we move. Next, I needed to deeply explore the potential variation common biomarkers of embodiment so that future research could be better designed, analyzed and interpreted. Through attending to both the interwoven cultural and biological aspects of potential embodiment I hope to challenge the typical analytical framework of biological anthropology, particularly in the context of continued binarizing of sex and gender.

REFERENCES

Abe, T., Wong, V., Dankel, S. J., Bell, Z. W., Spitz, R. W., Viana, R. B., & Loenneke, J. P. (2020a). Skeletal muscle mass in female athletes: The average and the extremes. *American Journal of Human Biology*, *32*(2), e23333. https://doi.org/10.1002/ajhb.23333

Aardal, E., & Holm, A. C. (1995). Cortisol in saliva-reference ranges and relation to cortisol in serum. *https://doi.org/10.1515/cclm.1995.33.12.927*

Abe, T., Bell, Z. W., Wong, V., Spitz, R. W., Yamada, Y., Song, J. S., & Loenneke, J. P. (2021). Skeletal muscle size distribution in large-sized male and female athletes. *American Journal of Human Biology*, *33*(2), e23473. https://doi.org/10.1002/ajhb.23473

Abe, T., Wong, V., Spitz, R. W., Viana, R. B., Bell, Z. W., Yamada, Y., … Loenneke, J. P. (2020b). Influence of sex and resistance training status on orofacial muscle strength and morphology in healthy adults between the ages of 18 and 40: A cross-sectional study. *American Journal of Human Biology*, *32*(6), e23401. https://doi.org/10.1002/ajhb.23401

ACLU, 2023. Mapping Attacks on LGBTQ Rights in U.S. State Legislatures. https://www.aclu.org/legislative-attacks-on-lgbtq-rights?state=TN

Ahn, R. S., Choi, J. H., Choi, B. C., Kim, J. H., Lee, S. H., & Sung, S. S. (2011). Cortisol, estradiol-17β, and progesterone secretion within the first hour after awakening in women with regular menstrual cycles. *Journal of Endocrinology*, *211*(3), 285–295. https://doi.org/10.1530/JOE-11-0247

Allison, P., Mnatsakanova, A., Fekedulegn, D. B., Violanti, J. M., Charles, L. E., Hartley, T. A., & Miller, D. B. (2019). Association of occupational stress with waking, diurnal, and bedtime cortisol response in police officers. American Journal of Human Biology, 31(6), e23296.

Allsworth, J.E., Clarke, J., Peipert, J.F., Hebert, M.R., Cooper, A. and Boardman, L.A. (2007) The Influence of Stress on the Menstrual Cycle among Newly Incarcerated Women. *Women's Health Issues*, **17**, 202–209.

Andreano, J. M., Arjomandi, H., & Cahill, L. (2008). Menstrual cycle modulation of the relationship between cortisol and long-term memory. *Psychoneuroendocrinology*, *33*(6), 874-882.

Apter, D., Reinilä, M. and Vihko, R. (1989) Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood. *Int J Cancer*, **44**, 783–787.

Aris, C., Mahoney, P., & Deter, C. (2020). Enamel growth rates of anterior teeth in males and females from modern and ancient British populations. *American Journal of Physical Anthropology*, *173*(2), 236–249. https://doi.org/10.1002/ajpa.24068

Arkansas SB43, https://www.arkleg.state.ar.us/Bills/Detail?id=SB43&ddBienniumSession=2023/2023R.

Arnegard, M. E., Whitten, L. A., Hunter, C., & Clayton, J. A. (2020). Sex as a biological variable: a 5-year progress report and call to action. Journal of Women's Health, 29(6), 858-864.

Astorino, C. M. (2019). Beyond dimorphism: Sexual polymorphism and research bias in biological anthropology. American Anthropologist, 121(2), 489-490.

Azlan, A., Salamonsen, L. A., Hutchison, J., & Evans, J. (2020). Endometrial inflammasome activation accompanies menstruation and may have implications for systemic inflammatory events of the menstrual cycle. Human Reproduction, 35(6), 1363-1376.

Bagepally, B. S., Majumder, J., & Kotadiya, S. M. (2020). Association between second to fourth digit ratio and personality among Indian men. *American Journal of Human Biology*, *32*(2), e23331. https://doi.org/10.1002/ajhb.23331

Bao, A. M., Ji, Y. F., Van Someren, E. J. W., Hofman, M. A., Liu, R. Y., & Zhou, J. N. (2004). Diurnal rhythms of free estradiol and cortisol during the normal menstrual cycle in women with major depression. *Hormones and Behavior*, *45*(2), 93–102. https://doi.org/10.1016/j.yhbeh.2003.09.004

Barrett, E. S., Thune, I., Lipson, S. F., Furberg, A. S., & Ellison, P. T. (2013). A factor analysis approach to examining relationships among ovarian steroid concentrations, gonadotrophin concentrations and menstrual cycle length characteristics in healthy, cycling women. Human Reproduction, 28(3), 801-811.

Bates, Douglas, Martin Mächler, Benjamin M. Bolker, and Steven C. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67 (1): 1–48. https://doi.org/10.18637/jss.v067.i01.

Beauchamp, T. (2019) "Bathrooms, Borders, and Biometrics," Going Stealth: Transgender Politics and U.S. Surveillance Practices (Durham: Duke University Press), 79-106.

Bederman, G. (2008). Manliness and civilization: A cultural history of gender and race in the United States, 1880-1917. University of Chicago Press.

Beretta, L., & Santaniello, A. (2016). Nearest neighbor imputation algorithms: a critical evaluation. BMC medical informatics and decision making, 16(3), 197-208.

Blume, C. A., Muller, B., Huber, P., Kraenzlin, M., Schindler, C., De Geyter, C., ... & Puder, J. J. (2005). Low-grade inflammation and estimates of insulin resistance during the menstrual cycle in lean and overweight women. The Journal of Clinical Endocrinology & Metabolism, 90(6), 3230-3235.

Bolker and Others. 2022. GLMM FAQ. http://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#model-selection-and-averaging (accessed 10 July 2022).

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in ecology & evolution*, *24*(3), 127-135.

Brooks, G. C., Blaha, M. J., & Blumenthal, R. S. (2010). Relation of C-reactive protein to abdominal adiposity. The American journal of cardiology, 106(1), 56-61.

Buikstra, J. E., and Ubelaker (1994). Standards for data collection from human skeletal remains. Arkansas archaeological survey research series, 44.

Colleran, H., & Mace, R. (2015). Social network-and community-level influences on contraceptive use: evidence from rural Poland. Proceedings of the Royal Society B: Biological Sciences, 282(1807), 20150398.

Carey, M. P., Deterd, C. H., De Koning, J., Helmerhorst, F., & De Kloet, E. R. (1995). The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. *Journal of Endocrinology*, *144*(2), 311–321. https://doi.org/10.1677/joe.0.1440311

Charrad, M., Ghazzali, N., Boiteau, V. and Niknafs, A. (2014) NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set. *Journal of Statistical Software*, **61**, 1–36.

Chida, Y., & Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biological psychology, 80(3), 265-278.

Childs, E., Dlugos, A., & De Wit, H. (2010). Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. *Psychophysiology*, *47*(3), 550-559.

Cipolla, C., Gupta, K., Rubin, D. A., & Willey, A. (Eds.). 2017. *Queer feminist science studies: A reader*. University of Washington Press.

Clancy, K. B. H., Baerwald, A. R., & Pierson, R. A. (2016). Cycle-phase dependent associations between CRP, leptin, and reproductive hormones in an urban, Canadian sample. *American Journal of Physical Anthropology*, *160*(3), 389–396. https://doi.org/10.1002/ajpa.22976

Clancy, K. B. H., Klein, L. D., Ziomkiewicz, A., Nenko, I., Jasienska, G., & Bribiescas, R. G. (2013). Relationships between biomarkers of inflammation, ovarian steroids, and age at menarche in a rural polish sample. *American Journal of Human Biology*, *25*(3), 389–398. https://doi.org/10.1002/ajhb.22386

Clancy, K. B., & Davis, J. L. (2019). Soylent is people, and WEIRD is white: Biological anthropology, whiteness, and the limits of the WEIRD. *Annual Review of Anthropology*, *48*, 169-186.

Clark, B. A., Veale, J. F., Townsend, M., Frohard-Dourlent, H., & Saewyc, E. (2018). Non-binary youth: Access to gender-affirming primary health care. *International Journal of Transgenderism*, *19*(2), 158–169. https://doi.org/10.1080/15532739.2017.1394954

Coburn, S. B., Stanczyk, F. Z., Falk, R. T., McGlynn, K. A., Brinton, L. A., Sampson, J., ... & Trabert, B. (2019). Comparability of serum, plasma, and urinary estrogen and estrogen metabolite measurements by sex and menopausal status. *Cancer Causes & Control*, *30*(1), 75-86.

Colleran, H. (2014). Farming in transition: Land and property inheritance in a rural polish population. Social Biology and Human Affairs, 78, 7–19.

Connell, R. 2012. Gender, health and theory: conceptualizing the issue, in local and world perspective. *Social science & medicine*. [74(11)]: 1675-1683.

Csordas, T. J. 1990. Embodiment as a Paradigm for Anthropology. *Etho.* [18(1)]: 5–47.

Danese, A., Moffitt, T. E., Harrington, H., Milne, B. J., Polanczyk, G., Pariante, C. M., ... & Caspi, A. (2009). Adverse childhood experiences and adult risk factors for age-related disease: depression, inflammation, and clustering of metabolic risk markers. Archives of pediatrics & adolescent medicine, 163(12), 1135-1143.

Darwin, C. (2004). *On the origin of species, 1859*. Routledge.

De Souza, M.J. and Williams, N.I. (2004) Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women. *Human Reproduction Update*, **10**, 433–448.

Del Bove, A., Profico, A., Riga, A., Bucchi, A., & Lorenzo, C. (2020). A geometric morphometric approach to the study of sexual dimorphism in the modern human frontal bone. *American Journal of Physical Anthropology*, *173*(4), 643–654. https://doi.org/10.1002/ajpa.24154

Desantis, A. S., Kuzawa, C. W., & Adam, E. K. (2015). Developmental origins of flatter cortisol rhythms: socioeconomic status and adult cortisol activity. *American Journal of Human Biology*, *27*(4), 458–467. https://doi.org/10.1002/ajhb.22668

Dreon DM, Slavin JL, Phinney SD. 2002. Oral contraceptive use and increased plasma concentration of C-reactive protein. Life Sci 73:1245–1252.

Dryden I.L., and K.V. Mardia. 2016. *Statistical Shape Analysis: With Applications in R*. John Wiley & Sons.

Dubois, L. Z. (2012). Associations between transition-specific stress experience, nocturnal decline in ambulatory blood pressure, and C-reactive protein levels among transgender men. *American Journal of Human Biology*, *24*(1), 52–61. https://doi.org/10.1002/ajhb.22203

DuBois, L. Z., & Shattuck-Heidorn, H. (2021). Challenging the binary: Gender/sex and the biologics of normalcy. American Journal of Human Biology, 33(5), e23623.

DuBois, L. Z., Gibb, J. K., Juster, R. P., & Powers, S. I. (2021). Biocultural approaches to transgender and gender diverse experience and health: Integrating biomarkers and advancing gender/sex research. American Journal of Human Biology, 33(1), e23555.

DuBois, L. Z., Powers, S., Everett, B. G., & Juster, R. P. (2017). Stigma and diurnal cortisol among transitioning transgender men. *Psychoneuroendocrinology*, *82*, 59-66.

DuBois, L. Z., Puckett, J. A., & Langer, S. J. (2022). Development of the Gender Embodiment Scale: trans Masculine Spectrum. Transgender health, 7(4), 287-291.

Duchesne, A., & Pruessner, J. C. (2013). Association between subjective and cortisol stress response depends on the menstrual cycle phase. *Psychoneuroendocrinology*, *38*(12), 3155-3159.

Edelman, E. A. (2014). Walking while transgender. Necropolitical regulations of trans feminine bodies of colour in the nation's capital. En J. Haritaworn, A. Kunstman and S. Posocco. Queer Necropolitics, 172-190.

Edwards, K. M., & Mills, P. J. (2008). Effects of estrogen versus estrogen and progesterone on cortisol and interleukin-6. *Maturitas*, *61*(4), 330–333. https://doi.org/10.1016/j.maturitas.2008.09.024

Edwards, Lloyd J., et al. "An R2 statistic for fixed effects in the linear mixed model." Statistics in medicine 27.29 (2008): 6137-6157.

Ehrlich, D. (2021) *MoRphomenses: Initial Release*. Zenodo.

Ehrlich, D. E., Rogers-Lavanne, M. P., Lee, K., Sgheiza, V., Wilson, M. A., & Clancy, K. B. (2022). Quantifying Curves: a geometric morphometric approach to analyze ovarian hormones. *American journal of biological anthropology* 177, 52-52.

Eisenhardt SU, Thiele JR, Bannasch H, Stark GB, Peter K. C-reactive protein: how conformational changes influence inflammatory properties. Cell Cycle. 2009 Dec;8(23):3885-92. doi: 10.4161/cc.8.23.10068. Epub 2009 Dec 14. PMID: 19887916.

Eisenlohr-Moul, T. A., & Owens, S. A. (2020). Hormones and personality. *Encyclopedia of Personality and Individual Differences*, 2010-2032.

Ellison PT, Lager C (1986) Moderate recreational running is associated with lowered salivary progesterone profiles in women. Am J Obst Gynecol 154: 1000–1003.

Ellison, P. T., Lipson, S. F., O'Rourke, M. T., Bentley, G. R., Harrigan, A. M., Panter-Brick, C., & Vitzthum, V. J. (1993). Population variation in ovarian function.

Ellison, P.T. (1990) Human Ovarian Function and Reproductive Ecology : New Hypotheses. *American Anthropologist*, **92**, 933–952.

Ertuğrul, B., & Özener, B. (2020). Relationship between parental consanguinity and digit ratios (2D:4D) among Turkish newborns in Sivas. *American Journal of Human Biology*, *32*(3), e23369. https://doi.org/10.1002/ajhb.23369

Evans, J., & Salamonsen, L. A. (2012). Inflammation, leukocytes and menstruation. Reviews in Endocrine and Metabolic Disorders, 13(4), 277-288.

Fassin D. 2009. "Another Politics of Life Is Possible." Theory, Culture, & Society 26 (5): 44–60.

Fausto-Sterling, A. (2019). Gender/Sex, Sexual Orientation, and Identity Are in the Body: How Did They Get There? *Journal of Sex Research*, *56*(4–5), 529–555. https://doi.org/10.1080/00224499.2019.1581883

Fausto-Sterling, A. 2008. *Myths of gender: Biological theories about women and men*. Basic Books.

Fausto-Sterling, Anne. (2012). The Dynamic Development of Gender Variability. Journal of Homosexuality 59, 398-421.

Fausto-Sterling, A. 1993. *The five sexes*. The Sciences. [*33*(2)]: 20-24.

Fernée, C., Zakrzewski, S., & Robson Brown, K. (2021). Dimorphism in dental tissues: Sex differences in archaeological individuals for multiple tooth types. *American Journal of Physical Anthropology*, *175*(1), 106–127. https://doi.org/10.1002/ajpa.24174

Fisher, J. A. (Ed.). 2011. *Gender and the science of difference: Cultural politics of contemporary science and medicine*. Rutgers University Press.

Fleming, C. M. (2018). How to be less stupid about race: On racism, white supremacy, and the racial divide. Beacon Press.

Flores, E., Tschann, J. M., Dimas, J. M., Bachen, E. A., Pasch, L. A., & de Groat, C. L. (2008). Perceived Discrimination, Perceived Stress, and Mental and Physical Health Among Mexican-Origin Adults. *Hispanic Journal of Behavioral Sciences*, *30*(4), 401–424. https://doi.org/10.1177/0739986308323056

Foucault, M. 1976. Right of death and power over life. In *Biopolitics. A Reader*, [2013] T. Campbell, and A. Sitze, eds. Durham and London: Duke UP.

Fuentes, Augustin. 2019. Identities, Experiences, and Beliefs: On Challenging Normativities in Biological Anthropology. American Anthropologist.

Frye, C. A., & Walf, A. A. (2004). Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. *Behavioral neuroscience*, *118*(2), 306.

Gaffey, A. E., & Wirth, M. M. (2014). Stress, rejection, and hormones: cortisol and progesterone reactivity to laboratory speech and rejection tasks in women and men. *F1000Research*, *3*.

García, A. R., Gurven, M., & Blackwell, A. D. (2017). A matter of perception: Perceived socio-economic status and cortisol on the island of Utila, Honduras. *American Journal of Human Biology*, *29*(5), e23031. https://doi.org/10.1002/ajhb.23031

Gaskins, A. J., Wilchesky, M., Mumford, S. L., Whitcomb, B. W., Browne, R. W., Wactawski-Wende, J., … Schisterman, E. F. (2012). Endogenous reproductive hormones and C-reactive protein across the menstrual cycle: The BioCycle Study. *American Journal of Epidemiology*, *175*(5), 423–431. https://doi.org/10.1093/aje/kwr343

Gettler, L. T., Mcdade, T. W., & Kuzawa, C. W. (2011). Cortisol and testosterone in Filipino young adult men: Evidence for co-regulation of both hormones by fatherhood and relationship status. American Journal of Human Biology, 23(5), 609–620. https://doi.org/10.1002/ajhb.21187

Gettler, L. T., Mcdade, T. W., Agustin, S. S., & Kuzawa, C. W. (2013). Progesterone and estrogen responsiveness to father-toddler interaction. American Journal of Human Biology, 25(4), 491–498. https://doi.org/10.1002/ajhb.22396

Gill-Peterson, J. 2018. *Histories of the transgender child*. U of Minnesota Press.

Gol M, Akan P, Dogan E, Karas C, Saygili U, Posaci C. 2006.Effects of estrogen, raloxifene, and hormone replacement therapy on serum C-reactive protein and homocysteine levels. Maturitas 53:252–259.

Goldberg, A. E., Kuvalanka, K. A., Budge, S. L., Benz, M. B., & Smith, J. Z. (2019). Health Care Experiences of Transgender Binary and Nonbinary University Students. *The Counseling Psychologist*, *47*(1), 59–97. https://doi.org/10.1177/0011000019827568

Goosby, B. J., Malone, S., Richardson, E. A., Cheadle, J. E., & Williams, D. T. (2015). Perceived discrimination and markers of cardiovascular risk among low-income African American youth. *American Journal of Human Biology*, *27*(4), 546–552. https://doi.org/10.1002/ajhb.22683

Gowland, R., Stewart, N. A., Crowder, K. D., Hodson, C., Shaw, H., Gron, K. J., & Montgomery, J. (2021). Sex estimation of teeth at different developmental stages using dimorphic enamel peptide analysis. *American Journal of Physical Anthropology*, *174*(4), 859–869. https://doi.org/10.1002/ajpa.24231

Graves Jr, J. L. 2015. Great is their sin: Biological determinism in the age of genomics. *The Annals of the American Academy of Political and Social Science*. [661(1)], 24-50.

Greven, S. and Kneib T. (2010) On the behaviour of marginal and conditional AIC in linear mixed models. Biometrika 97(4), 773-789.

Gröschl, M., Rauh, M., & Dörr, H.-G. (2003). Circadian Rhythm of Salivary Cortisol, 17α-Hydroxyprogesterone, and Progesterone in Healthy Children. *Clinical Chemistry*, *49*(10), 1688–1691. https://doi.org/10.1373/49.10.1688

Gröschl, M., Rauh, M., Schmid, P., & Dörr, H. G. (2001). Relationship between salivary progesterone, 17-hydroxyprogesterone, and cortisol levels throughout the normal menstrual cycle of healthy postmenarcheal girls. *Fertility and Sterility*, *76*(3), 615–617. https://doi.org/10.1016/S0015-0282(01)01960-4

Gupta, K., and D.A. Rubin. 2020. Queer science studies/queer science. In *The Routledge Handbook of Feminist Philosophy of Science*, S. Crasnow, and K. Intemann eds. Routledge.

Guthrie BJ, Young AM, Williams DR, Boyd CJ, Kintner EK. African American girls' smoking habits and day-to-day experiences with racial discrimination. Nurs Res. 2002;51:183–190.

Gutmann, M., Nelson, R. G., & Fuentes, A. (2021). Epidemic Errors in Understanding Masculinity, Maleness, and Violence An Introduction to Supplement 23. https://doi.org/10.1086/712485

Haas, R., Watson, J., Buonasera, T., Southon, J., Chen, J. C., Noe, S., ... & Parker, G. (2020). Female hunters of the early Americas. Science Advances, 6(45), eabd0310.

Halberstam, J. 2014. 28 Gender. In *Keywords for American Cultural Studies, Second Edition*, New York University Press, 116-118.

Hamidovic, A., Karapetyan, K., Serdarevic, F., Choi, S. H., Eisenlohr-Moul, T., & Pinna, G. (2020). Higher circulating cortisol in the follicular vs. luteal phase of the menstrual cycle: A meta-analysis. *Frontiers in endocrinology*, *11*, 311.

Hampson, E., & Duff-Canning, S. J. (2016). Salivary cortisol and explicit memory in postmenopausal women using hormone replacement therapy. *Psychoneuroendocrinology*, *64*, 99–107. https://doi.org/10.1016/j.psyneuen.2015.11.009

Handa, R. J., Burgess, L. H., Kerr, J. E., & O'keefe, J. A. (1994). Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Hormones and Behavior*, *28*(4), 464–476. https://doi.org/10.1006/hbeh.1994.1044

Haraway, D. (1988). Situated knowledges: The science question in feminism and the privilege of partial perspective. Feminist studies, 14(3), 575-599.

Harding, S. (1995). “Strong Objectivity”: A Response to the New Objectivity Question. *Synthese*, *104*(3,), 331–349.

Harding, S. G. (1986). The science question in feminism. Cornell University Press.

Heck, A. L., & Handa, R. J. (2019, January 1). Sex differences in the hypothalamic–pituitary–adrenal axis’ response to stress: an important role for gonadal hormones. *Neuropsychopharmacology*. Nature Publishing Group. https://doi.org/10.1038/s41386-018-0167-9

Herrera, A. Y., Nielsen, S. E., & Mather, M. (2016). Stress-induced increases in progesterone and cortisol in naturally cycling women. *Neurobiology of Stress*, *3*, 96–104. https://doi.org/10.1016/j.ynstr.2016.02.006

Hicks, K., & Leonard, W. R. (2014). Developmental systems and inequality: linking evolutionary and political-economic theory in biological anthropology. Current Anthropology, 55(5), 523-550.

Hill, E. C., Pearson, O. M., Durband, A. C., Walshe, K., Carlson, K. J., & Grine, F. E. (2020). An examination of the cross-sectional geometrical properties of the long bone diaphyses of Holocene foragers from Roonka, South Australia. American Journal of Physical Anthropology, 172(4), 682-697.

Hoke, M. K., & McDade, T. (2014). Biosocial inheritance: A framework for the study of the intergenerational transmission of health disparities. Annals of Anthropological Practice, 38(2), 187-213.

Hooks, B. 2013. *Teaching community: A pedagogy of hope*. Routledge.

Hutchinson, W. L., Koenig, W., Frohlich, M., Sund, M., Lowe, G. D., & Pepys, M. B. (2000). Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. *Clinical chemistry*, *46*(7), 934-938.

Hyde, J. S., Bigler, R. S., Joel, D., Tate, C. C., & van Anders, S. M. (2019). The future of sex and gender in psychology: Five challenges to the gender binary. *American Psychologist*, *74*(2), 171–193. https://doi.org/10.1037/amp0000307

Ingold, T., & Palsson, G. (Eds.). (2013). Biosocial becomings: integrating social and biological anthropology. Cambridge University Press.

Inoue, Y., Stickley, A., Yazawa, A., Li, D., Du, J., Jin, Y., … Watanabe, C. (2016). The association between economic development, lifestyle differentiation, and C-reactive protein concentration within rural communities in Hainan Island, China. *American Journal of Human Biology*, *28*(2), 186–196. https://doi.org/10.1002/ajhb.22771

Izawa, S., Miki, K., Liu, X., & Ogawa, N. (2013). The diurnal patterns of salivary interleukin-6 and C-reactive protein in healthy young adults. *Brain, Behavior, and Immunity*, *27*(1), 38–41. https://doi.org/10.1016/j.bbi.2012.07.001

Jaeger, Byron C., et al., "An R Squared Statistic for Fixed Effects in the Generalized Linear Mixed Model." Journal of Applied Statistics (2016).

James, S., Herman, J., Rankin, S., Keisling, M., Mottet, L., & Anafi, M. A. (2016). The report of the 2015 US transgender survey. https://www.transequality.org/sites/default/files/docs/USTS-Full-Report-FINAL.PDF

Jasienska, G. (2013). The Fragile Wisdon: an evolutionary view on women's biology and health. Harvard University Press.

Jasienska, G. and Ellison, P.T. (1998) Physical work causes suppression of ovarian function in women. *Proceedings. Biological sciences / The Royal Society*, **265**, 1847–51.

Jasienska, G., & Ellison, P. T. (2004). Energetic factors and seasonal changes in ovarian function in women from rural Poland. American Journal of Human Biology, 16(5), 563–580.

Jasienska, G., & Jasienski, M. (2008). Interpopulation, interindividual, intercycle, and intracycle natural variation in progesterone levels: a quantitative assessment and implications for population studies. *American Journal of Human Biology*, *20*(1), 35-42.

Jasienska, G., Bribiescas, R. G., Furberg, A. S., Helle, S., & Núñez-de la Mora, A. (2017). Human reproduction and health: an evolutionary perspective. *The Lancet*, *390*(10093), 510-520.

Jasienska, G., Ziomkiewicz, A., Thune, I., Lipson, S. F., & Ellison, P. T. (2006). Habitual physical activity and estradiol levels in women of reproductive age. European Journal of Cancer Prevention, 15(5), 439–445.

Jerjes, W. K., Cleare, A. J., Wood, P. J., & Taylor, N. F. (2006). Assessment of subtle changes in glucocorticoid negative feedback using prednisolone: Comparison of salivary free cortisol and urinary cortisol metabolites as endpoints. *Clinica Chimica Acta*, *364*(1–2), 279–286. https://doi.org/10.1016/j.cca.2005.07.011

Jha, M. (2015). The global beauty industry: Colorism, racism, and the national body. Routledge.

Jilma, B., Dirnberger, E., Löscher, I., Rumplmayr, A., Hildebrandt, J., Eichler, H. G., … Wagner, O. F. (1997). Menstrual cycle-associated changes in blood levels of interleukin-6, α1 acid glycoprotein, and C-reactive protein. *Journal of Laboratory and Clinical Medicine*, *130*(1), 69–75. https://doi.org/10.1016/S0022-2143(97)90060-3

Joel, D. 2012. Genetic-gonadal-genitals sex (3G-sex) and the misconception of brain and gender, or, why 3G-males and 3G-females have intersex brain and intersex gender. *Biology of sex differences*. [3(1)]: 1-6.

Joel, D., Z. Berman, I. Tavor, et al. 2015. Sex beyond the genitalia: The human brain mosaic. *Proceedings of the National Academy of Sciences*. [112(50)] 15468-15473.

Johnson, T. V., Abbasi, A., & Master, V. A. (2013, June 25). Systematic review of the evidence of a relationship between chronic psychosocial stress and C-reactive protein. *Molecular Diagnosis and Therapy*. Springer. https://doi.org/10.1007/s40291-013-0026-7

Jolicoeur P. 193. Note: The Multivariate Generalization of the Allometry Equation. *Biometrics* 1963; **19**: 497–499.

Jones, J. L. 2002. Performance ethnography: The role of embodiment in cultural authenticity. *Theatre Topics*. [12(1)]: 1-15.

Jordan-Young, R. M. 2011. *Brain storm: The flaws in the science of sex differences*. Harvard University Press.

Jordan-Young, R. M., & Karkazis, K. (2019). Testosterone: An unauthorized biography. Harvard University Press.

Jungers WL, Falsetti AB, Wall CE. Shape, relative size, and size-adjustments in morphometrics. *American Journal of Physical Anthropology* 1995; **38**: 137–161.

Kaiser, H.F. (1960) The application of electronic computers to factor analysis. *Educational and Psychological Measurement*, **20**, 141–151.

Kalichman, L., Batsevich, V., & Kobyliansky, E. (2020). Finger length ratio in longevity populations. *American Journal of Human Biology*, *32*(2), e23325. https://doi.org/10.1002/ajhb.23325

Kiecolt-Glaser, J. K., Gouin, J. P., Weng, N. P., Malarkey, W. B., Beversdorf, D. Q., & Glaser, R. (2011). Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. Psychosomatic medicine, 73(1), 16.

Kimmel, M. 2008. Properties of Cultural Embodiment: Lessons from the Anthropology of the Body. In *Body, language, and mind,* T. Ziemke, J. Zlatev, and R. M. Frank eds. [Volume 2]. Mouton de Gruyter, 77–108.

Kirchengast, S., Dottolo, E., Praxmarer, E., & Huber, J. (2020). Low digit ratio (2D:4D) is associated with early natural menopause. *American Journal of Human Biology*, *32*(3), e23374. https://doi.org/10.1002/ajhb.23374

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosomatic medicine, 61(2), 154-162.

Kirschbaum, C., Schommer, N., Federenko, I., Gaab, J., Neumann, O., Oellers, M., & Hellhammer, D. H. (1996). Short-term estradiol treatment enhances pituitary-adrenal axis and sympathetic responses to psychosocial stress in healthy young men. *The Journal of Clinical Endocrinology & Metabolism*, *81*(10), 3639–3643. https://doi.org/10.1210/jcem.81.10.8855815

Kline, W. (2001). Building a better race: Gender, sexuality, and eugenics from the turn of the century to the baby boom. Univ of California Press.

Kluft C., Gevers Leuven J.A., Helmerhorst F.M., Krans H.M.J. 2002. Pro-inflammatory effects of oestrogens during use of oral contraceptives and hormones replacement treatment. Vascul Pharmacol 39:149–154.

Kong, C., Dunn, M. & Parker, M. Psychiatric genomics and mental health treatment: setting the ethical agenda. Am. J. Bioeth. 17, 3–12 (2017).

Konishi, S., Parajuli, R. P., Takane, E., Maharjan, M., Tachibana, K. I., Jiang, H. W., ... & Watanabe, C. (2014). Significant sex difference in the association between C‐reactive protein concentration and anthropometry among 13‐to 19‐year olds, but not 6‐to 12‐year olds in Nepal. American journal of physical anthropology, 154(1), 42-51.

Kovacs A., Henriksson P., Hamsten A., Walle´n H., Bjorkegren J.,Tornvall P. 2005. Hormonal regulation of circulating C-reactive protein in men. Clin Chem 51:911–913.

Kranjac, A. W., Kranjac, D., & Lounsbury, O. (2022). Deconstructing sex differences in C‐reactive protein trends over time. American Journal of Human Biology, 34(5), e23705.

Krieger, N. 2005. Embodiment: a conceptual glossary for epidemiology. *Journal of Epidemiology & Community Health*. [59(5)]: 350-355.

Krieger, N. 2011. Epidemiology and People's Health: Theory and Context. New York: Oxford University Press.

Kudielka, B. M., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology*, *28*(1), 35–47. https://doi.org/10.1016/S0306-4530(02)00008-2

Kudielka, Brigitte M., & Kirschbaum, C. (2005, April 1). Sex differences in HPA axis responses to stress: A review. *Biological Psychology*. Elsevier. https://doi.org/10.1016/j.biopsycho.2004.11.009

Kuzawa, C. W., Gettler, L. T., Huang, Y. Y., & McDade, T. W. (2010). Mothers have lower testosterone than non-mothers: Evidence from the Philippines. Hormones and Behavior, 57(4-5), 441-447.

Laffranchi, Z., Charisi, D., Jiménez-Brobeil, S. A., & Milella, M. (2020). Gendered division of labor in a Celtic community? A comparison of sex differences in entheseal changes and long bone shape and robusticity in the pre-Roman population of Verona (Italy, third–first century BC). *American Journal of Physical Anthropology*, *173*(3), 568–588. https://doi.org/10.1002/ajpa.24111

Lamble, S. (2014). Queer investments in punitiveness. En J. Haritaworn, A. Kunstman and S. Posocco. Queer necropolitics, 151-171.

Larocque C, Foth T. Which lives are worth saving? Biolegitimacy and harm reduction during COVID-19. Nurs Inq. 2021 Dec;28(4):e12417. doi: 10.1111/nin.12417.

Larsen, C. S. 2014. *Our origins: Discovering physical anthropology*. WW Norton & Company.

Lee, K. M., Rogers, M. P., Galbarczyk, A., Jasienska, G., & Clancy, K. B. (2019). Physical activity in women of reproductive age in a transitioning rural polish population. American Journal of Human Biology, 31(3), e23231.

Lee, K. M., Rogers, M. P., Galbarczyk, A., Jasienska, G., & Clancy, K. B. (2020). Effects of physical activity and sedentary behavior on Bone density in healthy adult women. The FASEB Journal, 34(S1), 1-1.

Legatzke, H., & Gettler, L. T. (2020). Bilingual family communication and the impact of language anxiety on cortisol reactivity. *American Journal of Human Biology*. https://doi.org/10.1002/ajhb.23493

Leslie, M. (2019). Talk to the hand. Scientists try to debunk idea that finger length can reveal personality and health. *Science*. https://doi.org/10.1126/science.aay2735

Levine, A., Zagoory-Sharon, O., Feldman, R., Lewis, J. G., & Weller, A. (2007). Measuring cortisol in human psychobiological studies. *Physiology & behavior*, *90*(1), 43-53.

Lewis, T. T., Aiello, A. E., Leurgans, S., Kelly, J., & Barnes, L. L. (2010). Self-reported experiences of everyday discrimination are associated with elevated C-reactive protein levels in older African-American adults. *Brain, Behavior, and Immunity*, *24*(3), 438–443. https://doi.org/10.1016/j.bbi.2009.11.011

Lewontin, R. C. 1980. Sociobiology: another biological determinism. *International Journal of Health Services*. [10(3)]: 347-363.

Li, F., Qasim, S., Li, D., & Dou, Q. P. (2022, August). Updated review on green tea polyphenol epigallocatechin-3-gallate as a cancer epigenetic regulator. In Seminars in Cancer Biology (Vol. 83, pp. 335-352). Academic Press.

Lipson SF, Ellison PT. Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. Hum Reprod. 1996;11/10:2090–2096.

Lock, M. (2013). The lure of the epigenome. *The Lancet*, *381*(9881), 1896-1897.

Lock, M. M., & Palsson, G. (2016). Can science resolve the nature/nurture debate?. John Wiley & Sons.

Lombardo, M. P., & Otieno, S. (2021). The associations between digit ratio, aerobic fitness, physical skills, and overall physical fitness of elite youth distance runners. *American Journal of Human Biology*, *33*(1), e23448. https://doi.org/10.1002/ajhb.23448

Loponte, D., & Mazza, B. (2021). Breastfeeding and weaning in Late Holocene hunter-gatherers of the lower Paraná wetland, South America. American Journal of Physical Anthropology, 176(3), 504-520.

Maki, P. M., Mordecai, K. L., Rubin, L. H., Sundermann, E., Savarese, A., Eatough, E., & Drogos, L. (2015). Menstrual cycle effects on cortisol responsivity and emotional retrieval following a psychosocial stressor. *Hormones and Behavior*, *74*, 201–208. https://doi.org/10.1016/j.yhbeh.2015.06.023

Marino, R., Tanganelli, V., Pietrobelli, A., & Belcastro, M. G. (2021). Evaluation of the auricular surface method for subadult sex estimation on Italian modern (19th to 20th century) identified skeletal collections. *American Journal of Physical Anthropology*, *174*(4), 792–803. https://doi.org/10.1002/ajpa.24146

Mascie-Taylor, C. G. N., B. Bogin, and N.W. Solomons. 1996. Human Variability and Plasticity--Cambridge Studies in Biological Anthropology. *American Journal of Clinical Nutrition*, *63*(4), 616-616.

Matthews, K. A., Gump, B. B., & Owens, J. F. (2001). Chronic stress influences cardiovascular and neuroendocrine responses during acute stress and recovery, especially in men. *Health Psychology*, *20*(6), 403–410. https://doi.org/10.1037/0278-6133.20.6.403

McCann, E., Keogh, B., Doyle, L., & Coyne, I. (2019). The Experiences of Youth Who Identify as Trans* in Relation to Health and Social Care Needs: A Scoping Review. *Youth & Society*, *51*(6), 840–864. https://doi.org/10.1177/0044118X17719345

McCool, W. C., Tung, T. A., Coltrain, J. B., Accinelli Obando, A. J., & Kennett, D. J. (2021). The character of conflict: A bioarchaeological study of violence in the Nasca highlands of

McCutcheon, A. L. 1987. *Latent class analysis* [No. 64]. Sage.

McDade, T. W., Borja, J. B., Kuzawa, C. W., Perez, T. L. L., & Adair, L. S. (2015). C-reactive protein response to influenza vaccination as a model of mild inflammatory stimulation in the Philippines. Vaccine, 33(17), 2004-2008.

McDade, T. W., Hawkley, L. C., & Cacioppo, J. T. (2006). Psychosocial and Behavioral Predictors of Inflammation in Middle-Aged and Older Adults: The Chicago Health, Aging, and Social Relations Study. *Psychosomatic Medicine*, *68*(3), 376–381. https://doi.org/10.1097/01.psy.0000221371.43607.64

Measelle, E., McClure, H. H., Snodgrass, J. J., Martinez, C. R., Jiménez, R., & Isiordia, L. (2019). Climbing the ladder of decline: Income and acculturation associated with chronic inflammation among Mexican immigrants. *American Journal of Human Biology*, *31*(4), e23271. https://doi.org/10.1002/ajhb.23271

Medley, G., Lipari, R. N., Bose, J., Cribb, D. S., Kroutil, L. A., & McHenry, G. (2016). Sexual orientation and estimates of adult substance use and mental health: Results from the 2015 National Survey on Drug Use and Health. NSDUH Data Review, 10.

Meloni, M. (2015). Epigenetics for the social sciences: justice, embodiment, and inheritance in the postgenomic age. New Genetics and Society, 34(2), 125-151.

Meloni, M. (2016). Political biology: Science and social values in human heredity from eugenics to epigenetics (p. 295). Basingstoke: Palgrave Macmillan.

Meloni, M. (2017). Race in an epigenetic time: Thinking biology in the plural. The British Journal of Sociology, 68(3), 389-409.

Meloni, M. (2019). Impressionable biologies: From the archaeology of plasticity to the sociology of epigenetics. Routledge.

Meloni, M., & Testa, G. (2014). Scrutinizing the epigenetics revolution. BioSocieties, 9(4), 431-456.

Meloni, M., Moll, T., Issaka, A., & Kuzawa, C. W. (2022). A biosocial return to race? A cautionary view for the postgenomic era. American Journal of Human Biology, 34(7), e23742.

Melville, W., Verma, G., Campbell, T., & Park, B.-Y. (2022). Challenging the Hegemony of Western Scientism in Science Teacher Education. *Journal of Science Teacher Education*, *33*(7), 703–709. https://doi.org/10.1080/1046560X.2022.2112130

Meredith, S. L., & Schmitt, C. A. (2019). The outliers are in: Queer perspectives on investigating variation in biological anthropology. American Anthropologist, 121(2), 487-489.

Mikhael, S., Punjala-Patel, A., & Gavrilova-Jordan, L. (2019). Hypothalamic-Pituitary-Ovarian Axis Disorders Impacting Female Fertility. *Biomedicines*, *7*(1), 5. https://doi.org/10.3390/biomedicines7010005

Miller, E. M., & Costello, C. Y. (2001). The limits of biological determinism. *American Sociological Review*, *66*(4), 592-598.

Miller, G. E., Chen, E., & Zhou, E. S. (2007, January). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin*. https://doi.org/10.1037/0033-2909.133.1.25

Miller, R. C., Brindle, E., Holman, D. J., Shofer, J., Klein, N. A., Soules, M. R., & O'Connor, K. A. (2004). Comparison of specific gravity and creatinine for normalizing urinary reproductive hormone concentrations. *Clinical chemistry*, *50*(5), 924-932.

Misiak, M., Butovskaya, M., Oleszkiewicz, A., & Sorokowski, P. (2020). Digit ratio and hand grip strength are associated with male competition outcomes: A study among traditional populations of the Yali and Hadza. *American Journal of Human Biology*, *32*(2), e23321. https://doi.org/10.1002/ajhb.23321

Mississippi HB1125, http://billstatus.ls.state.ms.us/2023/pdf/history/HB/HB1125.xml.

Montero-López, E., Santos-Ruiz, A., García-Ríos, M. C., Rodríguez-Blázquez, M., Rogers, H. L., & Peralta-Ramírez, M. I. (2018). The relationship between the menstrual cycle and cortisol secretion: Daily and stress-invoked cortisol patterns. *International Journal of Psychophysiology*, *131*, 67–72. https://doi.org/10.1016/j.ijpsycho.2018.03.021

Munro, C. J., Stabenfeldt, G. H., Cragun, J. R., Addiego, L. A., Overstreet, J. W., & Lasley, B. L. (1991). Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. *Clinical chemistry*, *37*(6), 838-844.

Murray K. 2018. "Epigenetics and Politics in the Colonial present." Canadian Journal of Sociology 43 (3): 225–63.

Nakagawa, Shinichi, and Holger Schielzeth. "A general and simple method for obtaining R2 from generalized linear mixed effects models." Methods in Ecology and Evolution 4.2 (2013): 133-142.

Nazmi, A., & Victora, C. G. (2007). Socioeconomic and racial/ethnic differentials of C-reactive protein levels: A systematic review of population-based studies. *BMC Public Health*, *7*(1), 212. https://doi.org/10.1186/1471-2458-7-212

Neary, J. P., Malbon, L., & McKenzie, D. C. (2002). Relationship between serum, saliva and urinary cortisol and its implication during recovery from training. *Journal of science and medicine in sport*, *5*(2), 108-114.

Nepomnaschy, P. A., Welch, K., McConnell, D., Strassmann, B. I., & England, B. G. (2004). Stress and female reproductive function: a study of daily variations in cortisol, gonadotrophins, and gonadal steroids in a rural Mayan population. *American Journal of Human Biology: The Official Journal of the Human Biology Association*, *16*(5), 523-532.

Nettle, D., and M. Bateson. 2015. Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve?. *Proceedings of the Royal Society B: Biological Sciences*. [282(1812)]: 20151005.

Niewöhner, J. 2011 Epigenetics: Embedded bodies and the molecularisation of biography and milieu. BioSocieties 6 : 279 – 298.

Niewöhner, J., & Lock, M. (2018). Situating local biologies: Anthropological perspectives on environment/human entanglements. BioSocieties, 13(4), 681-697.

Nunez-De La Mora, A., Chatterton, R. T., Choudhury, O. A., Napolitano, D. A., & Bentley, G. R. (2007). Childhood conditions influence adult progesterone levels. *PLoS Medicine*, *4*(5), e167.

Nurden, A. T. (2011). Platelets, inflammation and tissue regeneration. *Thrombosis and haemostasis*, *105*(S 06), S13-S33.

Nylund-Gibson, K., and A.Y. Choi. 2018. Ten frequently asked questions about latent class analysis. *Translational Issues in Psychological Science*. [4(4)]: 440.

O'Connor, K. A., Brindle, E., Holman, D. J., Klein, N. A., Soules, M. R., Campbell, K. L., ... & Wood, J. W. (2003). Urinary estrone conjugate and pregnanediol 3-glucuronide enzyme immunoassays for population research. *Clinical chemistry*, *49*(7), 1139-1148.

Ordover, N. (2003). American eugenics: Race, queer anatomy, and the science of nationalism. U of Minnesota Press.

Oreskes, N. (2019). Why trust science?. In Why Trust Science?. Princeton University Press.

Owen, N., Poulton, T., Hay, F. C., Mohamed-Ali, V., & Steptoe, A. (2003). Socioeconomic status, C-reactive protein, immune factors, and responses to acute mental stress. *Brain, Behavior, and Immunity*, *17*(4), 286–295. https://doi.org/10.1016/S0889-1591(03)00058-8

Pannacciulli, N., Cantatore, F. P., Minenna, A., Bellacicco, M., Giorgino, R., & De Pergola, G. (2001). C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. International journal of obesity, 25(10), 1416-1420.

Pascoe, E. A., & Richman, L. S. (2009). Perceived Discrimination and Health: A Meta-Analytic Review. *Psychological Bulletin*, *135*(4), 531–554. https://doi.org/10.1037/a0016059

Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update. *Journal of Clinical Investigation*, *111*(12), 1805–1812. https://doi.org/10.1172/jci18921

Pfennig, D. W. (2021). Phenotypic plasticity & evolution: causes, consequences, controversies (p. 436). Taylor & Francis.

Picazo, O., & Ferna, A. (1995). Anti-anxiety effects of progesterone and some of its reduced metabolites: an evaluation using the burying behavior test. *Brain research*, *680*(1-2), 135-141.

Pigg, S.L., and V. Adams. 2005. Introduction: The Moral Object of Sex. In *Sex and Development: Science, Sexuality, and Morality in Global Perspective*, V. Adams and S.L. Pigg eds. Durham: Duke University Press, 1-38.

Pitts-Taylor, V. 2016. Feminism, Science, and Corporeal Politics. *Mattering: Feminism, science, and materialism*, *1*, 1.

Pollard, T. M. (1995). Use of cortisol as a stress marker: Practical and theoretical problems. *American Journal of Human Biology*, **7**(2), 265– 274. https://doi.org/10.1002/ajhb.1310070217

Prinz, P., Bailey, S., Moe, K., Wilkinson, C., & Scanlan, J. (2001). Urinary free cortisol and sleep under baseline and stressed conditions in healthy senior women: effects of estrogen replacement therapy. *Journal of sleep research*, *10*(1), 19-26.

Reed BG, Carr BR. The Normal Menstrual Cycle and the Control of Ovulation. In: Endotext. MDText.com, Inc., South Dartmouth (MA); 2000. PMID: 25905282

Reed, S. A. 1998. The politics and poetics of dance. *Annual review of anthropology*. [27(1)]: 503-532.

Rees, E. 2017. Talking Bodies: Interdisciplinary Perspectives on Embodiment, Gender and Identity. Springer.

Rethlefsen, M. L., Kirtley, S., Waffenschmidt, S., Ayala, A. P., Moher, D., Page, M. J., & Koffel, J. B. (2021). PRISMA-S: an extension to the PRISMA statement for reporting literature searches in systematic reviews. Systematic reviews, 10(1), 1-19.

Reynolds, T. A., Makhanova, A., Marcinkowska, U. M., Jasienska, G., McNulty, J. K., Eckel, L. A., ... & Maner, J. K. (2018). Progesterone and women's anxiety across the menstrual cycle. *Hormones and behavior*, *102*, 34-40.

Richardson, Sarah S. 2017. Plasticity and Programming: Feminism and the Epigenetic Imaginary. *Signs: Journal of Women in Culture and Society*. [43(1)]: 29-52.

Richey, C. R., Lee, K. M. N., Rodgers, E., & Clancy, K. B. (2019). Gender and sexual minorities in astronomy and planetary science face increased risks of harassment and assault. The Bulletin of the American Astronomical Society.

Rohlf F.J. 1999. Shape Statistics: Procrustes Superimpositions and Tangent Spaces. *J of Classification.* [16]: 197–223.

Rohlf, F. J., & Corti, M. (2000). Use of two-block partial least-squares to study covariation in shape. *Systematic biology*, *49*(4), 740-753.

Ronan, W. 2021. Officially becomes worst year in recent history for LGBTQ state legislative attacks as unprecedented number of states enact record-shattering number of anti-LGBTQ measures into law. *The Human Rights Campaign.* https://www. hrc. org/press-releases/2021-officially-becomes-worst-year-in-recent-history-for-lgbtq-state-legislative-attacks-as-unprecedented-number-of-states-enact-record-shattering-number-of-anti-lgbtq-measures-into-law.

Rostovtseva, V. V., Mezentseva, A. A., Windhager, S., & Butovskaya, M. L. (2021). Sexual dimorphism in facial shape of modern Buryats of Southern Siberia. *American Journal of Human Biology*, *33*(2), e23458. https://doi.org/10.1002/ajhb.23458

Saboowala, S., Wilson, M.A., Vilchis, S., Rogers- LaVanne, M.P. Embodiment in anthropological epigenetic research. Human Biology.

Saefken, B., Kneib T., van Waveren C.-S. and Greven, S. (2014) A unifying approach to the estima- tion of the conditional Akaike information in generalized linear mixed models. Electronic Journal Statistics Vol. 8, 201-225.

Saefken, B., Ruegamer, D., Kneib, T. and Greven, S. (2021): Conditional Model Selection in Mixed-Effects Models with cAIC4. <doi:10.18637/jss.v099.i08>

Salvante, K. G., Brindle, E., McConnell, D., O'connor, K., & Nepomnaschy, P. A. (2012). Validation of a new multiplex assay against individual immunoassays for the quantification of reproductive, stress, and energetic metabolism biomarkers in urine specimens. American Journal of Human Biology, 24(1), 81-86.

Sapolsky, R. M. (2021). Glucocorticoids, the evolution of the stress-response, and the primate predicament. *Neurobiology of Stress*, **14**, 100320. https://doi.org/10.1016/j.ynstr.2021.100320

Sarkar, P. L., Zeng, L., Chen, Y., Salvante, K. G., & Nepomnaschy, P. A. (2013). A longitudinal evaluation of the relationship between first morning urinary and salivary cortisol. *American Journal of Human Biology*, *25*(3), 351–358. https://doi.org/10.1002/ajhb.22376

Schlager, S. (2017) Morpho and Rvcg -- shape analysis in r: R-packages for geometric morphometrics, shape analysis and surface manipulations. *Statistical shape and deformation analysis* (eds G. Zheng), S. Li), & G. Székely), pp. 217–256. Academic Press.

Schuller, K. (2018). The biopolitics of feeling: Race, sex, and science in the nineteenth century. Duke University Press.

Schuller, K., and J. Gill-Peterson. 2020. Introduction: Race, the State, and the Malleable Body. *Social Text*. [38]: 1-17.

Shattuck-Heidorn, H., & Richardson, S. S. (2019). Sex/Gender and the Biosocial Turn. The Scholar and Feminist Online, 15. http://sfonline.barnard.edu/neurogenderings/sex-gender-and-the-biosocial-turn/

Shattuck-Heidorn, H., and S.S. Richardson. 2019. Sex/gender and the biosocial turn. *Neurogenderings.* [15(2)].

Shattuck‐Heidorn, H., Eick, G. N., Kramer, K. L., Sugiyama, L. S., Snodgrass, J. J., & Ellison, P. T. (2021). Variability of C‐reactive protein in first‐generation Ecuadorian immigrants living in the United States. American Journal of Human Biology, 33(6), e23547.

Siobhan Somerville. 1997. Scientific Racism and the Invention of the Homosexual Body. In *The Gender/Sexuality Reader: Culture, History, Political Economy*, R.N. Lancaster and M. di Leonardo eds. New York: Routledge, 37-52.

Skouby SO, Gram J, Andersen LF, Sidelmann J, Petersen KR, Jespersen J. 2002. Hormone replacement therapy: estrogen and progestin effects on plasma C-reactive protein concentrations. Am J Obstet Gynecol 186:969–977.

Smith, R. W. 2021. Imperial terroir: Toward a queer molecular ecology of colonial masculinities. *Current Anthropology*. [62(S23)] S000-S000.

Smith, R. W., & Archer, S. M. (2019). Bisexual science. American Anthropologist, 121(2), 491-492.

Snorton, C. R., and J. Haritaworn. 2013. Trans necropolitics: A transnational reflection on violence, death, and the trans of color afterlife. In *The Transgender Studies Reader Remix*, Routledge, 305-316.

Spillers, H. J. 1994. *Mamas baby, papas maybe: An American grammar book*, Duke University Press, 454-481.

Stanford, C., J.S. Allen, and S.C. Antón. 2011. *Biological anthropology: the natural history of humankind*. Fourth Ed. Pearson Education.

Stibbard-Hawkes, D. N., Attenborough, R. D., Mabulla, I. A., & Marlowe, F. W. (2020). To the hunter go the spoils? No evidence of nutritional benefit to being or marrying a well-reputed Hadza hunter. American Journal of Physical Anthropology, 173(1), 61-79.

Stinson, S., B. Bogin, and D.H. O'Rourke. 2012. *Human biology: an evolutionary and biocultural perspective*. John Wiley & Sons.

Strimbu, K., and J.A. Tavel. 2010. What are Biomarkers? *Current Opinion in HIV and AIDS*. [5(6)]: 463–466.

Strings, S. (2019). Fearing the black body. In Fearing the Black Body. New York University Press.

Stryker S. and A.Z. Aizura. 2013. Transgender Studies 2.0. In *The Transgender Studies Reader 2*, Stryker S., and Aizura A.Z. eds. New York: Routledge.

Stryker, S. 2008. Transgender history, homonormativity, and disciplinarity. *Radical History Review*. [100]: 145-157.

Stryker, S. 2008. *Transgender History*. Berkley, CA: Seal Press.

Takkouche, B., & Norman, G. (2011). PRISMA statement. Epidemiology, 22(1), 128.

Tallbear, Kim. 2019. Feminist, Queer, and Indigenous Thinking as an Antidote to Masculinst Objectivity and Binary Thinking in Biological Anthropology. American Anthropologist.

Tate, C. C., C.P. Youssef, and J.N. Bettergarcia. 2014. Integrating the study of transgender spectrum and cisgender experiences of self-categorization from a personality perspective. *Review of General Psychology*. [*18*(4)]: 302-312.

Tchernof, A., Nolan, A., Sites, C. K., Ades, P. A., & Poehlman, E. T. (2002). Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation, 105(5), 564-569.

Tennessee SB0001, https://wapp.capitol.tn.gov/apps/BillInfo/Default.aspx?BillNumber=SB0001&GA=113.

Tennessee SB3, https://wapp.capitol.tn.gov/apps/BillInfo/default.aspx?BillNumber=SB0003&ga=113.

Thayer, Z. M., & Kuzawa, C. W. (2014). Early origins of health disparities: Material deprivation predicts maternal evening cortisol in pregnancy and offspring cortisol reactivity in the first few weeks of life. American Journal of Human Biology, 26(6), 723–730. https://doi.org/10.1002/ajhb.22532

Thayer, Z. M., Wilson, M. A., Kim, A. W., & Jaeggi, A. V. (2018). Impact of prenatal stress on offspring glucocorticoid levels: A phylogenetic meta-analysis across 14 vertebrate species. Scientific reports, 8(1), 1-9.

Thiele JR, Habersberger J, Braig D, Schmidt Y, Goerendt K, Maurer V, Bannasch H, Scheichl A, Woollard KJ, von Dobschütz E, Kolodgie F, Virmani R, Stark GB, Peter K, Eisenhardt SU. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: in vivo proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. Circulation. 2014 Jul 1;130(1):35-50. doi: 10.1161/CIRCULATIONAHA.113.007124. Epub 2014 Apr 28. PMID: 24982116.

Torres, J.B., & Torres Colón, G.A. (2020). Racial Experience as an Alternative Operationalization of Race. Human Biology 92(3), 181-187. https://www.muse.jhu.edu/article/793971.

Uy, J., Hawks, J., & VanSickle, C. (2020). Sexual dimorphism of the relationship between the gut and pelvis in humans. *American Journal of Physical Anthropology*, *173*(1), 130–140. https://doi.org/10.1002/ajpa.24084

van Anders, S. M., Tolman, R. M., & Volling, B. L. (2012). Baby cries and nurturance affect testosterone in men. Hormones and Behavior, 61(1), 31-36.

Verma, S., Szmitko, P. E., & Yeh, E. T. (2004). C-reactive protein: structure affects function. *Circulation*, *109*(16), 1914-1917.

Viau, V. (2002). Functional cross-talk between the hypothalamic-pituitary-gonadal and -Adrenal axes. *Journal of Neuroendocrinology*, *14*(6), 506–513. https://doi.org/10.1046/j.1365-2826.2002.00798.x

Viciano, J., Tanga, C., D'Anastasio, R., Belcastro, M. G., & Capasso, L. (2021). Sex estimation by odontometrics of nonadult human remains from a contemporary Italian sample. American Journal of Physical Anthropology, 175(1), 59–80. https://doi.org/10.1002/ajpa.24132.

Villada, C., Espin, L., Hidalgo, V., Rubagotti, S., Sgoifo, A., & Salvador, A. (2017). The influence of coping strategies and behavior on the physiological response to social stress in women: The role of age and menstrual cycle phase. *Physiology and Behavior*, *170*, 37–46. https://doi.org/10.1016/j.physbeh.2016.12.011

Vitzthum, V.J. (2009) The ecology and evolutionary endocrinology of reproduction in the human female. *American Journal of Physical Anthropology*, **140**, 95–136.

Walsh BW, Paul S, Wild RA, Dean RA, Tracy RP, Cox DA, Anderson PW. 2000. The effects of hormone replacement therapy and raloxifene on C-reactive protein and homocysteine in healthy postmenopausal women: a randomized, controlled trial. J Clin Endocrinol Metab 85:214–218.

Wander, K., Brindle, E., & O'Connor, K. A. (2008). C-reactive protein across the menstrual cycle. *American Journal of Physical Anthropology. 136*(2), 138–146. https://doi.org/10.1002/ajpa.20785

Wanta, J. W., Niforatos, J. D., Durbak, E., Viguera, A., & Altinay, M. (n.d.). Mental Health Diagnoses Among Transgender Patients in the Clinical Setting: An All-Payer Electronic Health Record Study. https://doi.org/10.1089/trgh.2019.0029

Ward, J.H. (1963) Hierarchical Grouping to Optimize an Objective Function.

Warin, M., Kowal, E., & Meloni, M. (2020). Indigenous knowledge in a postgenomic landscape: The politics of epigenetic hope and reparation in Australia. Science, Technology, & Human Values, 45(1), 87-111.

Warren MP (1980) The effects of exercise on pubertal progression and reproductive function in girls. J Clin Endocrinol Metab 51: 1150–1157.

Weasel, L. H. 2016. Embodying Intersectionality. *Mattering: Feminism, science, and materialism*. [1]: 104.

Weidman, A. 2014. Anthropology and voice. *Annual Review of Anthropology*. [43] 37-51.

West-Eberhard, M. J. (2003). Developmental plasticity and evolution. Oxford University Press.

Williams, N.I., Reed, J.L., Leidy, H.J., Legro, R.S. and De Souza, M.J. (2010) Estrogen and progesterone exposure is reduced in response to energy deficiency in women aged 25–40 years. *Human Reproduction*, **25**, 2328–2339.

Wingfield, J. C., & Kitaysky, A. S. (2002). Endocrine responses to unpredictable environmental events: Stress or anti-stress hormones? In *Integrative and Comparative Biology* (Vol. 42, pp. 600–609). Society for Integrative and Comparative Biology. https://doi.org/10.1093/icb/42.3.600

Wirth, M. M., Meier, E. A., Fredrickson, B. L., & Schultheiss, O. C. (2007). Relationship between salivary cortisol and progesterone levels in humans. *Biological Psychology*, *74*(1), 104–107. https://doi.org/10.1016/j.biopsycho.2006.06.007

Wolfram, M., Bellingrath, S., & Kudielka, B. M. (2011). The cortisol awakening response (CAR) across the female menstrual cycle. *Psychoneuroendocrinology*, *36*(6), 905–912. https://doi.org/10.1016/j.psyneuen.2010.12.006

Worthman, C. M., and E.J. Costello. 2009. Tracking biocultural pathways in population health: The value of biomarkers. *Annals of Human Biology*. [36(3)]: 281–297.

Wunder, D. M., Yared, M., Bersinger, N. A., Widmer, D., Kretschmer, R., & Birkhäuser, M. H. (2006). Serum leptin and C-reactive protein levels in the physiological spontaneous

menstrual cycle in reproductive age women. *European Journal of Endocrinology*, *155*(1), 137–142. https://doi.org/10.1530/eje.1.02178

Wynter, S. 2003. Unsettling the coloniality of being/power/truth/freedom: Towards the human, after man, its overrepresentation—An argument. *CR: The new centennial review*. [*3*]: 257-337.

Zannas, A. S., Arloth, J., Carrillo-Roa, T., Iurato, S., Röh, S., Ressler, K. J., ... & Mehta, D. (2015). Lifetime stress accelerates epigenetic aging in an urban, African American cohort: relevance of glucocorticoid signaling. Genome biology, 16(1), 1-12.

Żelaźniewicz, A., Nowak, J., Studzińska, I., & Pawłowski, B. (2020). Do adipokines levels influence facial attractiveness of young women? *American Journal of Physical Anthropology*, *173*(2), 250–257. https://doi.org/10.1002/ajpa.24114

Zimman, L., and K. Hall. 2009. Language, embodiment, and the 'third sex'. *Language and identities*. [166]: 178.

APPENDIX A

IRB APPROVAL LETTER FOR CHAPTERS 3 AND 4

UNIVERSITY OF ILLINOIS URBANA-CHAMPAIGN

Office of the Vice Chancellor for Research & Innovation

Office for the Protection of Research Subjects
805 W. Pennsylvania Ave., MC-095
Urbana, IL 61801-4822

Notice of Approval: Continuing – Data Analysis Only

March 14, 2022

Principal Investigator	Kathryn Clancy
CC	Mary Rogers
	Katharine Lee
	Meredith Wilson
	Maria Cox
Protocol Title	*Ecological determinants of luteal reproductive function among rural Polish women*
Protocol Number	13856
Funding Source	Unfunded
Review Type	Expedited 8
Status	Data Analysis Only
Risk Determination	No more than minimal risk
Approval Date	March 14, 2022
Expiration Date	March 13, 2027

This letter authorizes the use of human subjects in the above protocol. The University of Illinois at Urbana-Champaign Institutional Review Board (IRB) has reviewed and approved the research study as described.

The Principal Investigator of this study is responsible for:

- Conducting research in a manner consistent with the requirements of the University and federal regulations found at 45 CFR 46.
- Using the approved consent documents, with the footer, from this approved package.
- Requesting approval from the IRB prior to implementing modifications.
- Notifying OPRS of any problems involving human subjects, including unanticipated events, participant complaints, or protocol deviations.
- Notifying OPRS of the completion of the study.

APPENDIX B

CHAPTER 1 TEXTUAL ANALYSIS RESULTS

The file “Wilson_Dissertation_AppendixB_Spring2023.docx” contains the results of textual analysis of American Journal of Human Biology and American Journal of Biological Anthropology.

APPENDIX C

CHAPTER 3 SUPPLEMENTAL MATERIALS

Inter and Intra assay CVs

Laboratory Protocol: We measured multiple hormones over the course of each participant's menstrual cycle using two types of Quansys multiplex enzyme linked immunosorbent assays (ELISA). Hormone data was generated for samples collected in Poland and the United States during the 2014-2017 field seasons. The measured hormones in this analysis included: the urinary estradiol metabolite (estrone-3-glucuronide, E1G), C-reactive protein (CRP), and urinary progesterone metabolite (pregnanediol glucuronide, PdG). All hormones except PdG were run on a custom Quansys 8-Plex ELISA or 9-Plex ELISA, and PdG was run individually on a 1-plex Quansys ELISA. All samples were run in duplicate.

140 total ELISA plates were run for the PdG assay, ninety-five ELISA plates were run for the 9-Plex, and forty-four ELISA plates were run for the 8-Plex. The intra- and inter- assay variation for each measured hormones was calculated. The intra-assay variation was as follows: E1G (8.83%), CRP (6.51%), and PdG (31%). The inter-assay variation is located in Tables C.1 and C.2. While the inter-assay variation is high, it is within the range typically seen in multiplex assays (Chowdhury et al., 2009; Bastarache et al., 2011). Further, for analytes with extremely low concentrations in the control, the CV is inflated due to comparing trace amounts (presence) to zero (absence).

	CNTL_A	**CNTL_B**	**CNTL_C**	**CNTL_D**	**CNTL_E**
Batch Number (n plates)	*3 (10)*	*2 (34), 3 (76)*	*3 (88)*	*3 (88)*	*1 (104), 2 (76)*
E1G_CV	39.01	28.02	22.28	22.93	35.53
CRP_CV	25.26	40.90	48.23	60.59	19.16

Table C.1: The CV for each control for each analyte run on the custom 8 or 9 Plex is displayed here. Note that some controls were run across batches, and multiple controls were present on a single plate. All assays run by same technician.

	CNTL 1	CNTL2	CNTL3	CNTL4	H1to1	H1to10	L1to1	L1to10	CNTL7	CNTL8	CNTL9	CNTL1 0
Tech:	MPR	MPR	MPR	MPR	MAW	MAW	MAW	MAW	MAW	MAW	MAW	MAW
Batch Numbers (n)	1 (11)	47	2 (18)	2 (12)	2 (3)	2 (10)	2 (13)	2 (23)	2 (0)	2 (6)	2 (1)	2 (17)
PdG_CV	41.36	28.37	18.67	35.85	66.35	30.34	21.2	29.82	NA	19.5	NA	26.71

Table C.2: The CV for each control for PdG is displayed here. Note that some controls were run across batches, and multiple controls were present on a single plate. The NA represents if the analyte was not present in that particular control.

LMMs and Cycle phase unscaled results for Polish and Polish American analysis

Model #		cAIC
Model 0	Raw CRP ~ 1+ (1|id)	22,456.8
Model 1	Raw CRP ~ menses + (1|id)	22,458.4
Model 2	Raw CRP ~ menses + cycle day + (1|id)	22,459.8
*Model 3	Raw CRP ~ menses + cycle day + scaled E1G + (1|id)	22,453.6
Model 4	Raw CRP ~ menses + cycle day + scaled E1G + scaled PdG + (1|id)	22,454.1
Model 5	Raw CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + (1|id)	22,454.2
Model 6	Raw CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + body fat % (1|id)	22,454.2
Model 7	Raw CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + body fat % + waist to hip ratio + (1|id)	22,454.2

Table C.3: cAIC results for Polish unscaled CRP models. *model chosen by cAIC.

Model #		cAIC
Model 0	Raw CRP ~ 1+ (1|id)	5704.7
Model 1	Raw CRP ~ menses + (1|id)	5692.8
Model 2	Raw CRP ~ menses + cycle day + (1|id)	5693.6
Model 3	Raw CRP ~ menses + cycle day + scaled E1G + (1|id)	5679.5
*Model 4	Raw CRP ~ menses + cycle day + scaled E1G + scaled PdG + (1|id)	5678.6
Model 5	Raw CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + (1|id)	5678.7
Model 6	Raw CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + body fat % (1|id)	5678.9
Model 7	Raw CRP ~ menses + cycle day + scaled E1G + scaled PdG + age + body fat % + waist to hip ratio + (1|id)	5679.0

Table C.4: cAIC results for Polish American unscaled CRP models. *model chosen by cAIC.

	Estimate	Std. error	t value	95% CI	r^2beta
Intercept	234.7	19.1	12.3	197.1 – 272.2	.005 (full model)
menses	-9.5	6.5	-1.5	-22.2 - 3.2	.001
Scaled day	2.0	3.1	.6	-4.1 - 8.1	.000
Scaled E1G	17.9	6.4	2.8	5.3 - 30.4	.004

Table C.5: LMM results for the Polish unscaled CRP model 3.

	Estimate	Std. error	t value	95% CI	r^2beta
Intercept	223.3	6.5	34.3	210.6 – 236.0	.051 (full model)
menses	-22.9	5.0	-4.5	-32.7 – -13.0	.031
Scaled day	4.9	3.1	1.6	-1.05 – 10.94	.004
Scaled E1G	20.5	5.2	3.9	10.25 – 30.7	.026
Scaled PdG	-10.1	5.9	-1.7	-21.6 – 1.33	.005

Table C.6: LMM results for the Polish American unscaled CRP model 4.

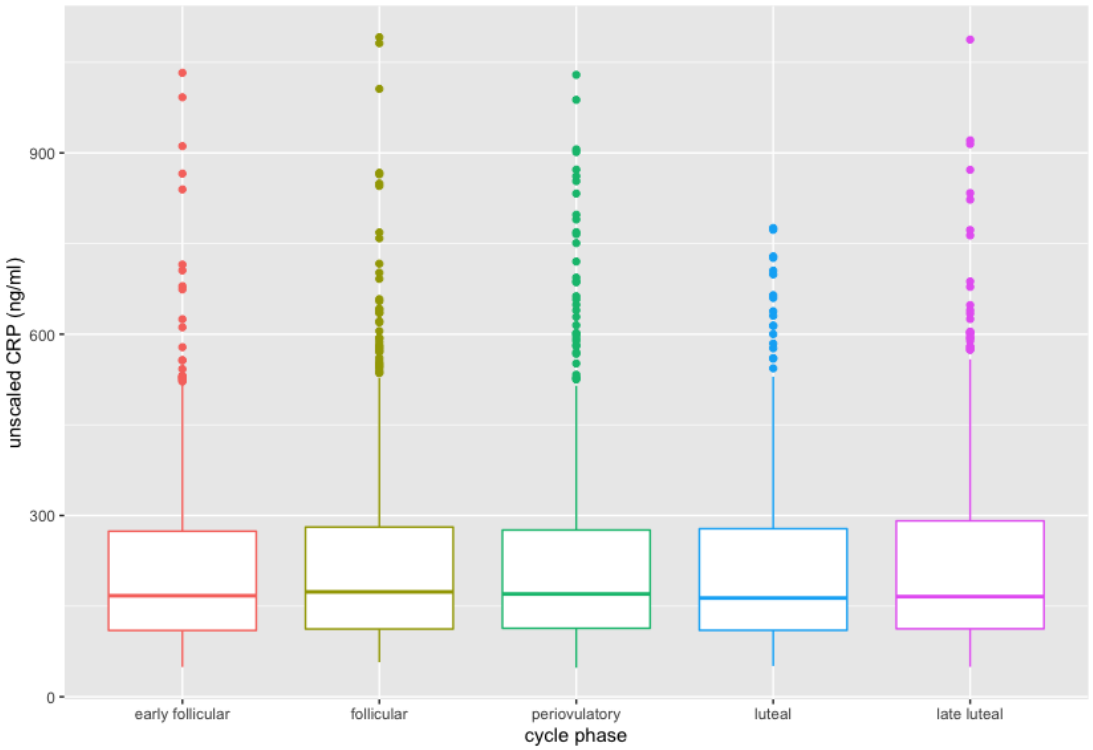


Figure C.1: Box plots of Polish unscaled CRP by cycle phase. No significant differences were found.

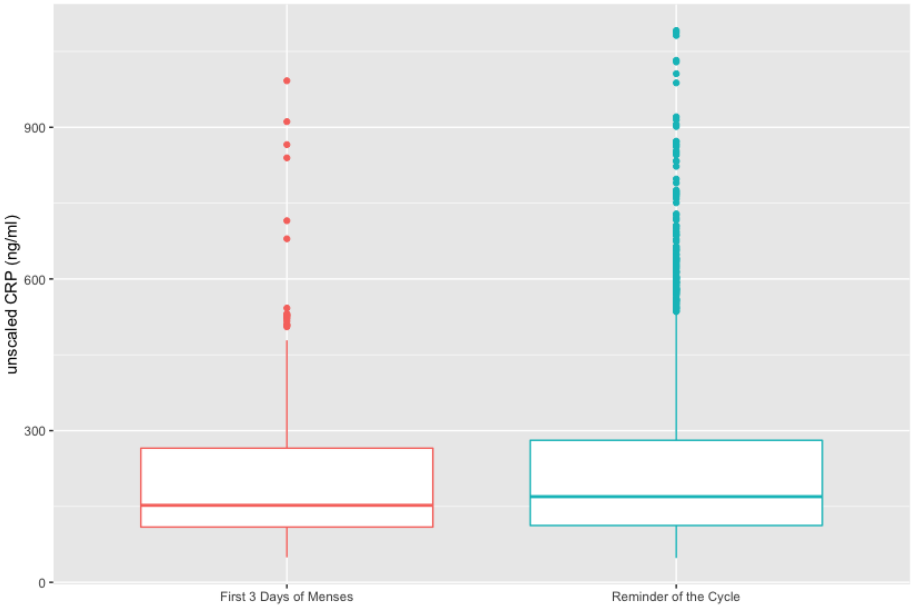


Figure C.2: Boxplot of Polish unscaled CRP between active bleeding (menses) and no active bleeding. No significant differences were found.

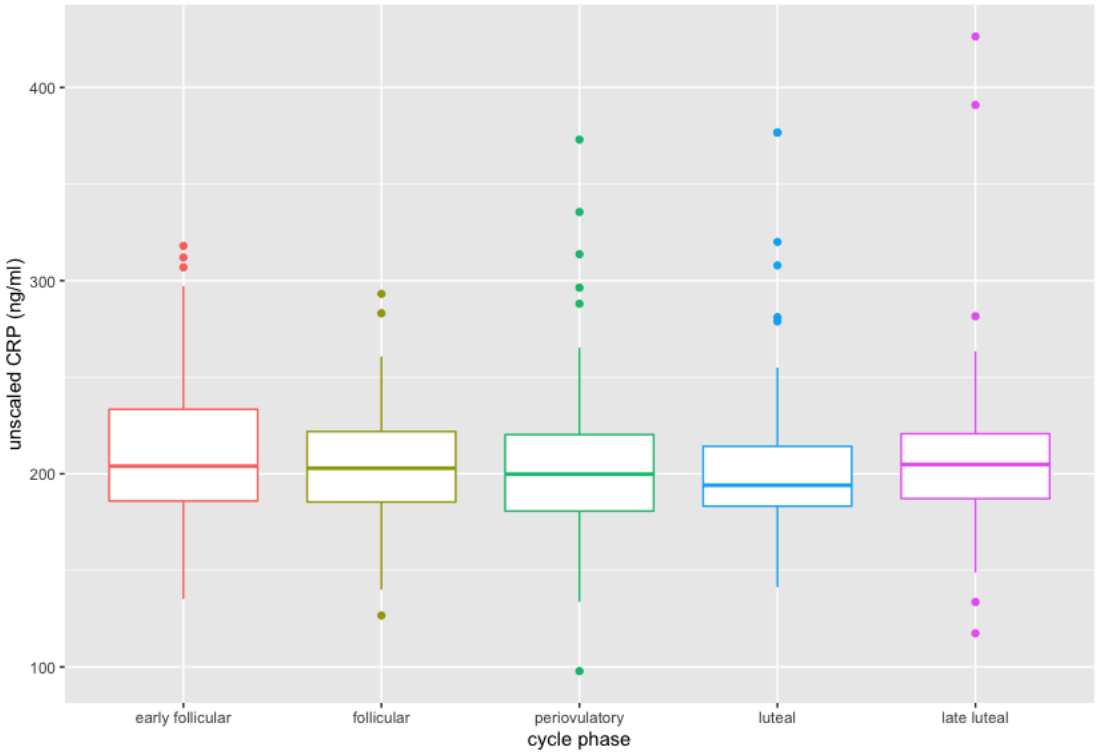


Figure C.3: Box plots of Polish American unscaled CRP by cycle phase. No significant differences were found.

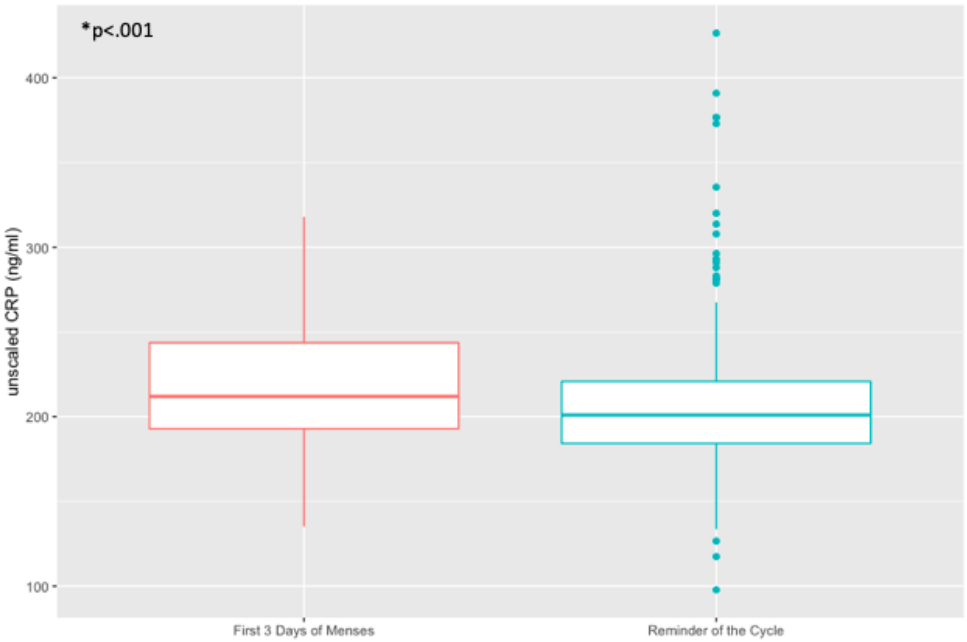


Figure C.4: Box plots of Polish American unscaled CRP comparing the first three days of menses (median=212(ng/ml)) with the rest of the cycle (median = 201(ng/ml)). A Kruskal-Wallace test showed significant group difference (p<.001).

	Polish unscaled CRP concentrations (ng/ml)	Polish American unscaled CRP concentrations (ng/ml)
Early follicular	167	204
Follicular	174	203
Periovulatory	170	200
Luteal	163	194
Late luteal	166	205
First three days of menses	152	212^
Rest of the cycle	169	201^

Table C.7: The median unscaled CRP values for the Polish and Polish American samples. *denotes significant difference by Kruskal-Wallace (p<.01).

Exploring age as a possible source of CRP phenotype variation

The same analyses were conducted on the entire sample set (polish and polish American combined) after splitting into a younger and older group. The younger group and older group were determined by averaging total sample (mean= 32, n=98) age and individuals younger than 32 years were assigned the younger group and individuals older than 32 were assigned the older group. We found no cycle effects or cycle phase differences in both groups. Below are the average line graphs and boxplots for the age analysis.

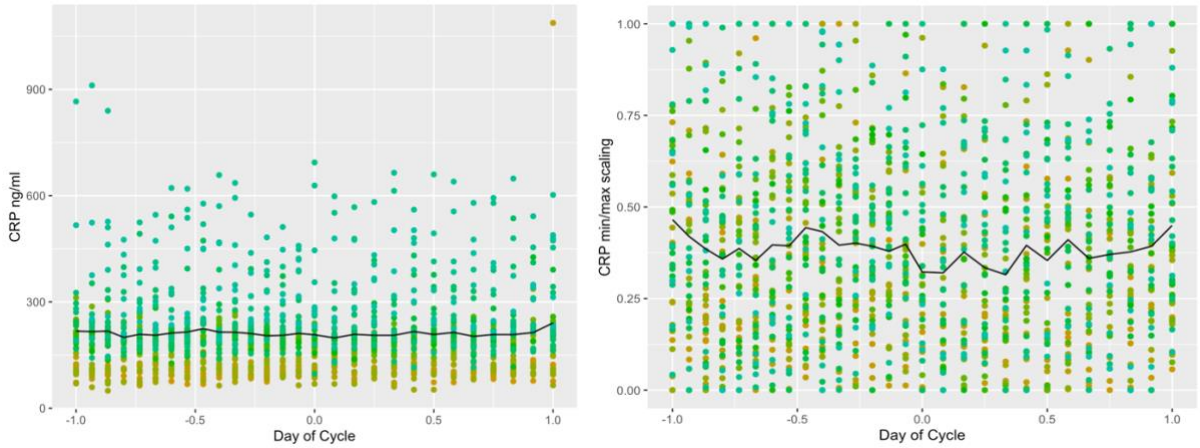


Figure C.5: The scaled and unscaled CRP average cycle shape for the younger group (<32 years).

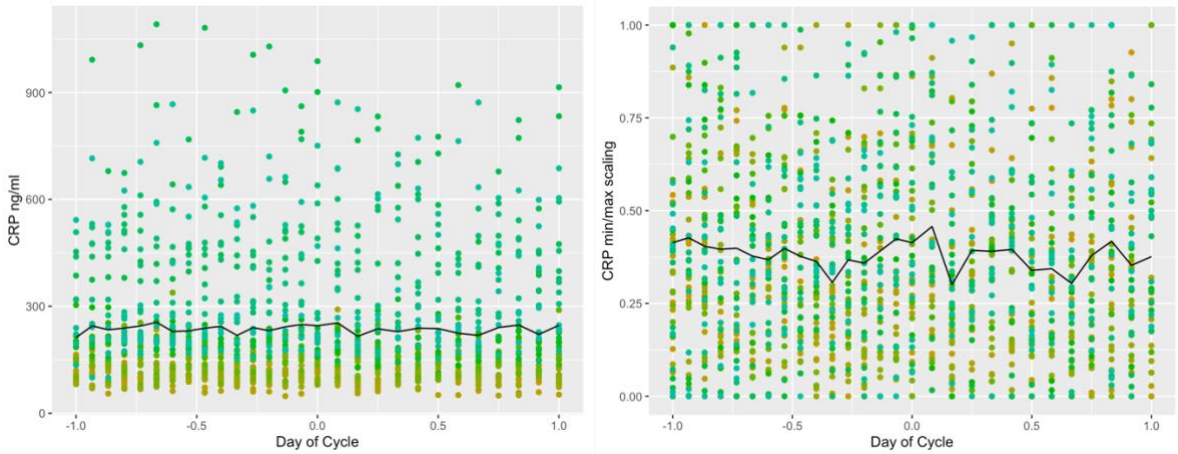


Figure C.6: The scaled and unscaled CRP average cycle shape for the older group (<32 years).

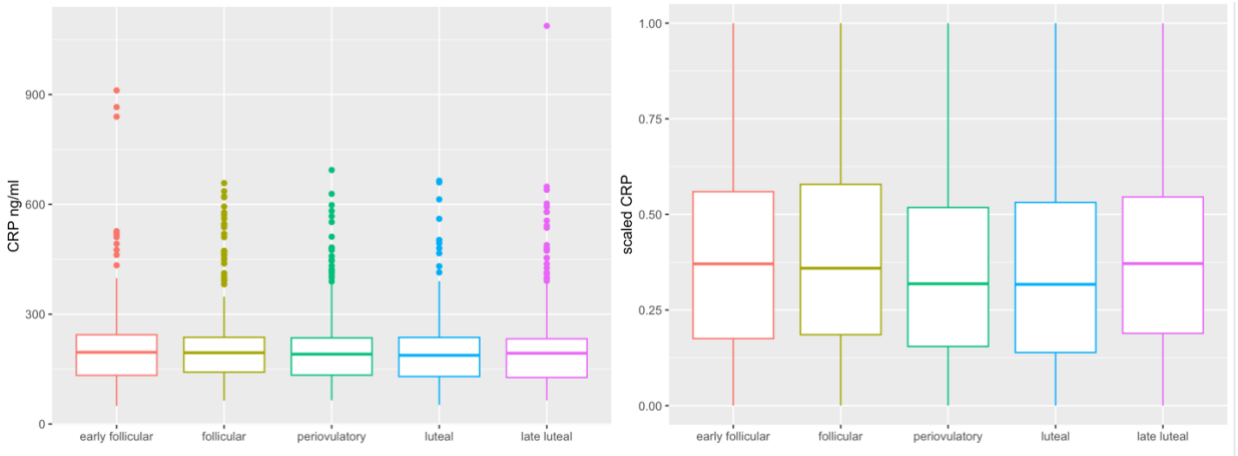


Figure C.7: The unscaled and scaled CRP box plots comparing CRP concentration distributions by cycle phase of the young group. There were no significant differences.

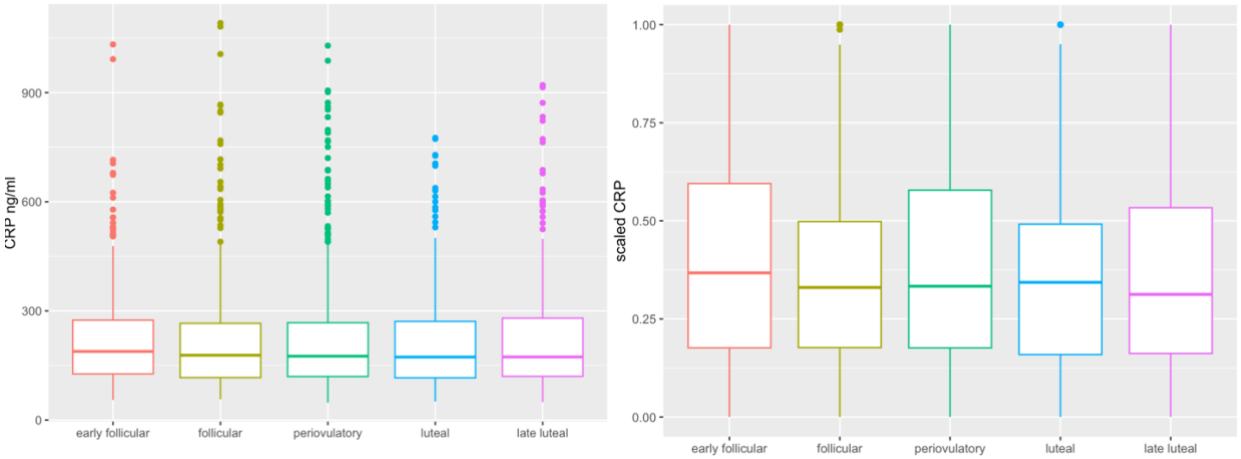


Figure C.8: The unscaled and scaled CRP box plots comparing CRP concentration distributions by cycle phase of the older group. There were no significant differences.

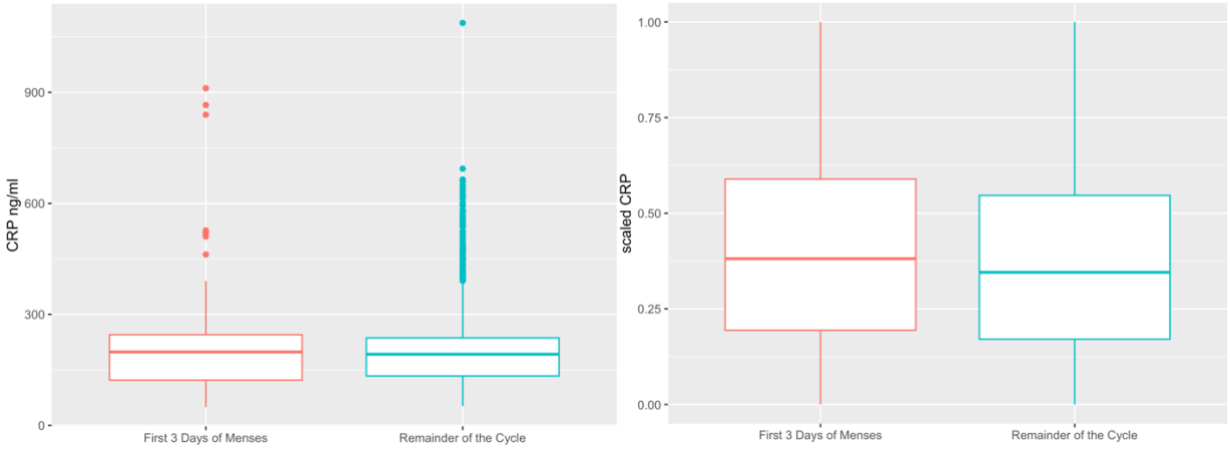


Figure C.9: The unscaled and scaled CRP box plots comparing CRP concentration distributions by first three days of menses and the remainder of the cycle of the younger group. There were no significant differences.

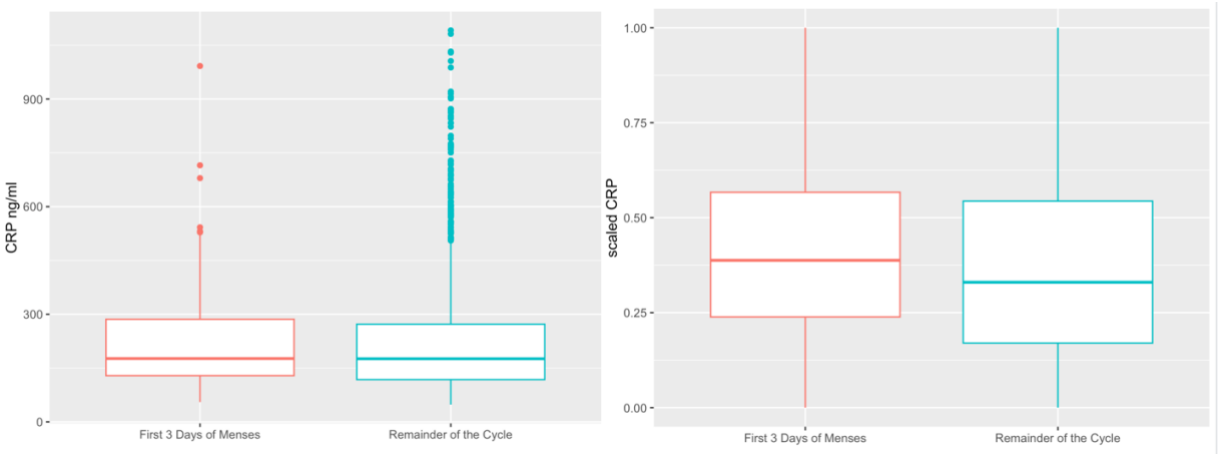


Figure C.10: The unscaled and scaled CRP box plots comparing CRP concentration distributions by first three days of menses and the remainder of the cycle of the older group. There were no significant differences.

	Young age group unscaled CRP	Young age group scaled CRP	Old age group unscaled CRP	Old age group scaled CRP
Early follicular	196	.371	189	.367
Follicular	195	.359	178	.330
Periovulatory	191	.319	176	.333
Luteal	188	.317	173	.343
Late luteal	193	.371	173	.313
First three days of menses	199	.381	177	.388
Rest of the cycle	192	.346	176	.330

Table C.8: The median unscaled and scaled CRP values for the young and older groups. There were no significant differences.

Exploring body fat percentage as a possible source of CRP phenotype variation

The same analyses were conducted on the entire sample set (polish and polish American combined) after splitting into a low body fat (n=14), average body fat (n=33), and high body fat (n=51). Groups were determined by using the American Journal of Clinical Nutrition cut offs for low (<21%), average (21-32%), and high body fat (>32%) percentage for individuals assigned female at birth. While the average body fat group had the lowest CRP concentrations (for the unscaled analyses) compared to the low and high ($p<.01$), we found no cycle effects or cycle phase differences in any of the groups, except when comparing scaled CRP between the beginning of menses and the remainder of menses of the low body fat group ($p<.05$). However, this relationship is no longer significant after removing the Polish American individuals (n=4) from the low body fat group, suggesting the difference is driven by population. Below are the average line graphs and boxplots for the body fat analyses.

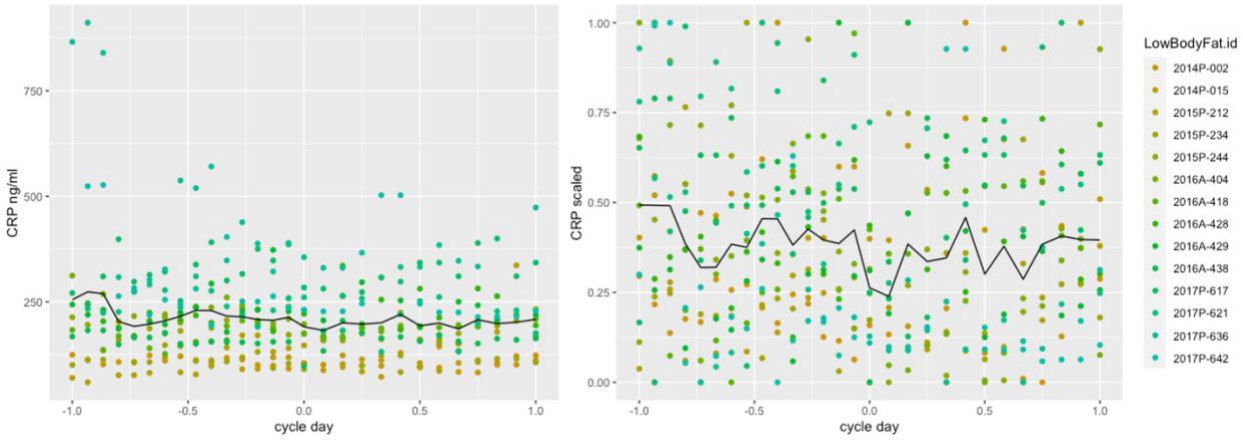


Figure C.11: The scaled and unscaled CRP average cycle shape for the low body fat percentage group (< 21%).

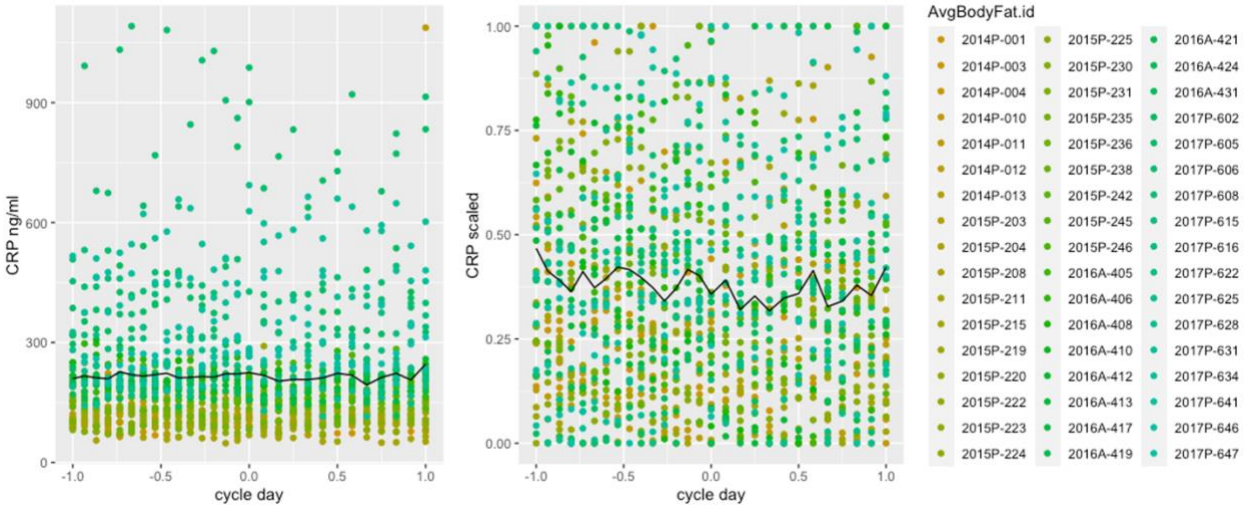


Figure C.12: The scaled and unscaled CRP average cycle shape for the average body fat percentage group (21% - 32%).

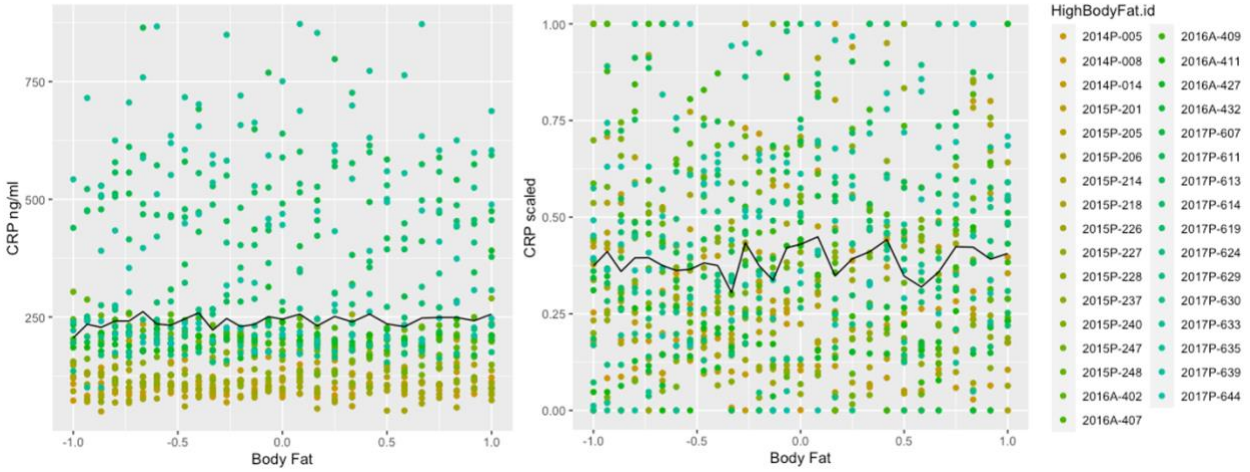


Figure C.13: The scaled and unscaled CRP average cycle shape for the high body fat percentage group (>32%).

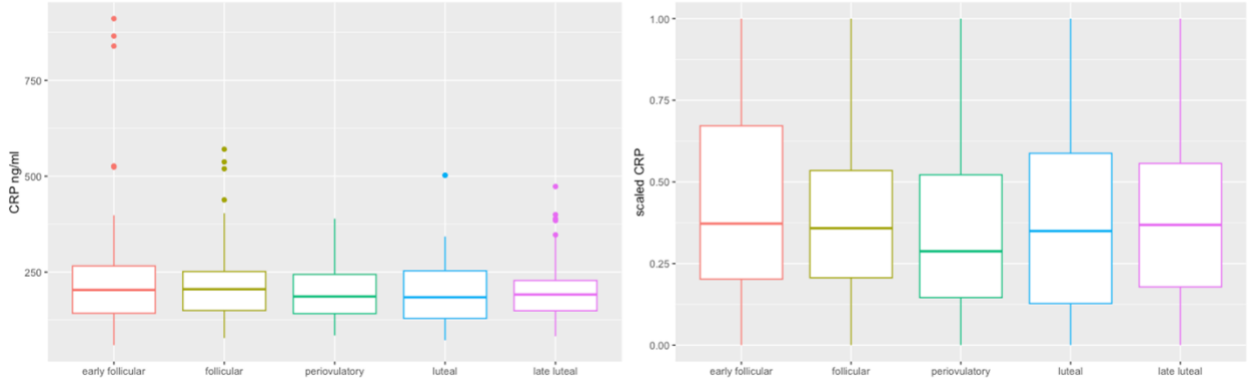


Figure C.14: The unscaled and scaled CRP box plots comparing CRP concentration distributions by cycle phase of the low body fat group. There were no significant differences.

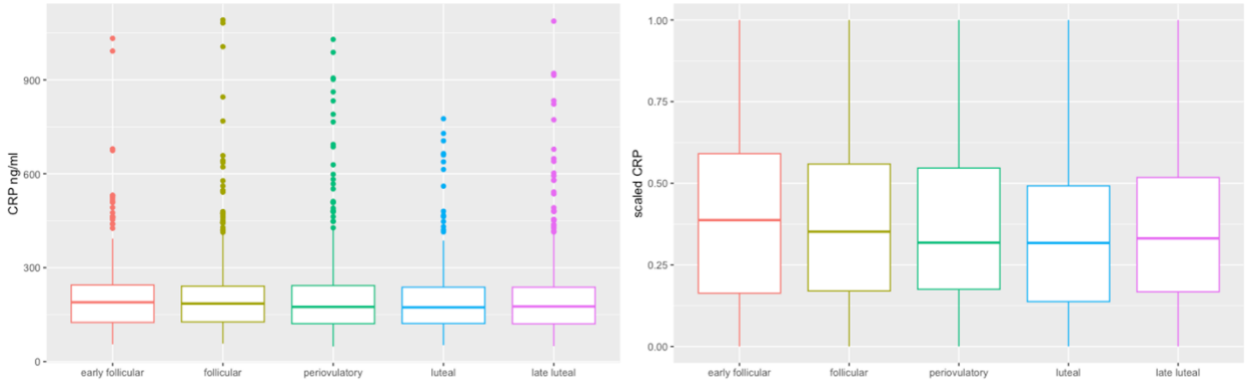


Figure C.15: The unscaled and scaled CRP box plots comparing CRP concentration distributions by cycle phase of the average body fat group. There were no significant differences.

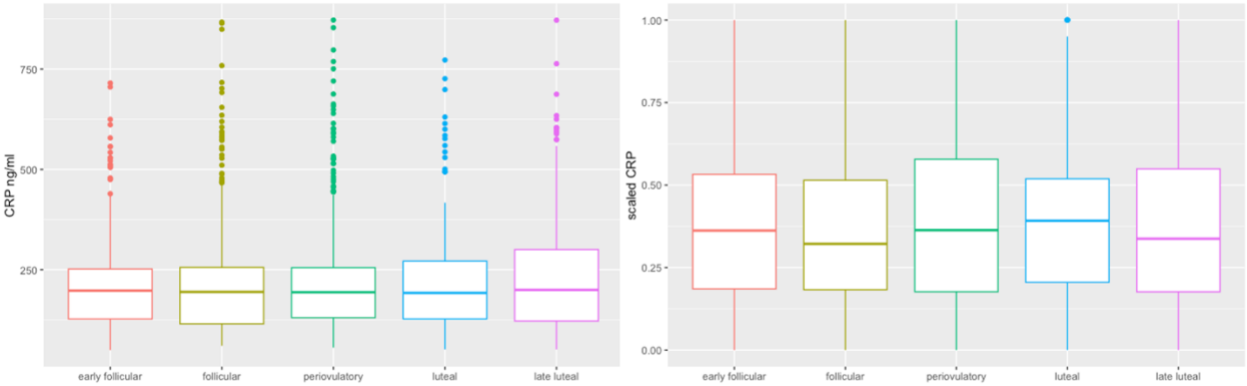


Figure C.16: The unscaled and scaled CRP box plots comparing CRP concentration distributions by cycle phase of the high body fat group. There were no significant differences.

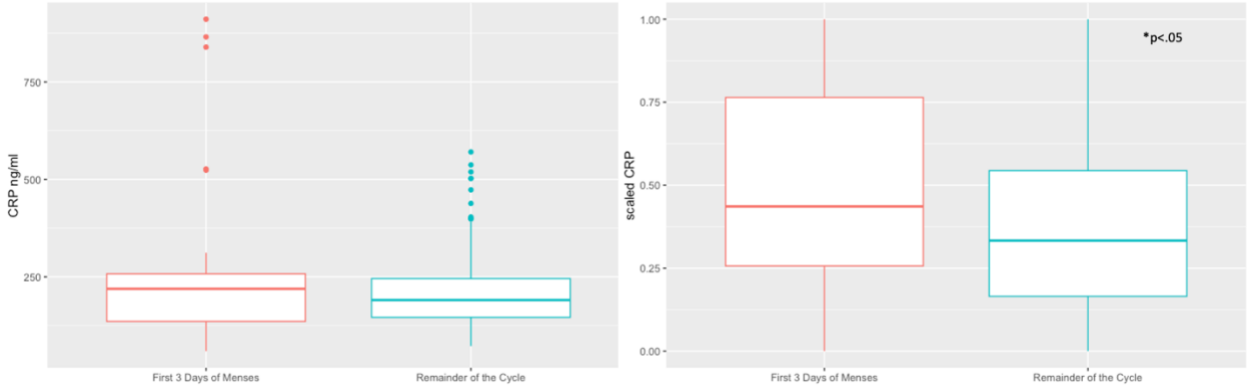


Figure C.17: The unscaled and scaled CRP box plots comparing CRP concentration distributions by first three days of menses and the remainder of the cycle of the low body fat group. The first three days of menses was significantly higher than the remainder of the cycle in this analysis ($p<.05$).

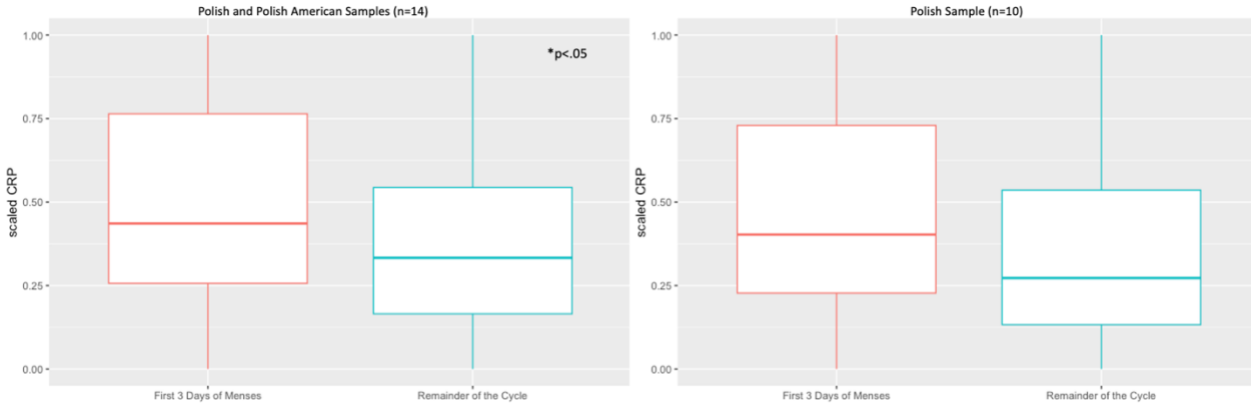


Figure C.18: The scaled CRP box plots comparing CRP concentration distributions by first three days of menses and the remainder of the cycle of the low body fat group (n=14) and the low body fat group, Polish only (n=10). The first three days of menses was significantly higher than the remainder of the cycle in this analysis for the combined sample ($p<.05$) but is no longer significant after removing Polish American individuals.

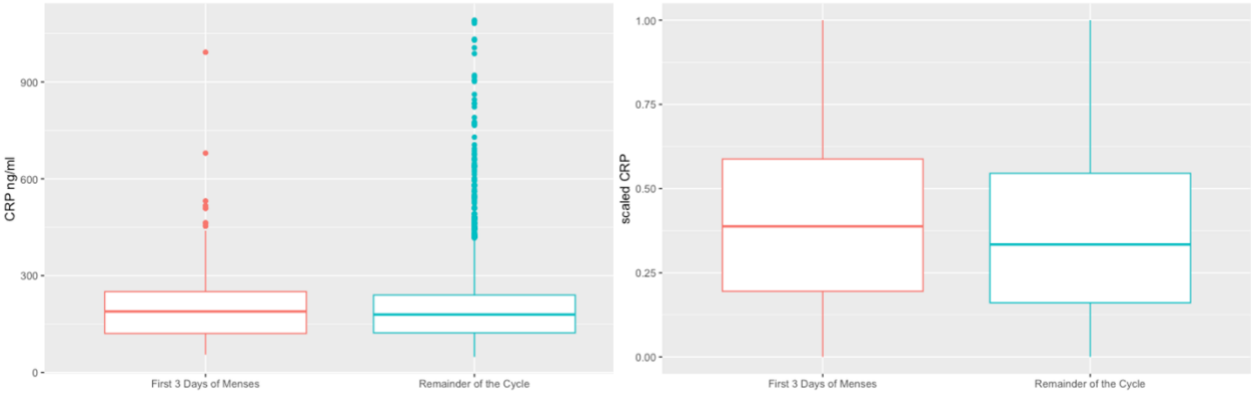


Figure C.19: The unscaled and scaled CRP box plots comparing CRP concentration distributions by first three days of menses and the remainder of the cycle of the average body fat group. There are no significant differences.

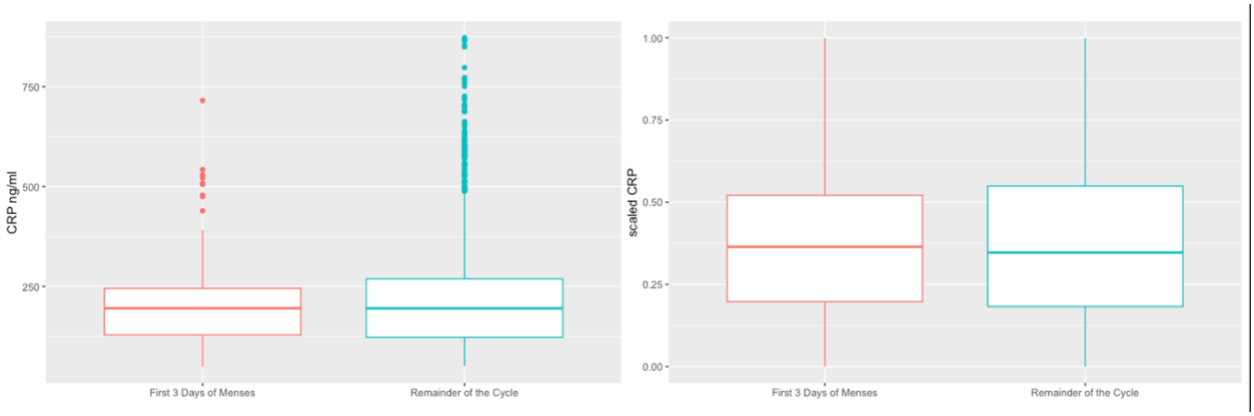


Figure C.20: The unscaled and scaled CRP box plots comparing CRP concentration distributions by first three days of menses and the remainder of the cycle of the high body fat group. There were no significant differences

	Low body fat unscaled CRP	Low body fat scaled CRP	Average body fat unscaled CRP	Average body fat scaled CRP	High body fat unscaled CRP	High body fat scaled CRP
Early follicular	203	.372	189	.387	198	.362
Follicular	205	.358	185	.352	194	.322
Periovulatory	186	.288	175	.318	194	.363
Luteal	184	.350	173	.317	192	.392
Late luteal	191	.368	176	.331	199	.337
First three days of menses	219	.436*	189	.388	195	.364
Rest of the cycle	190	.333*	180	.334	195	.347

Table C.9: The median unscaled and scaled CRP values for the low, average, and high groups. * denotes significant differences by Kruskall-Wallace.

Exploring waist to hip ratio as a possible source of CRP phenotype variation

The same analyses were conducted on the entire sample set (polish and polish American combined) after splitting into a low waist to hip ratio (n=71) and moderate/high waist to hip ratio (n=27). Groups were determined by using the World Health Organization cut offs for low and moderate/ high waist to hip ratios for individuals assigned female at birth (ratio <.80 assigned to the low). While the low waist to hip ratio group (median = 193.7 ng/ml) had higher CRP concentrations for the unscaled analyses compared to the moderate (median = 164.1ng/ml, p<.01), we found no cycle effects or cycle phase differences in any of the groups. Below are the average line graphs and boxplots for the waist to hip ratio analyses.

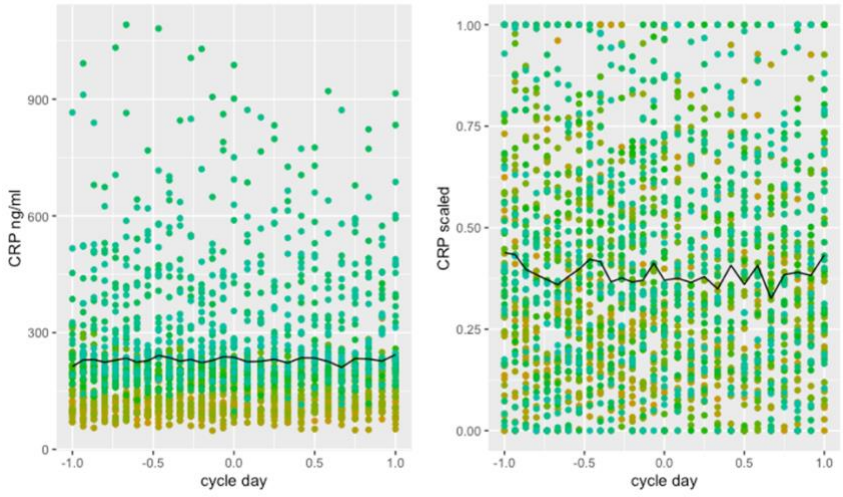


Figure C.21: The scaled and unscaled CRP average cycle shape for the low waist to hip ratio group (<.8).

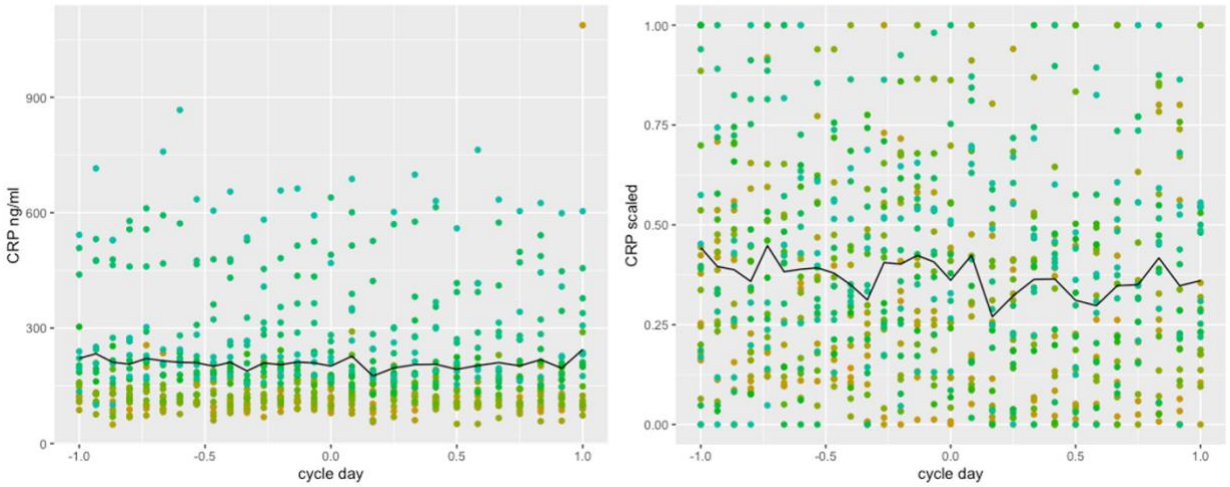


Figure C.22: The scaled and unscaled CRP average cycle shape for the moderate waist to hip ratio group (>.8).

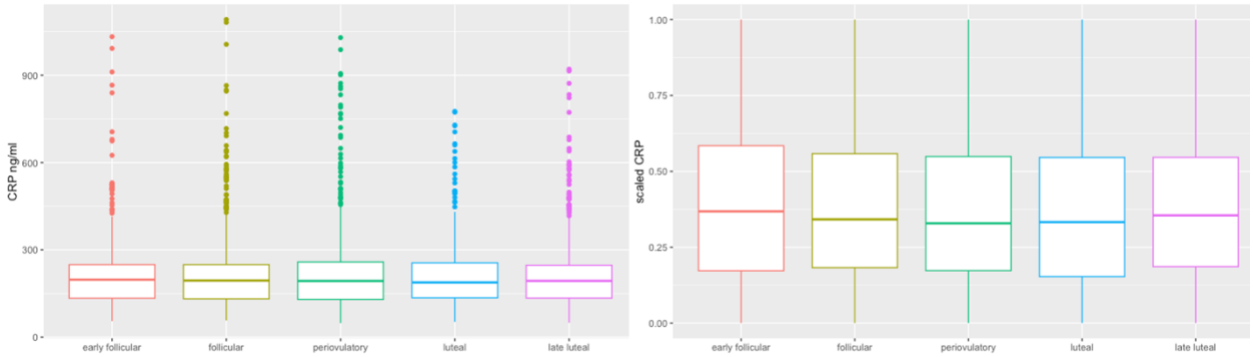


Figure C.23: The unscaled and scaled CRP box plots comparing CRP concentration distributions by cycle phase of the low waist to hip ratio group. There were no significant differences.

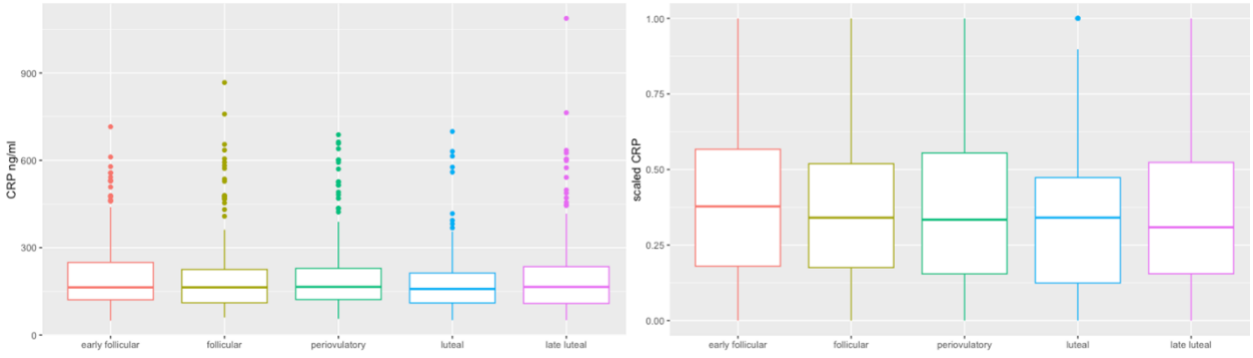


Figure C.24: The unscaled and scaled CRP box plots comparing CRP concentration distributions by cycle phase of the moderate to high waist to hip ratio group. There were no significant differences.

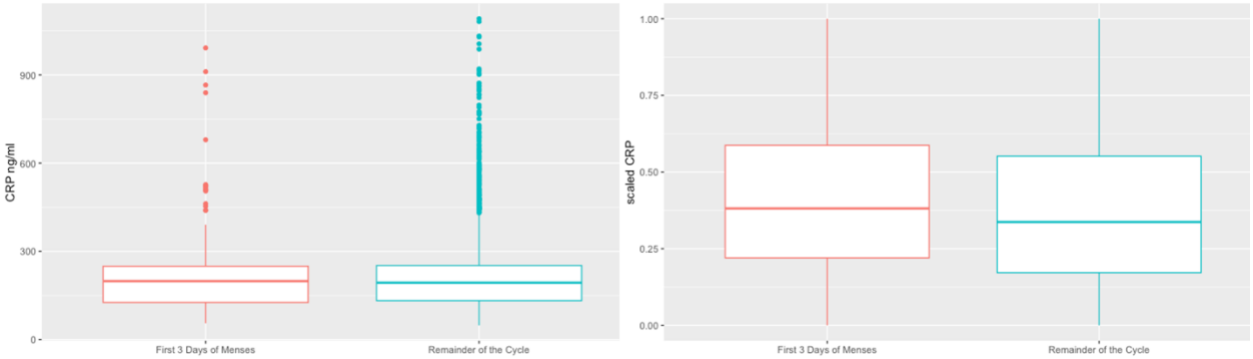


Figure C.25: The unscaled and scaled CRP box plots comparing CRP concentration distributions by first three days of menses and the remainder of the cycle of the low waist to hip ratio group. There are no significant differences.

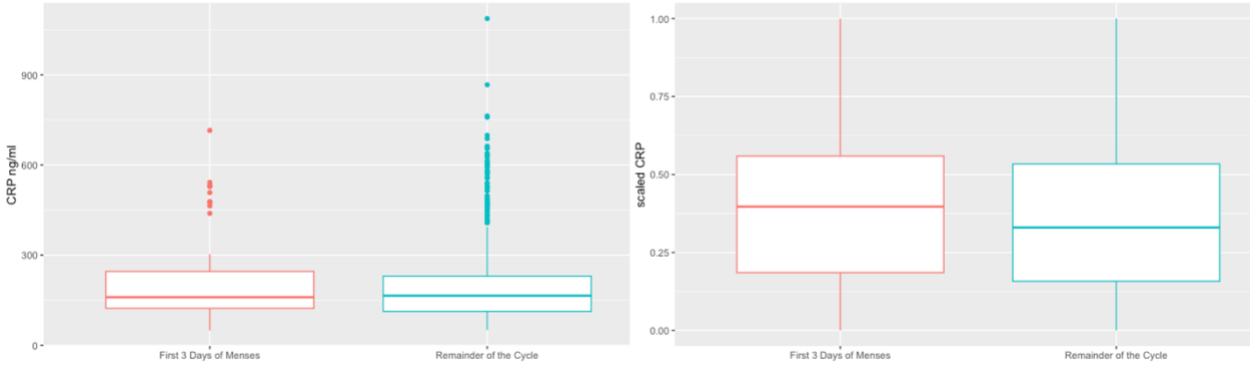


Figure C.26: The unscaled and scaled CRP box plots comparing CRP concentration distributions by first three days of menses and the remainder of the cycle of the moderate to high waist to hip ratio group. There are no significant differences.

	Low waist 2 hip ratio unscaled CRP	Low waist to hip ratio scaled CRP	Moderate or high waist to hip ratio CRP	Moderate or high waist to hip ratio CRP
Early follicular	198	.368	164	.378
Follicular	194	.342	164	.341
Periovulatory	193	.329	165	.334
Luteal	188	.333	158	.341
Late luteal	193	.355	165	.309
First three days of menses	199	.381	160	.397
Rest of the cycle	193	.337	165	.330

Table C.10: The median unscaled and scaled CRP values for the low waist to hip ratio and the moderate/high waist to hip ratio groups. There were no significant differences.

Analysis of data missingness

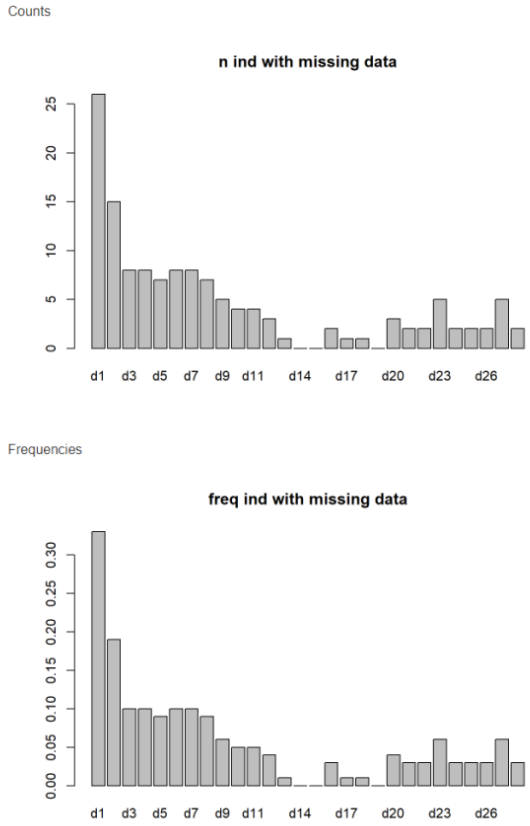


Figure C.27: Distribution of number of missing data points by cycle day

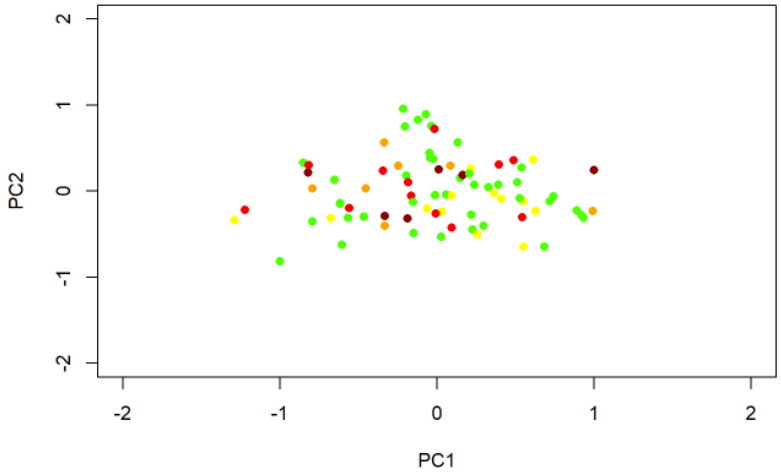


Figure C.28: PCA analysis of how we actually quantify shape variation. The distribution of this plot shows that individuals with no missing (green) cover the same range as those with missing data (yellow missing 1, orange missing 2, red missing 3 days, dark red 5+) and while a few individuals extend just past the range of the no-missing, it is not a significant difference.

Analyses with both samples combined

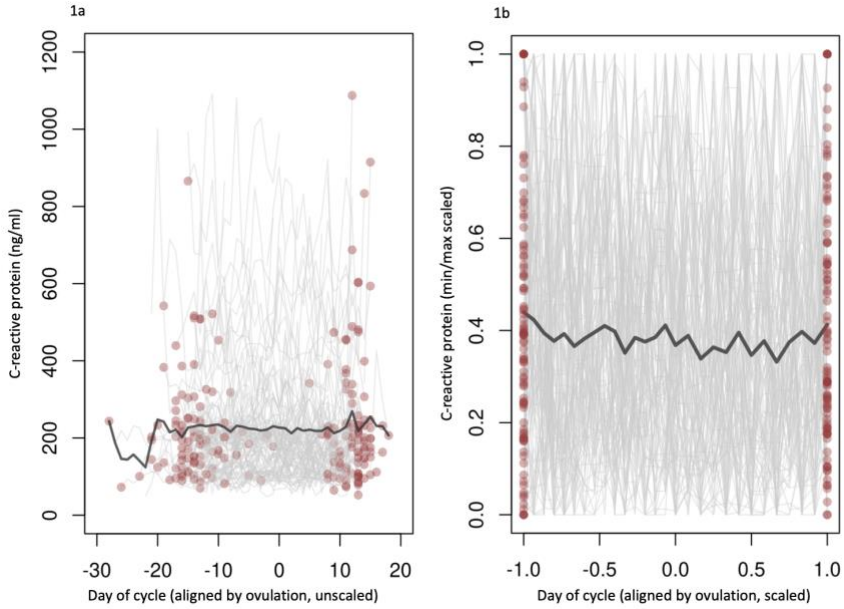


Figure C.29: The scaled and unscaled CRP average cycle shape for combined sample of Polish and Polish American.

```
crp.Scaledlmm0 <- lmer(filled_CRP ~
                       1 + (1|id), data=scaled_output)
crp.Scaledlmm1 <- lmer(filled_CRP ~
                       menses + (1|id), data=scaled_output)
crp.Scaledlmm2 <- lmer(filled_CRP ~
                       menses + scaled_day + (1|id), data=scaled_output)
crp.Scaledlmm3 <- lmer(filled_CRP ~
                       menses + scaled_day  + filled_E1G + (1|id), data=scaled_output)
crp.Scaledlmm4 <- lmer(filled_CRP ~
                       menses + scaled_day  + filled_E1G + filled_PDG +  (1|id), data=scaled_output)
crp.Scaledlmm5 <- lmer(filled_CRP ~
                       menses + scaled_day  + filled_E1G + filled_PDG + age_calculated_manually +  (1|id), data=scaled_output)
crp.Scaledlmm6 <- lmer(filled_CRP ~
                       menses + scaled_day  + filled_E1G + filled_PDG + age_calculated_manually + BodyFatAvg + (1|id), data=scaled_output)
crp.Scaledlmm7 <- lmer(filled_CRP ~
                       menses + scaled_day  + filled_E1G + filled_PDG + age_calculated_manually + BodyFatAvg + Waist2Hip + (1|id), data=scaled_output)
```

```
filled_CRP ~ 1 + (1 | id)                                                                                    49.50 84.36 69.73 FALSE
filled_CRP ~ menses + (1 | id)                                                                               53.46 85.40 63.87 FALSE
filled_CRP ~ menses + scaled_day + (1 | id)                                                                  53.73 86.39 65.33 FALSE
filled_CRP ~ menses + scaled_day + filled_E1G + (1 | id)                                                     68.55 87.68 38.26 FALSE
filled_CRP ~ menses + scaled_day + filled_E1G + filled_PDG +      (1 | id)                                   71.12 88.83 35.41 FALSE
filled_CRP ~ menses + scaled_day + filled_E1G + filled_PDG +      age_calculated_manually + (1 | id)         71.27 89.11 35.69 FALSE
filled_CRP ~ menses + scaled_day + filled_E1G + filled_PDG +      age_calculated_manually + BodyFatAvg + (1 | id)          71.41 89.40 35.97 FALSE
filled_CRP ~ menses + scaled_day + filled_E1G + filled_PDG +      age_calculated_manually + BodyFatAvg + Waist2Hip + (1 | id) 71.44 89.46 36.05 FALSE
```

Figure C.30: cAIC results and linear mixed models for combined sample. Model 4 had the lowest cAIC.

```
Linear mixed model fit by REML ['lmerMod']
Formula: filled_CRP ~ menses + scaled_day + filled_E1G + filled_PDG +      (1 | id)
   Data: scaled_output

REML criterion at convergence: 160

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.4597 -0.6599 -0.1526  0.5224  3.7218

Random effects:
 Groups   Name        Variance Std.Dev.
 id       (Intercept) 0.01170  0.1082
 Residual             0.05739  0.2396
Number of obs: 2744, groups:  id, 98

Fixed effects:
                             Estimate Std. Error t value
(Intercept)                  0.416490   0.020972  19.859
mensesRemainder of the Cycle -0.058296   0.017800  -3.275
scaled_day                   0.002086   0.010987   0.190
filled_E1G                   0.100120   0.018690   5.357
filled_PDG                  -0.042048   0.021210  -1.982

Correlation of Fixed Effects:
            (Intr) mnRotC scld_d fl_E1G
mnssRmndotC -0.760
scaled_day   0.528 -0.489
filled_E1G  -0.076 -0.200 -0.031
filled_PDG  -0.343  0.172 -0.579 -0.060
```

Figure C.31: Summary results of model 4.

Effect	Rsq	upper.CL	lower.CL
Model	0.017	0.030	0.009
filled_E1G	0.012	0.023	0.005
mensesRemainder of the Cycle	0.004	0.011	0.001
filled_PDG	0.002	0.007	0.000
scaled_day	0.000	0.002	0.000

Figure C.32: r2Beta values for model 4.

	2.5 %	97.5 %
.sig01	0.09144715	0.1273255134
.sigma	0.23308202	0.2459876495
(Intercept)	0.37542803	0.4575589271
mensesRemainder of the Cycle	-0.09315855	-0.0234077521
scaled_day	-0.01945928	0.0236009181
filled_E1G	0.06346983	0.1367156910
filled_PDG	-0.08357465	-0.0004176284

Figure C.33: 95% confidence intervals for variables in model 4.

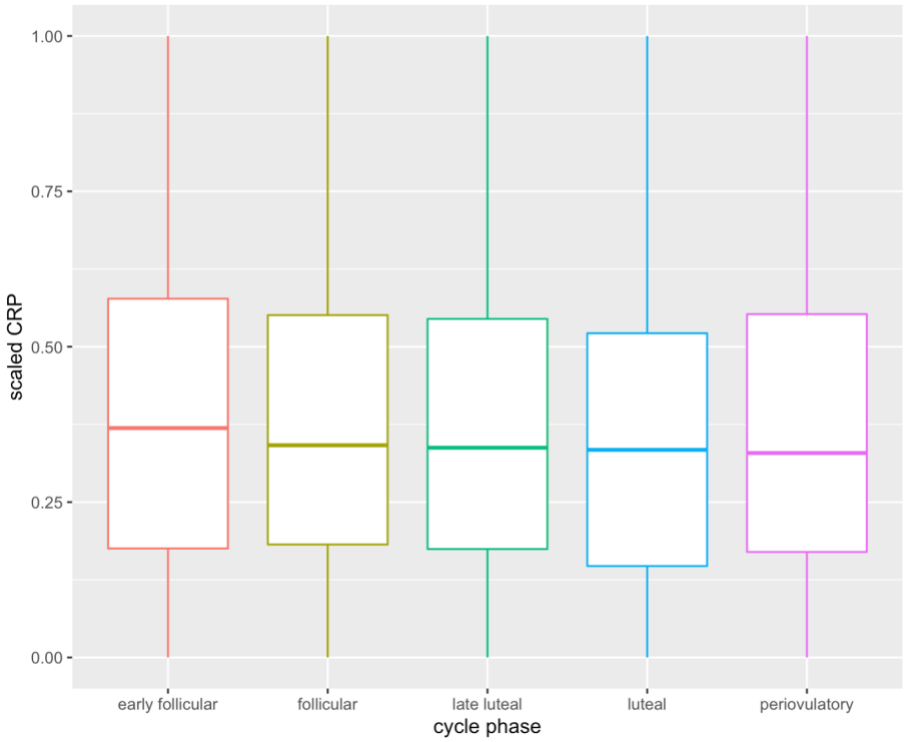


Figure C.34: Box plot of combined sample showing medium and distribution of CRP for each cycle phase.

```
        Kruskal-Wallis rank sum test

data:  scaled_output$filled_CRP and scaled_output$cyclephase
Kruskal-Wallis chi-squared = 4.514, df = 4, p-value = 0.3409
```

Figure C.35: Kruskal-Wallis results for combined sample comparing CRP concentrations across cycle phases. No significant differences found.

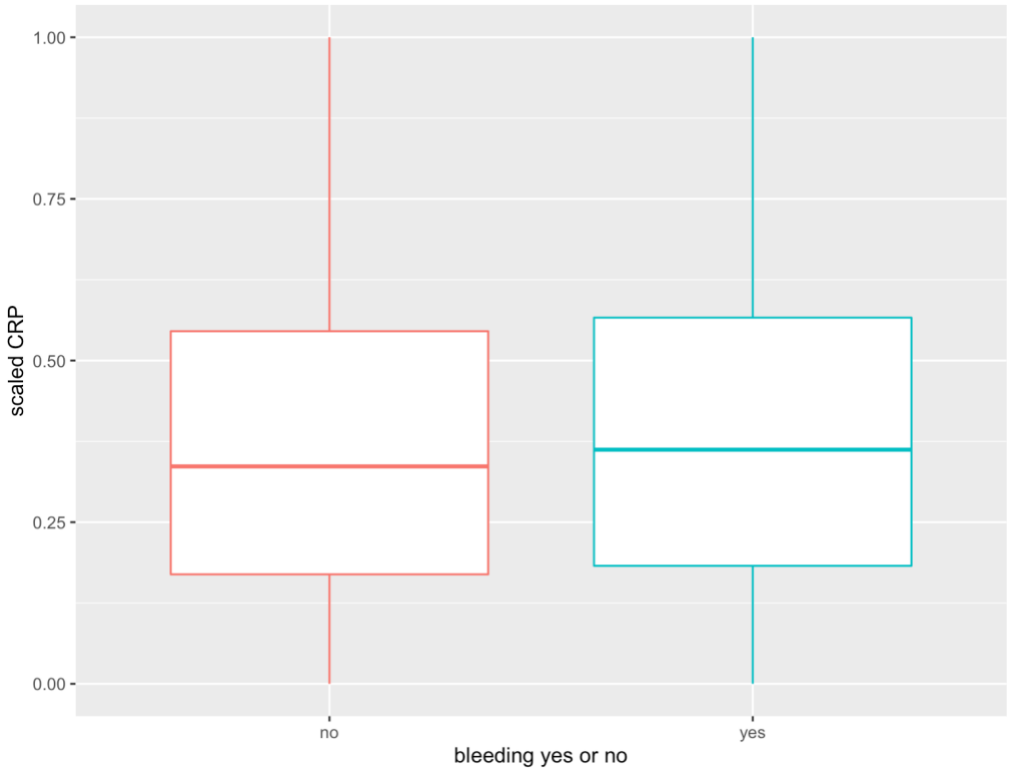


Figure C.36: Box plot of combined sample showing medium and distribution of CRP for first three days of menses (yes) and reminder of cycle (no).

```
        Kruskal-Wallis rank sum test

data:  scaled_output$filled_CRP and scaled_output$bleeding
Kruskal-Wallis chi-squared = 2.2028, df = 1, p-value = 0.1378
```

Figure C.37: Kruskal-Wallis results for combined sample comparing CRP concentrations for first three days of menses (yes) and reminder of cycle (no). No significant differences found.

APPENDIX D

CHAPTER 3 R code

The file “Wilson_Dissertation_AppendixD_Spring2023.R” contains all the code written for the analyses for chapter 3.

APPENDIX E

CHAPTER 4 SUPPLEMENTAL MATERIALS

Inter and Intra assay CVs

Laboratory Protocol: We measured multiple hormones over the course of each participant's menstrual cycle using two types of Quansys multiplex enzyme linked immunosorbent assays (ELISA). Hormone data was generated for samples collected in Poland and the United States during the 2014-2017 field seasons. The measured hormones in this analysis included: the urinary estradiol metabolite (estrone-3-glucuronide, E1G), cortisol, and urinary progesterone metabolite (pregnanediol glucuronide, PdG). All hormones except PdG were run on a custom Quansys 8-Plex ELISA or 9-Plex ELISA, and PdG was run individually on a 1-plex Quansys ELISA. All samples were run in duplicate.

140 total ELISA plates were run for the PdG assay, ninety-five ELISA plates were run for the 9-Plex, and forty-four ELISA plates were run for the 8-Plex. The intra- and inter- assay variation for each measured hormones was calculated. The intra-assay variation was as follows: E1G (8.83%), cortisol (9.53%), and PdG (31%). The inter-assay variation is located in Tables D.1 and D.2. While the inter-assay variation is high, it is within the range typically seen in multiplex assays (Chowdhury et al., 2009; Bastarache et al., 2011). Further, for analytes with extremely low concentrations in the control, the CV is inflated due to comparing trace amounts (presence) to zero (absence).

	CNTL_A	**CNTL_B**	**CNTL_C**	**CNTL_D**	**CNTL_E**
Batch Number (n plates)	*3 (10)*	*2 (34), 3 (76)*	*3 (88)*	*3 (88)*	*1 (104), 2 (76)*
E1G_CV	39.01	28.02	22.28	22.93	35.53
Cortisol_CV	24.42	17.47	18.56	21.15	24.93

Table D.1: The CV for each control for each analyte run on the custom 8 or 9 Plex is displayed here. Note that some controls were run across batches, and multiple controls were present on a single plate. All assays run by same technician.

	CNTL 1	CNTL2	CNTL3	CNTL4	H1to1	H1to10	L1to1	L1to10	CNTL7	CNTL8	CNTL9	CNTL1 0
Tech:	MPR	MPR	MPR	MPR	MAW	MAW	MAW	MAW	MAW	MAW	MAW	MAW
Batch Numbers (n)	1 (11)	47	2 (18)	2 (12)	2 (3)	2 (10)	2 (13)	2 (23)	2 (0)	2 (6)	2 (1)	2 (17)
PdG_CV	41.36	28.37	18.67	35.85	66.35	30.34	21.2	29.82	NA	19.5	NA	26.71

Table D.2: The CV for each control for PdG is displayed here. Note that some controls were run across batches, and multiple controls were present on a single plate. The NA represents if the analyte was not present in that particular control.

Additional PCA shapes.

Figure D.1: PC shapes of PCs 1-9. These PCs account for a total of 62% of the variation

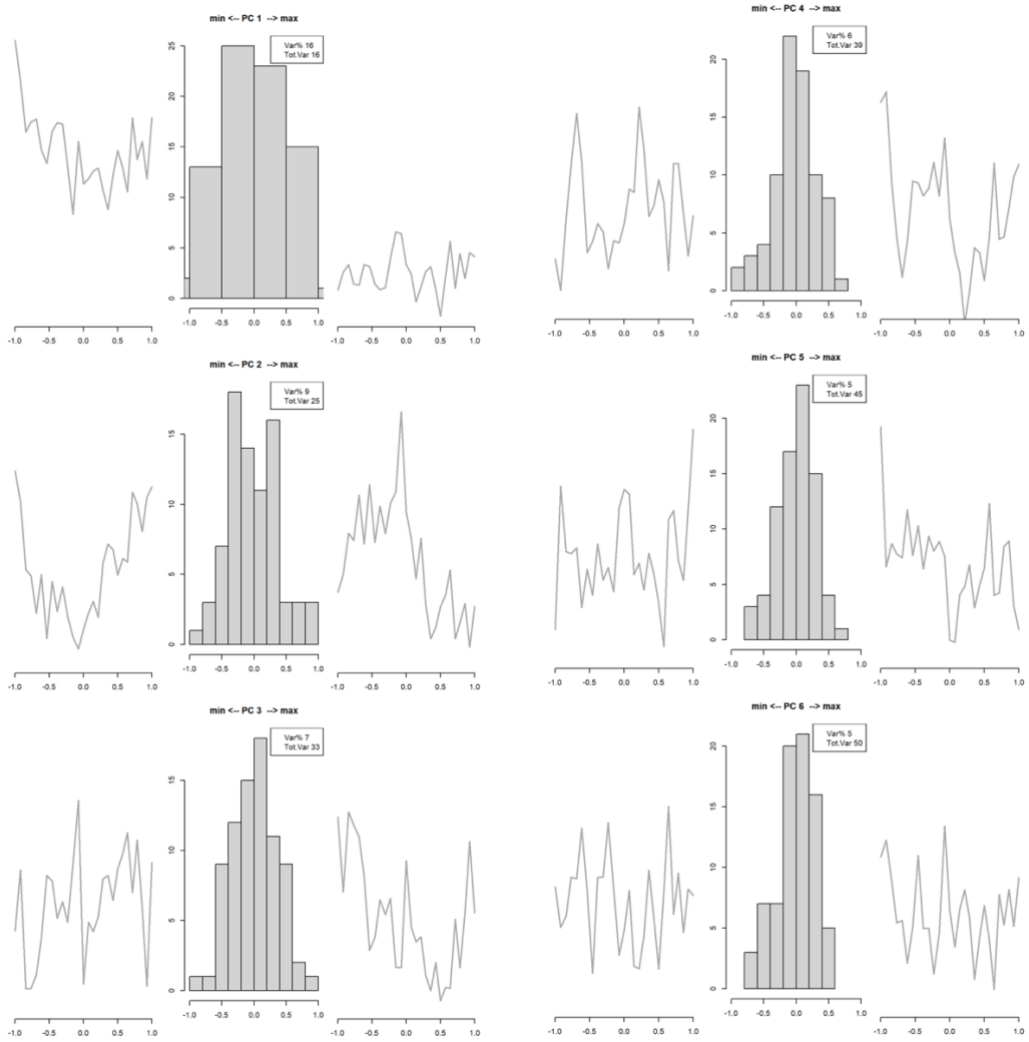
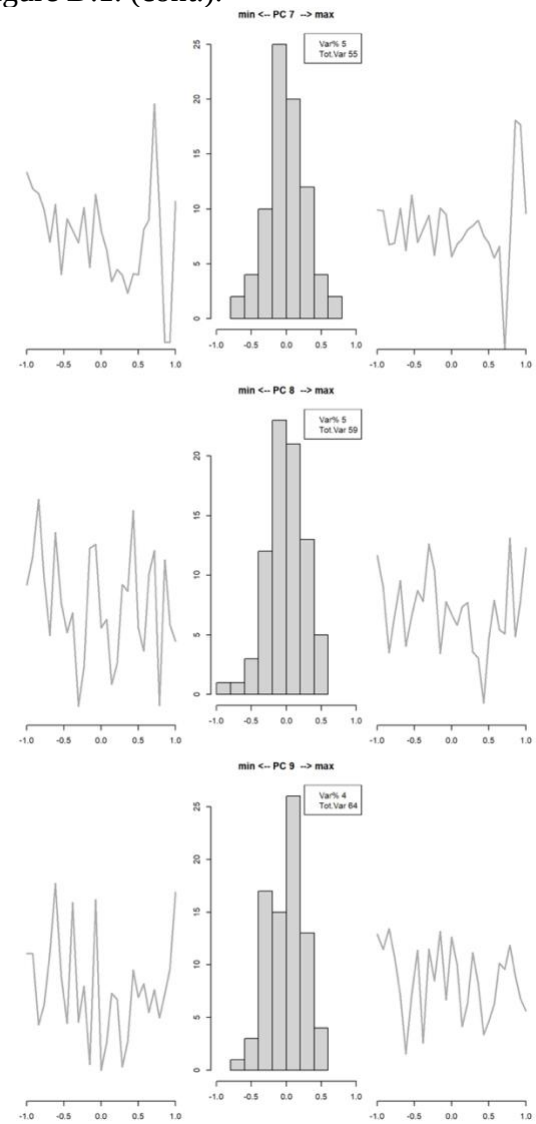


Figure D.1. (cont.).



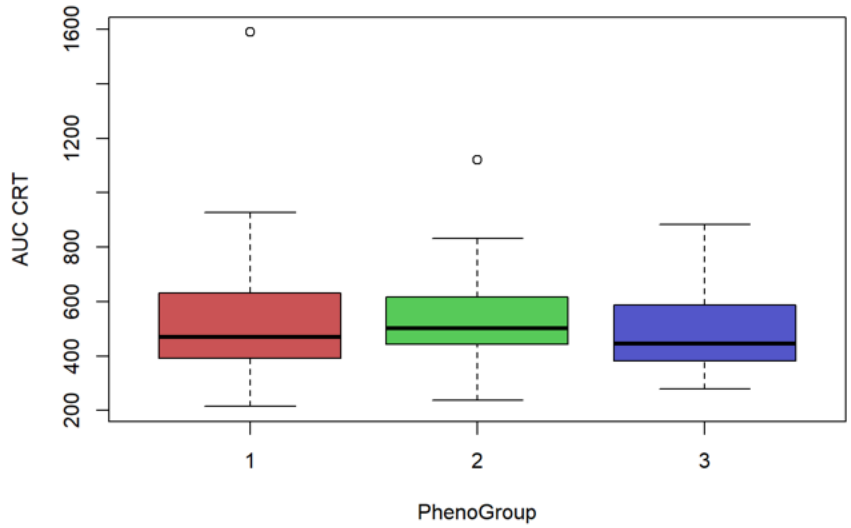
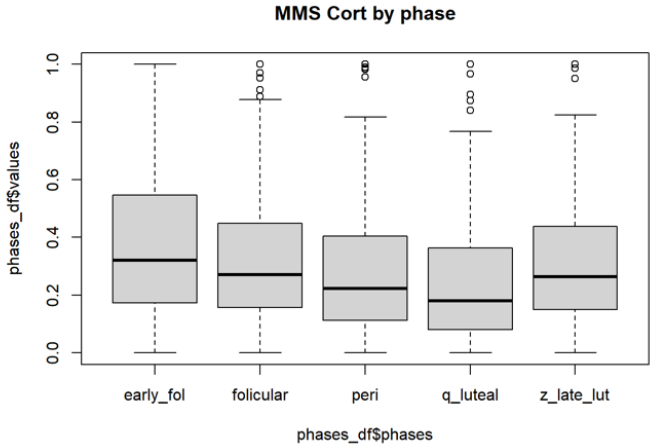


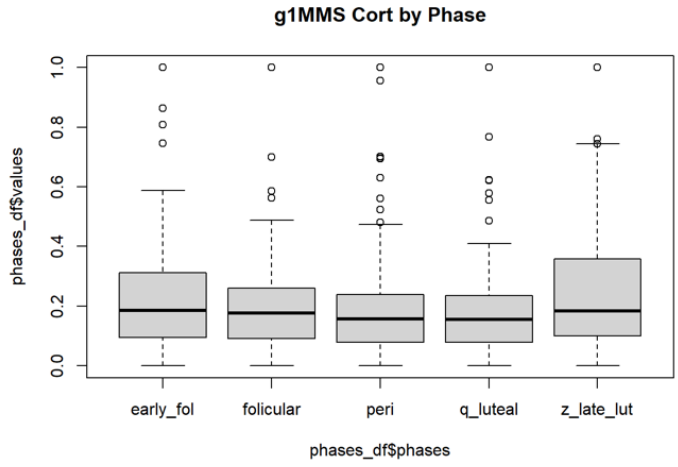
Figure D.2: Cortisol AUC difference by phenotype group, pairwise t-tests not significant, p=.87 for all groups

Average cortisol difference by cycle phase



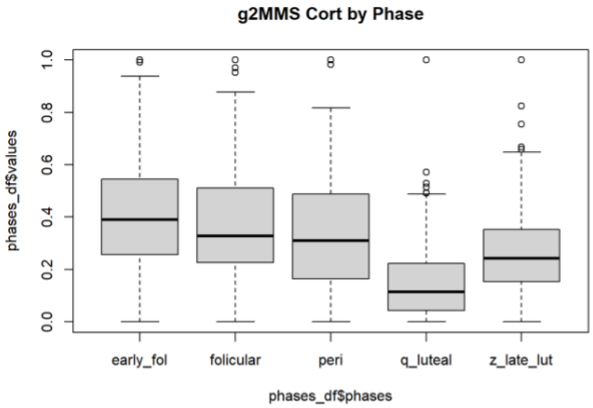

```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  phases_df$values and phases_df$phases
##
##            early_fol folicular peri    q_luteal
## folicular  0.00052   -         -       -
## peri       4.4e-07   0.03934   -       -
## q_luteal   6.8e-12   1.8e-05   0.01549 -
## z_late_lut 0.00083   0.83935   0.02694 1.1e-05
##
## P value adjustment method: fdr
```

Figure D.3: Whole sample group average scaled cortisol values by cycle phase. Pairwise t-tests conducted. Distinctly different cortisol pattern emerges.



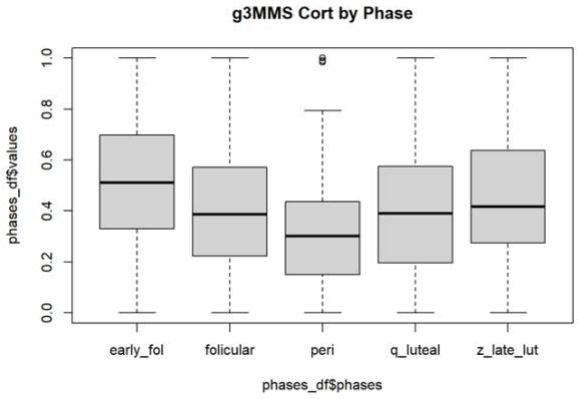

```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  phases_df$values and phases_df$phases
##
##           early_fol folicular peri    q_luteal
## folicular 0.20457   -         -       -
## peri      0.17523   0.84423   -       -
## q_luteal  0.27933   0.93348   0.84423 -
## z_late_lut 0.17523   0.00073   0.00073 0.00524
##
## P value adjustment method: fdr
```

Figure D.4: Variable group average scaled cortisol by cycle phase, pairwise t-tests conducted. Only late luteal had significantly higher cortisol.




```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  phases_df$values and phases_df$phases
##
##            early_fol folicular peri    q_luteal
## folicular  0.15689   -         -       -
## peri       0.03038   0.33782   -       -
## q_luteal   4.5e-16   4.1e-15   6.1e-12 -
## z_late_lut 7.6e-09   2.9e-07   6.4e-05 0.00033
##
## P value adjustment method: fdr
```

Figure D.5: Responsive group average scaled cortisol values by cycle phase. Pairwise t-tests conducted. Distinctly different cortisol pattern emerges.




```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  phases_df$values and phases_df$phases
##
##            early_fol folicular peri    q_luteal
## folicular  0.00064   -         -       -
## peri       6.5e-08   0.01904   -       -
## q_luteal   0.00110   0.81269   0.06388 -
## z_late_lut 0.05840   0.06811   5.2e-05 0.06811
##
## P value adjustment method: fdr
```

Figure D.6: Consistent group average scaled cortisol values by cycle phase. Pairwise t-tests conducted. Distinctly different cortisol pattern emerges.

Cortisol phenotypes by daily stress measures

We conducted pls regression to test whether daily stress measurement across the cycle differed by phenotype group. Results were non significant (p=.4) but there is a slight pattern emerging especially in the variable group.

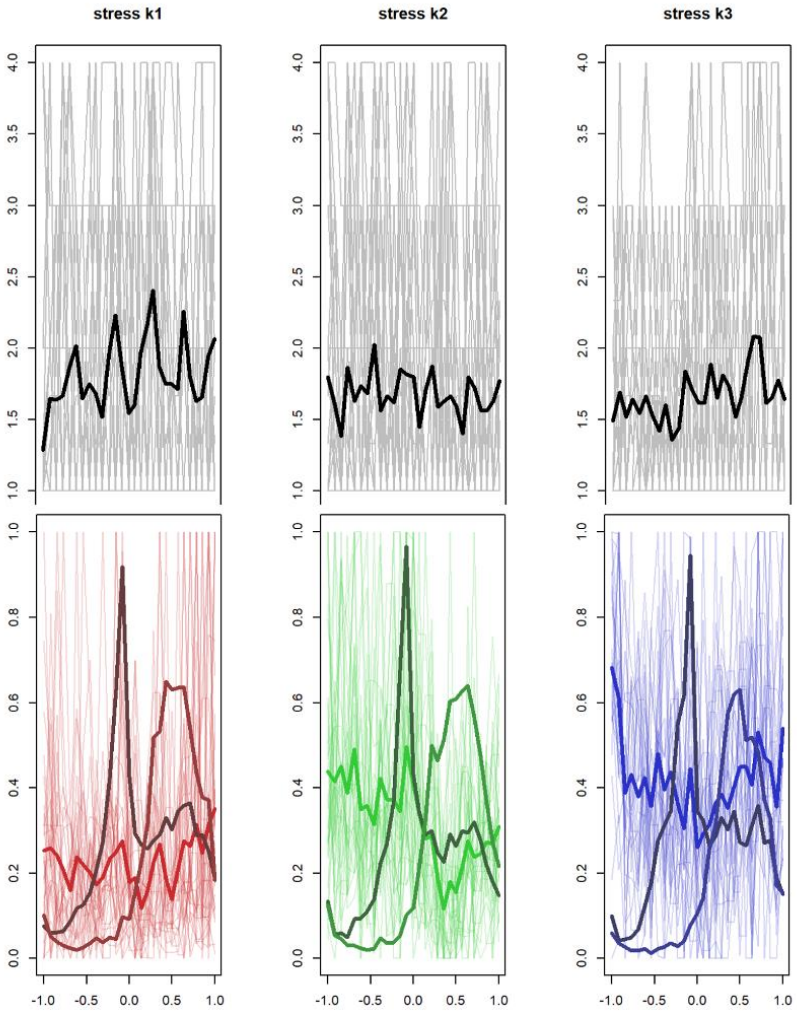


Figure D.7: Partial least squared regression to test whether daily stress measurement across the cycle differed by phenotype group. Results were non significant (p=.4) but there is a slight pattern emerging especially in the variable group.

Age distributions by phenotype

Age distributions were not significantly different by. However, below are histograms of age distribution for each phenotype and boxplots showing age for each phenotype.

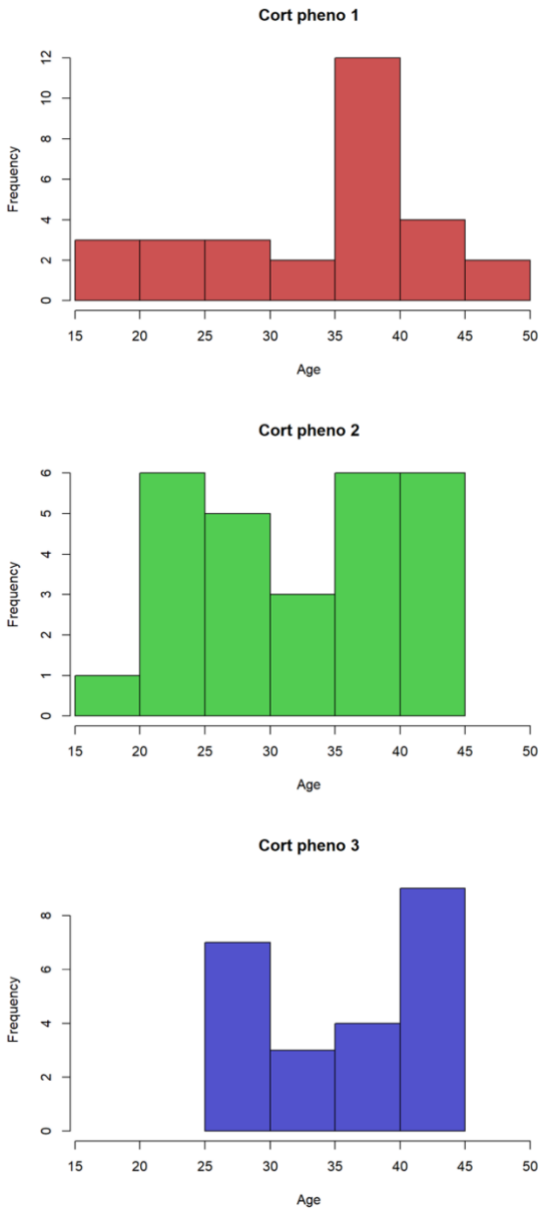


Figure D.8: Histograms of age distribution for each phenotype and boxplots showing age for each phenotype.

```
 Pairwise comparisons using t tests with pooled SD

data:  sclas2$Age and k_grps$k3

  1    2
2 0.37 -
3 0.37 0.24

P value adjustment method: fdr
```

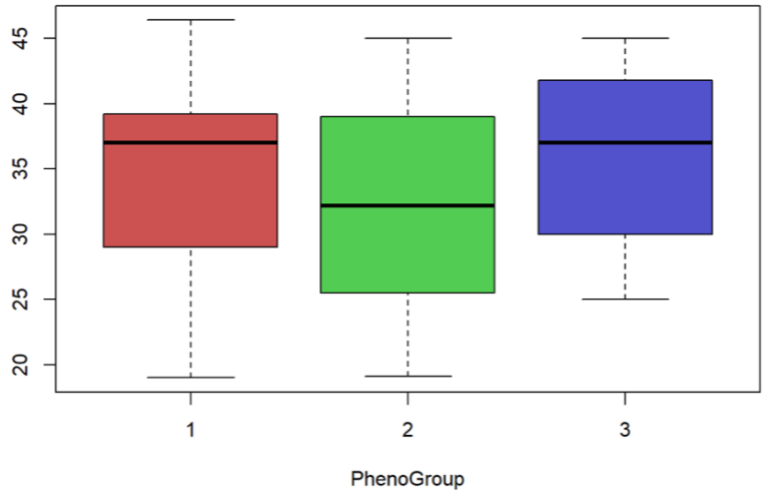


Figure D.9: Pairwise t-test results and box plot of age for each phenogroup. No significant differences were found.

Analysis of data missingness

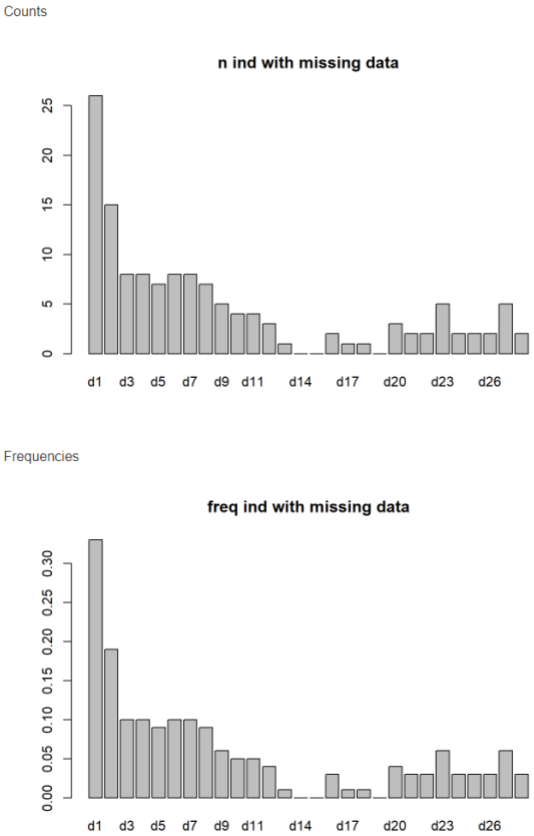


Figure D.10: Distribution of number of missing data points by cycle day.

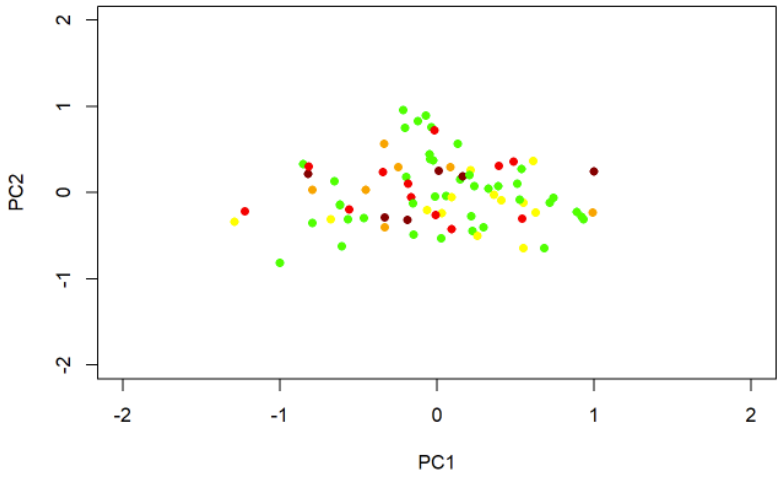


Figure D.11: PCA analysis of how we actually quantify shape variation. The distribution of this plot shows that individuals with no missing (green) cover the same range as those with missing data (yellow missing 1, orange missing 2, red missing 3 days, dark red 5+) and while a few individuals extend just past the range of the no-missing, it is not a significant difference.

APPENDIX F

CHAPTER 4 R code

The file “Wilson_Dissertation_AppendixF_Spring2023.Rmd” contains all the code written for the analyses for chapter 4.