UNIVERSITY OF CALIFORNIA

Santa Barbara

Effects of socio-ecological variation on female health and immune status and consequences for sexual dimorphism in immune function

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Anthropology

by

Carmen Marie Hové

Committee in charge:

Professor Amy Boddy, Chair

Professor Michael Gurven

Professor David Lawson

Professor Aaron Blackwell

Professor Melanie Martin

March 2022

The dissertation of Carmen Marie Hové is approved.

_____

Michael Gurven

_____

David Lawson

_____

Aaron Blackwell

_____

Melanie Martin

_____

Amy Boddy, Committee Chair

March 2022

Effects of socio-ecological variation on female health and immune status and consequences for sexual dimorphism in immune function

by

Carmen Marie Hové

ACKNOWLEDGEMENTS

Thank you to everyone who contributed to this dissertation – my committee, my co-authors, my mentors, my fellow graduate students, and all the anonymous study participants who volunteered time and effort for the advancement of scientific research. Many thanks to my friends and family who supported me throughout the years. You know who you are.

"Giving up is not in the plot" - Nims Purja

CURRICULUM VITAE OF CARMEN M. HOVÉ
March 2022

EDUCATION

2018-2022 **PhD** **Biological Anthropology**, University of California – Santa Barbara
2015-2018 **MA** **Biological Anthropology**, University of California – Santa Barbara
2010-2014 **BS** **Applied Human Biology**, Seattle Pacific University

PROFESSONAL POSITIONS

2016-21 **Graduate Research Fellow**, NSF Graduate Research Fellowship Program
2014-15 **Post-Baccalaureate Research Fellow**, Seattle Pacific University
2013-14 **Data Technician**, Women's Health Initiative, Fred Hutchinson Cancer Research Center
2011-13 **Laboratory Assistant**, Chemistry Department, Seattle Pacific University

PUBLISHED PAPERS

1. **Hové C**, Trumble BC, Anderson AS, Stieglitz J, Kaplan H, Gurven MD, Blackwell AD. 2020. Flexibility of fetal tolerance: Immune function during pregnancy varies between ecologically distinct populations. Evol Med Public Heal.
2. Anderson AS, Trumble BC, **Hové C**, Kraft TS, Kaplan H, Gurven M, Blackwell AD. 2019. Old friends and friendly fire: Pregnancy, hookworm infection, and anemia among tropical horticulturalists. Am J Hum Biol:1–15.

PUBLISHED ABSTRACTS

1. **Hové CM,** Trumble BC, Stieglitz J, Gurven M, Kaplan H, Boddy AM, Blackwell AD. 2019. Socioecological conditions shape postpartum immune trajectories. Journal of Human Biology Abstracts.
2. **Hové CM**, Trumble BC, Anderson AS, Stieglitz J, Gurven M, Kaplan H, Blackwell AD. 2019. The flexibility of fetal tolerance: how immune function during pregnancy varies between two ecologically distinct populations. Journal of Human Biology Abstracts.
3. **Hové CM**, Blackwell AD, Trumble BC, Suarez IM, Stieglitz J, Beheim B, Snodgrass JJ, Gurven M, Kaplan H. 2017. Immune Modulation during Pregnancy for Women in a High Pathogen Environment. Journal of Human Biology Abstracts.
4. **Hové CM**, Wall-Scheffler CM. 2015. Comparing optimal and preferred walking speeds. American Journal of Physical Anthropology Suppl. 60:170.
5. **Hové CM**, Wagnild JM, Wall-Scheffler CM. 2014. The Humor gender gap: How gender and humor interact to influence social behavior. American Journal of Physical Anthropology Suppl. 58:145-6.
6. Jones C, **Hové CM**, Wagnild JM, Wall-Scheffler CM. 2014. How travel time influences sexual dimorphism. American Journal of Physical Anthropology Suppl. 58:153.

RESEARCH GRANTS

2021 **Elings Wells Dissertation Fellowship** ($8000), UCSB
2020 **Dissertation Fieldwork Grant** ($19,998), Wenner-Gren Foundation
2020 **Dissertation Improvement Grant** ($30,934), National Science Foundation

2019 **Graduate Student Research and Travel Grant** ($1,258), UCSB Broom Center for Demography
2018 **Graduate Student Research and Travel Grant** ($2,000), UCSB Broom Center for Demography
2018 **Summer Research Grant** ($630), UCSB Anthropology Department
2017 **Summer Travel Grant** ($400), UCSB Anthropology Department
2016 **Graduate Research Fellowship Program** ($138,000), National Science Foundation
2016 **Summer Research Grant** ($2,000), UCSB Anthropology Department

FELLOWSHIPS & AWARDS
2019 **Edward E. Hunt Jr. Student Award,** Human Biology Association Annual Meeting
2017 **2nd Place Flash-Talk Award**, Anthropology Grad-Slam, University of California – Santa Barbara
2017 **Conference Travel Award** ($500), Human Biology Association
2016 **Conference Travel Fellowship** ($700), Int'l Society for Evolution, Medicine & Public Health
2016 **William S. Pollitzer Travel Award** ($500), American Association of Physical Anthropology
2016 **2nd Place Poster Prize**, California Workshop for Evolutionary Social Science
2014 ***Cum laude***, Department of Biology, Seattle Pacific University
2014 **Best Oral Presentation**, Erickson Undergraduate Research Conference, Seattle Pacific University
2015 **UC Regents in the Disciplines Fellowship** ($59,784), University of California – Santa Barbara
2011 **Vereide Award** ($2,024), Seattle Pacific University
2010 **President's Scholar Award** ($48,000), Seattle Pacific University

INVITED LECTURES
2019 **University of Washington BASS Seminar Series,** Seattle, WA
Podium: *The flexibility of fetal tolerance: how immune function during pregnancy varies between two ecologically distinct populations*

CONFERENCE PRESENTATIONS
2019 **Human Biology Association,** Cleveland, OH
Podium: *The flexibility of fetal tolerance: how immune function during pregnancy varies between two ecologically distinct populations*
2017 **UCSB Anthropology Grad-Slam**, Santa Barbara, CA
Flash-Talk: *Immune Modulation during Pregnancy for Women in a High Pathogen Environment*
2017 **Human Biology Association,** New Orleans, LA
Poster: *Immune Modulation during Pregnancy for Women in a High Pathogen Environment*
2016 **International Society for Evolution, Medicine & Public Health,** Raleigh, NC
Poster: *Immune Modulation during Pregnancy for Women in a High Pathogen Environment*
2016 **California Workshop for Evolutionary Social Science,** San Luis Obispo, CA

Poster: *Immune Modulation during Pregnancy for Women in a High Pathogen Environment*

2014 **SPU Summer Research Symposium,** Seattle, WA
Podium: *Comparing optimal and preferred walking speeds*

2014 **Murdock College Science Research Conference,** Vancouver, WA
Poster: *Comparing optimal and preferred walking speeds*

2014 **SPU Erickson Undergraduate Research Conference,** Seattle, WA
Podium: *The humor gender gap: how gender and humor interact to influence social behavior*

2014 **American Association for Physical Anthropology,** Calgary, Canada
Poster: *How gender and humor interact to influence social behavior*

2013 **Murdock College Science Research Conference,** Vancouver, WA
Poster: *How gender and humor interact to influence social behavior*

TEACHING EXPERIENCE

2020 **Research Mentorship Program**, University of California – Santa Barbara
Mentored 4 high school students throughout 6-week intensive course on original research design and Bayesian analysis using R.

2016-17 **Teaching Assistant**, University of California – Santa Barbara

Course: *ANTH 5 Introduction to Biological Anthropology*

2014-15 **Adjunct Laboratory Instructor**, Seattle Pacific University

Courses: *BIO 2102 General Biology; BIO 2130 Human Anatomy & Physiology II; BIO 2129 Human Anatomy & Physiology I*

2013-14 **Teaching Assistant**, Seattle Pacific University
Courses: *PPHS 1200 Introduction to the Health Professions; CHEM 1212 General Chemistry II; CHEM 1330 Organic and Biological Chemistry*

PROFESSIONAL DEVELOPMENT

2018 **Course in Basic Blood Collection,** North Seattle Community College
Intensive 5-week course covering blood collection techniques; GPA 3.9

RELEVANT SKILLS

Clinical Licensed Medical Assistant – Phlebotomist

Laboratory Experience performing enzyme-linked immunosorbent assays, flow cytometry, RNA interference, DNA extraction using gel electrophoresis, portable respirometry, temperature telemetry

Software Fluent in R, LaTex, Microsoft Word/Excel/PowerPoint, Qualtrics
Proficient in JMP, SPSS, Adobe Photoshop, Adobe Illustrator

SERVICE & OUTREACH

Apr 2019 **Invited Speaker**, Sausage of Science Podcast, Human Biology Association

2016-18 **STEM Tutor**, Teen Center, Girls Inc. of Greater Santa Barbara
Assisted 7-9th grade girls with math and science homework on a weekly basis

2016-18 **Graduate Mentor**, American Association of Physical Anthropologists

Provided feedback for undergraduates submitting posters to the undergraduate symposium at annual AAPA meetings

2017-18 **Colloquium Co-Chair**, UCSB Anthropology Graduate Student Association
Coordinated quarterly on-campus lectures by faculty, post-docs and graduate students

2016-17 **Social Events Chair**, UCSB Anthropology Graduate Student Association
Orchestrated quarterly social gatherings for anthropology graduate students

2015-16 **Graduate Symposium Co-Chair**, UCSB Anthropology Graduate Student Association
Organized department-wide symposium showcasing graduate student research

2014- **STEM Blogger**, The Informal Scientist, www.theinformalscientist.com
Publish entries on general science topics, advice for early-stage students, and resources for current graduate students

2013 **University Ambassador**, Junior Science and Humanities Symposium
Panel member for outreach event geared towards high school students interested in pursuing science careers

2013 **Peer Mentor**, Navigating Health Professions Mentor Program, Seattle Pacific University
Matched with freshman student in a pre-profession health program; met bi-quarterly to discuss challenges, study techniques, overall progress

2011-12 **Hospital Volunteer**, Day Surgery Unit, Swedish Medical Center
Admitted and discharged patients, retrieved medications, delivered specimens to lab, maintained hygienic standards

2011 **ESL Instructor/Team Leader**, SPU Reach-Out International, Vietnam
Taught English to a wide range of students, elementary through undergraduate; managed team finances, directed team meetings, drafted teaching plans, organized travel itinerary

PEER REVIEWER
PLOS ONE, Medical Hypotheses, Journal of Human Evolution

MEMBERSHIPS / AFFILIATIONS
National Science Foundation
UCSB Broom Center for Demography
Human Biology Association
International Society for Evolution, Medicine, & Public Health
American Association of Physical Anthropologists

ABSTRACT

Effects of socio-ecological variation on female health and immune status and consequences for sexual dimorphism in immune function

by

Carmen Marie Hové

Among humans, female reproduction requires significant immune modulation. Invasive placentation results in exposure to fetal antigens and corresponding shifts in female immune function and disease susceptibility, which vary in magnitude in response to ecological conditions (e.g., pathogen exposure). By comparison, there have been very few studies on how time since delivery affects immune recovery or how variation in infant feeding behavior (e.g., breastfeeding, formula feeding, pumping) might moderate postpartum immune recovery. Of the few studies that have been conducted on postpartum women, most have come from post-industrial populations experiencing evolutionarily novel conditions (e.g., obesity, low pathogen exposure). Consequently, current understanding of sexual dimorphism in immune function, a phenomenon commonly observed in humans and other mammalian species, is skewed by absence of data from postpartum females as well as disproportionate sampling of populations experiencing environmental cues (e.g., obesity) that may exacerbate sex hormone production and amplify sex bias in immune function and disease risk.

In this dissertation, I aim to address these gaps in knowledge via three distinct yet interconnected studies. I utilize pre-existing data from the Tsimane Health and Life History Project (THLHP) and the National Health and Nutrition Examination Survey (NHANES) to

characterize and compare the effects of month since delivery on immune outcomes among Tsimane and USA women (two ecologically distinct populations) (Study 1) and compare the effects of sex and female reproductive phase on immune function across the lifespan in both the Tsimane and the USA (Study 2). Lastly, I investigate the impact of infant feeding behavior (e.g., at-the-nipple breastfeeding, pumping, other supplementation) on maternal immune function, health, and wellbeing among postpartum women in Seattle, Washington, USA using data I collected between October 2020 and July 2021 as part of the Seattle Postpartum Health Study (Study 3).

My findings indicate that the postpartum period is a unique immunological state, with month of gestation and month since delivery exerting opposing effects on most immune markers. Observed differences between Tsimane and USA women point towards the role of environment in shaping immunological recovery and may specifically reflect differences in pathogen clearance requirements following pregnancy. My results also show that sex bias in immune status is comparatively attenuated among the Tsimane, indicating that ecological conditions in the USA (e.g., increased energetic budget) may exacerbate evolved mechanisms underlying sexual dimorphism in disease risk. Within each population, pregnancy was generally associated with increased sexual dimorphism while the postpartum period was often associated with attenuated sex bias, highlighting the underappreciated role of female reproductive phase in generating or dampening sex differences in immune function. Lastly, I provide evidence that both at-the-nipple breastfeeding and pumping confer benefits on postpartum maternal outcomes (e.g., fewer depressive symptoms, reduced inflammation) compared to reliance on formula/other forms of supplementation, but that heavy reliance on pumping is associated with increased symptoms of physical illness.

TABLE OF CONTENTS

I. Introduction 1

A. Female reproduction and maternal offspring conflict 1

B. Immunological demands of pregnancy 3

C. Ecological conditions moderate immune response 5

D. What about the postpartum period? 7

E. Effects of ecological context on sexual dimorphism? 8

F. Dissertation objectives 9

Figures 12

II. Effects of time since delivery on female immune status in two ecologically dinstinct populations 17

A. Introduction 17

B. Materials and Methods 23

C. Results 27

D. Discussion 30

Figures and Tables 34

III. Effects of ecological context and female reproductive phase on sexual dimorphism in immune status 51

A. Introduction 51

B. Materials and Methods 56

C. Results 59

D. Discussion 64

Figures and Tables 68

IV. The relationship between infant feeding behavior and maternal inflammation, physical health, and mental wellbeing 78
A. Introduction 78
B. Materials and Methods 86
C. Results 91
D. Discussion 94
Figures and Tables 99
V. Conclusions and future directions 108
References 114

I. Introduction

A. Female reproduction, maternal investment, and maternal-offspring conflict

In mammalian species, reproduction requires that mothers successfully gestate, deliver, and care for offspring for an extended period thereafter, all while simultaneously balancing concurrent survival needs and investment in future offspring. This high degree of maternal investment required for offspring survival introduces numerous opportunities for maternal-offspring conflict, given that the fitness "interests" of the paternal genome often differ from those of the maternal genome (Trivers, 1974; Hollegaard et al., 2013; Haig, 2014), with paternal genes favoring aggressive growth and resource extraction from the mother and maternal genes restricting resource extraction (Haig, 2010). Maternal-offspring conflict begins during placentation, a process necessary for successful reproduction that can also pose serious risks to the mother (e.g., hemorrhage) if not regulated properly (Haig, 1993; Washecka and Behling, 2002). There are three types of placentation found across mammalian species: epitheliochorial (least invasive), endotheliochorial (intermediate invasiveness), and haemochorial (most invasive) (Figure 1). Faster life history traits (e.g., shorter lifespan, high offspring quantity) correspond to heightened maternal-offspring conflict, due to more competition between siblings for maternal resources, and may therefore have put selection pressure on females that favored the evolution of less invasive, more restrictive placental structures (Garratt et al., 2013). Conversely, slower pace of life (e.g., fewer offspring, more investment in individual offspring) is associated with comparatively reduced maternal-offspring conflict and more invasive placentation (Garratt et al., 2013). Following this pattern, humans and other great apes exhibit slow life history and possess haemochorial

placentation (Chuong et al., 2013). Humans also exhibit villous interdigitation, the most invasive type of placental morphology, which allows for an even greater surface area to volume ratio for exchange between mother and fetus – a trait which sets us apart even from other great apes (Moffett and Loke, 2006).

During human placentation, fetal-derived placental cells invade the maternal endometrium, break down maternal tissue (Carter et al., 2015), and remodel the spiral arteries (Lyall, 2005; Osol and Mandala, 2009). This process establishes close contact between maternal and fetal-derived tissue at two levels. Fetal trophoblast cells forming the outer surface of the placenta interact with cells of the maternal decidua and cells in the maternal bloodstream interact with fetal cells inside the placenta (Erlebacher, 2013). Once established, the placenta provides oxygen and nutrients to the fetus, removes carbon dioxide and other waste products, protects the fetus against harmful agents, and acts as an endocrine organ by producing high concentrations of progesterone, human chorionic gonadotropin, estrogens, placental lactogen, placental growth hormone, and other growth factors (Gude et al., 2004). In this way, the placenta acts as a highly specialized organ that supports fetal growth and development and primes the maternal body for the transition to lactation after delivery.

After delivery, the specific demands exerted by placentation are removed but offspring requirements for maternal investment continue. Throughout most of human evolution, pathogen exposure and resource restriction posed considerable threats to infant survival, especially considering that human babies are highly altricial. Such constraints placed strong selection pressure on sustained lactation following delivery. In humans, breastfeeding is even more energetically costly than gestation (Butte and King, 2005a) and the combined effects of

reduced energy budget and hormone cycling induced by frequent infant suckling produce lactational amenorrhea, a period when ovarian function is inhibited (Neville and Neifert, 1983; Valeggia and Eellison, 2009). Lactational amenorrhea effectively extends time between delivery and time to next pregnancy, creating a potential "battle ground" of maternal-offspring conflict as time since delivery increases.

B. Immunological demands of pregnancy

A key feature of female reproduction is that it requires a significant degree of immune modulation (Figure 2). The immune system is an extremely complex system that has evolved to identify and eliminate foreign threats and remove harmful debris generated by processes such as cell turnover. In very general terms, the immune system can be broken down into innate and acquired responses (Figure 3), with the acquired immune response further separated into cellular and humoral immunity (Figure 4). The innate system does not require priming and responds to all types of pathogenic challenges using a suite of highly conserved defense mechanisms (e.g., release of antibacterial lysozymes, fever, inflammation). Upon exposure to a pathogenic invader, the innate system also primes the acquired immune response via professional antigen presenting cells (APCs). Upon antigen exposure, B cells differentiate into plasma and memory B cells, which produce antigen-specific antibodies that freely circulate in the blood (humoral immunity). Once activated, T cells generate antigen-specific cellular immunity by phagocytosing and inducing apoptosis of infected cells. In the case of female reproduction, the immune system must negotiate the tension between maintaining these core competencies and tolerating foreign paternal and offspring antigens.

Noticeable vacillations in immunological regulation emerge after menarche, with ovarian cycling organized around the potential for conception and transient tolerance of sperm and

paternal antigens within the female reproductive tract (Schuberth et al., 2008; Lorenz et al., 2015, 2018; Robertson and Sharkey, 2016). Such oscillations are regulated by shifting production of female sex hormones, namely estradiol and progesterone (Wira et al., 2015). In the event of fertilization, gestation is then marked by sustained maternal exposure to semi-allogeneic fetal-derived antigens (Moffett and Loke, 2006). In humans, a high degree of placental invasiveness results in extensive maternal exposure to fetal antigens (Petroff, 2011). Such exposure requires sustained localized tolerance at the maternal-placental interface, largely mediated by highly specialized tissue-specific immune cells (e.g., uterine NK cells) (Moffett and Loke, 2006; Kane et al., 2009; Erlebacher, 2013), and peripheral tolerance of fetal cells and cell-free fetal DNA (cffDNA) that escape the placental barrier (Lo et al., 1997; Erlebacher, 2013; Phillippe, 2015). While localized *in vivo* mechanisms of fetal tolerance are difficult to study in humans, peripheral tolerance is easier to characterize. On a systemic level, the most reliable markers of pregnancy are increased neutrophils, elevated markers of inflammation (e.g., erythrocyte sedimentation rate, C-reactive protein), and reduced total lymphocyte count (Belo et al., 2005; Purohit et al., 2015; Elsayed Azab, 2017; Bakrim et al., 2018). These shifts presumably reflect a reorganization of immune response patterning that limits immune targeting of the fetus via reduced antigen-specific cellular cytotoxicity while maintaining basic immune competence via heavier reliance on non-antigen-specific defense mechanisms and humoral immunity (Monteiro et al., 2021). These shifts are heavily regulated by hormonal shifts during pregnancy (Schumacher et al., 2014) and have been associated with increased susceptibility to viral pathogens and temporary amelioration of cellular-immunity mediated autoimmune disease (e.g., rheumatoid arthritis) (Thong et al., 1973; Jamieson et al., 2009; Khashan et al., 2011). Although less well-studied,

there is also evidence that pregnancy induces an immunological state similar to that favored by large, multicellular parasites (e.g., helminths). Data from Sub-Saharan Africa indicate that *Ascaris lumbricoides* (roundworm) and *Trichuris trichiura* (whipworm) infection is significantly more common among pregnant women compared to cycling women (Adegnika et al., 2007). Among the Tsimane of Bolivia, infection with roundworm has been associated with earlier age at first birth and reduced interbirth intervals, suggesting that infection with certain helminth species *before* pregnancy may increase the chance of conception by inducing immunological tolerance (Blackwell et al., 2015).

C. Ecological conditions moderate immune response

Throughout the vast majority of human evolution, females negotiated the immunological requirements of pregnancy against a backdrop of chronic pathogen burden, stringent constraints on energetic budget, and the absence of modern interventions (e.g., C-section, infant formula). Based on data from contemporary hunting-gathering societies, such harsh ecological conditions can exact high costs including elevated infectious disease mortality, energetic depletion, and death in childbirth (Gurven et al., 2007a). In contrast, current understanding of "normal" immune function is predominantly based on post-industrial populations experiencing reduced pathogen exposure and curbed fertility. Such ecological conditions are examples of evolutionary mismatch, a phenomenon characterized by rapid environmental change that outpaces genetic evolution. The effects of environmental mismatch are not always deleterious. For example, reduction in infectious disease burden within industrialized populations has resulted in longer lifespans, less energetic stress, and reduced maternal mortality. On the flip side, there are also costs associated with these evolutionarily novel conditions. Microbial deprivation (especially of low

virulence/commensal microbes), obesity, and other keystones of industrialization have been linked to a constellation of immunological disorders (e.g., diabetes, allergy) characterized by impaired immune calibration and chronic inflammation (Bloomfield et al., 2016; Rook et al., 2017). In the USA, one of the wealthiest countries in the world, maternal mortality rates have been steadily *rising* over the last several decades, with the etiology becoming increasingly ambiguous and therefore more difficult to treat (Figure 2) (Joseph et al., 2021). Atopy and autoimmune diseases, disproportionately common among women, have also been on the rise across wealthy countries (Jacobson et al., 1997; Løvås and Husebye, 2002; Takahashi et al., 2020a). To date, however, few studies have investigated the effects of ecological variability on female immune function throughout different phases of reproduction.

Findings generated by my master's research indicate that ecological conditions moderate the effects of pregnancy on peripheral immune status (Hové et al., 2020). In this initial foray, I estimated and compared the effects of trimester on immune cell counts and acute inflammation among Tsimane women, a natural fertility subsistence population in the Bolivian Amazon, and women in the USA. Results indicated that, despite comparatively elevated immune non-pregnant baselines due to chronic pathogen exposure (Blackwell et al., 2016a; Hové et al., 2020), pregnant Tsimane women exhibit comparatively attenuated increase in neutrophils and acute inflammation but relatively larger reductions in eosinophils and total lymphocytes compared to pregnant women in the USA. Such findings could reflect higher energetic constraints and concurrent infection risk among Tsimane women. On the other hand, these population-level differences could indicate that the Tsimane have comparatively better calibration to fetal antigens while the inflammatory response among pregnant USA women may be an underappreciated example of environmental mismatch.

D. What about the postpartum period?

Compared to the immunological effects of placentation and pregnancy, far less is known regarding female immune function, health, and wellbeing following delivery – even in otherwise heavily studied populations. From an evolutionary and mechanistic perspective, the postpartum period should be a time of unique immune modulation distinct from both regular ovarian cycling and pregnancy. Delivery marks an end to fetal tolerance (Thomas et al., 1995; Lo et al., 1999) and removal of the placenta precipitates large reductions in estradiol and progesterone production to below pre-pregnancy baselines (Neville and Neifert, 1983), allowing for the transition to lactogenesis and the potential for long-term breastfeeding (Figure 2). Potential retention of infant cells trafficked during pregnancy (Bianchi et al., 1996; Boddy et al., 2015), transfer of both microbial and infant cells during active breastfeeding (Molès et al., 2018), and secondary infections associated with delivery may result in a unique pathogen exposure profile during the postpartum period. Furthermore, there has likely been selection on enhanced pathogen clearance after delivery to compensate for transiently increased susceptibility to certain pathogens during pregnancy. While pregnancy is associated with elevated susceptibility to viral and parasitic infections (due to downregulation of cellular immunity) (Belo et al., 2005; Purohit et al., 2015; Elsayed Azab, 2017; Bakrim et al., 2018), limited evidence indicates that postpartum women exhibit enhanced viral and parasite clearance and preponderance of bacterial infections (Nobbenhuis et al., 2002; Boel et al., 2012; Coss et al., 2020). These patterns suggest that, in addition to alleviating fetal tolerance requirements, delivery prompts compensatory immunological defense against pathogens that may have been tolerated during pregnancy – a pattern that may be more robust in populations where relative pathogen exposure and infection risk is

higher across gestation. To date, however, a scarcity of data precludes a firm understanding of the effects of time-since-delivery of female immune function and, consequently, limits understanding of how these effects might be mediated by variation in breastfeeding behavior (a key form of maternal investment that has been primary studied from an infant-centric perspective).

E. What are the effects of ecological context and female reproductive phase on sexual dimorphism?

Sex differences in disease morbidity and mortality have been observed among humans, with dimorphism becoming most apparent after puberty. In general, female immunity has been characterized as more vigilant and responsive to both foreign and self-antigens, exemplified by elevated antibody response to infectious agents and vaccines, lower risk of non-reproductive cancers, and substantially elevated incidence of autoimmunity (Klein and Huber, 2010; Ortuna et al., 2016; Jaillon et al., 2019; Takahashi et al., 2020b). Sex chromosomes are thought to be a primary mechanism through which sex differences in immune function and disease risk are established, via developmental mechanism such as incomplete X-inactivation in females and determination of sex-specific hormone production profiles established during puberty (Klein and Flanagan, 2016). The observation that sex differences tend to emerge after puberty highlights the outsized role of hormones in mediating sexual dimorphism in immune function and disease risk, with testosterone generally suppressing various aspects of immune function while estradiol and progesterone exhibit dose-dependent stimulatory effects (Klein, 2004; Klein and Flanagan, 2016; Kadel and Kovats, 2018).

To date, however, understanding of sex biases in human immune function is limited by skewed sampling across multiple dimensions. Sexual dimorphism in overall *disease risk* has been primarily documented in industrialized populations, with very little consideration for the role of female reproductive phase (e.g., cycling, pregnant, postpartum) in moderating the degree of observed sex bias, while most data used to determine sex biases in *immune function* come from rodent models (Klein and Flanagan, 2016). There is strong evidence that growing up in an industrialized context results in elevated adult testosterone levels in men (Bribiescas, 1996) and higher progesterone production among women (Núñez-de la Mora et al., 2007), indicating that the dimorphic effects of sex hormones on immune function may be exacerbated under such conditions. Furthermore, female reproductive phase is likely to impact the degree of sex bias observed *within* populations due to substantial hormonal changes that occur across female reproduction (Neville and Neifert, 1983).

F. Dissertation objectives

In **Chapter II**, I utilize pre-existing data collected by the Tsimane Health and Life History Project (THLHP) and the National Health and Nutrition Examination Survey (NHANES) to investigate how month-since-delivery affects maternal immune status among the Tsimane, a natural fertility population inhabiting the Amazonian River basin, and women in the USA. I also leverage variation in breastfeeding behavior within the NHANES dataset to test the prediction that breastfeeding absence will be associated with blunted immune recovery and faster return to cycling baselines among postpartum women in the USA.

In **Chapter III**, I again utilize THLHP and NHANES data to investigate the how female and male immune status vary across the lifespan among the Tsimane and the USA. By drawing these population-level comparisons, I test the prediction that evolutionarily novel

environmental conditions common in the USA and other post-industrialized societies (e.g., reduced parity, increased sedentism) exacerbate evolved sex differences in immune function by increasing lifetime exposure to sex hormones and, among females, reducing the time spent either pregnant or lactating (Natri et al., 2019). I also predict that sexual dimorphism *within* populations will be greatest between males and pregnant females and least accentuated between males and postpartum females, due to hormonal changes and shifting immunological requirements that occur before and after parturition. To test these hypotheses, I investigate the population-specific effects of female reproductive phase (i.e., cycling, pregnancy, postpartum period) on sexual dimorphism on immune outcomes. Lastly, I test the prediction that sexual dimorphism in immune status will be reduced among post-menopausal women and age-matched men due to changes in female hormone profiles, but that increased lifetime exposure to sex hormones in the USA will result in comparatively greater magnitude of enduring sex bias.

In **Chapter IV**, I investigate the effects of within-population variation in infant feeding behavior on maternal immune status, mental health, and reported physical health in a sample of mothers in the USA. Due to the introduction of myriad breastfeeding alternatives (e.g., breast pumps, formula), the range of infant feeding behavior exhibited by mothers in the USA (and other post-industrial societies) is much wider than existed throughout most of human history. I predict that replacement or heavy supplementation of at-the-nipple breastfeeding is linked to dysregulated maternal immune recalibration, increased risk of sickness behavior (e.g., depression) and more symptoms of physical illness, due to environmental mismatch. On the other hand, I predict that mothers who sustain exclusive at-the-nipple breastfeeding for extended periods may experience diminishing returns on

immune and health benefits due to increasing maternal-offspring conflict. From this perspective, moderating the degree of skin-to-skin breastfeeding using evolutionarily novel alternatives may, to some degree and in a time-dependent manner, help mothers optimize their own immune status and health. The pre-existing NHANES dataset used in Chapter II highlighted current limitations in metrics used to assess breastfeeding success (e.g., simple binary presence/absence of *any* breastfeeding), therefore I explicitly designed more nuanced measurements of infant feeding behavior (e.g., proportion of infant feedings conducted via at-the-nipple breastfeeding) which were used to test these predictions.

Finally, in **Chapter V**, I summarize key findings from this dissertation and pinpoint areas for future research.

Chapter I: Figures

Figure 1. The difference types of placentation, indicating the varying degrees of separation between fetal tissue (in pink) and maternal tissue (in green/blue). Adapted from Montiel et al, 2013.

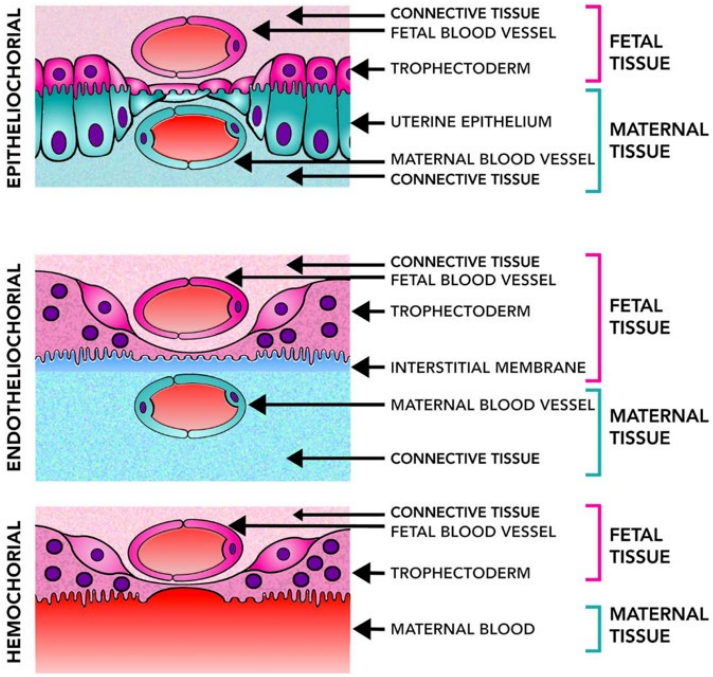


Figure 2. Graphic of the different phases of female reproduction, each requiring dynamic immune modulation. cffDNA = cell-free fetal DNA.

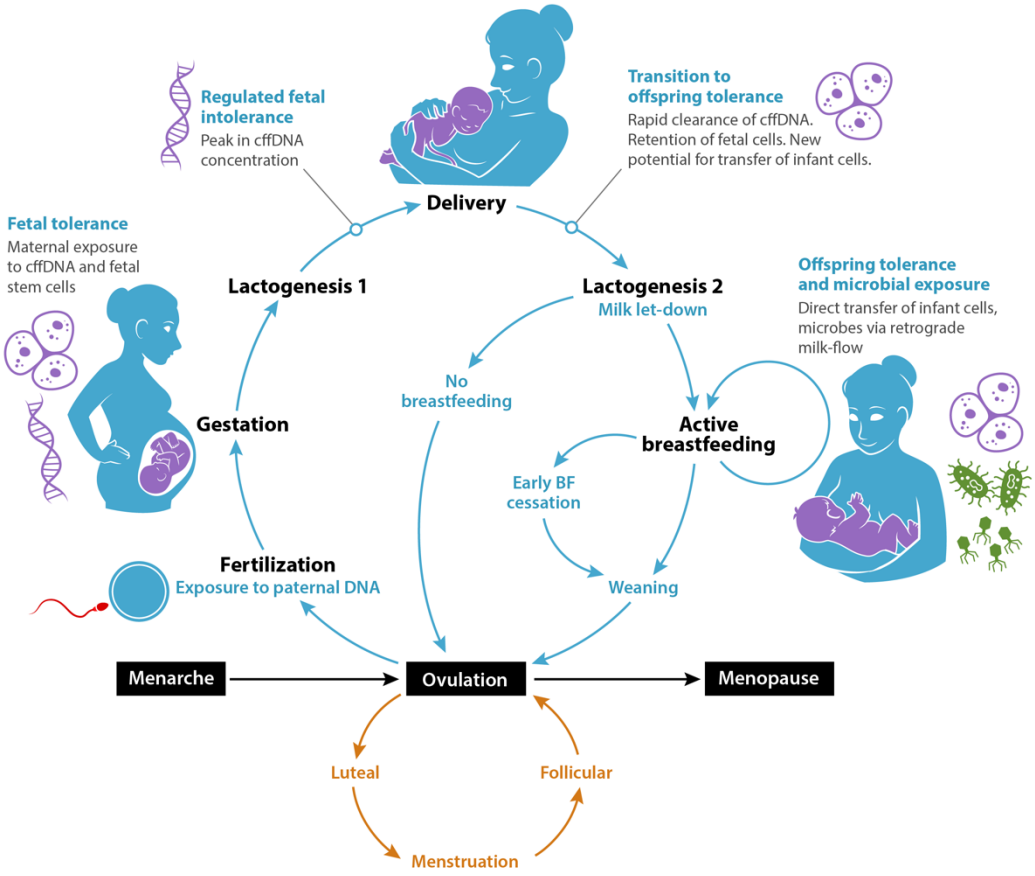


Figure 3. Generalized overview of the immune components of the innate versus acquired (i.e., adaptive) immune system, adopted from Sharpe & Mount, 2015. Granulocytes include neutrophils, eosinophils, and basophils. Lymphocytes include B cells, T cells, and NK T cells.

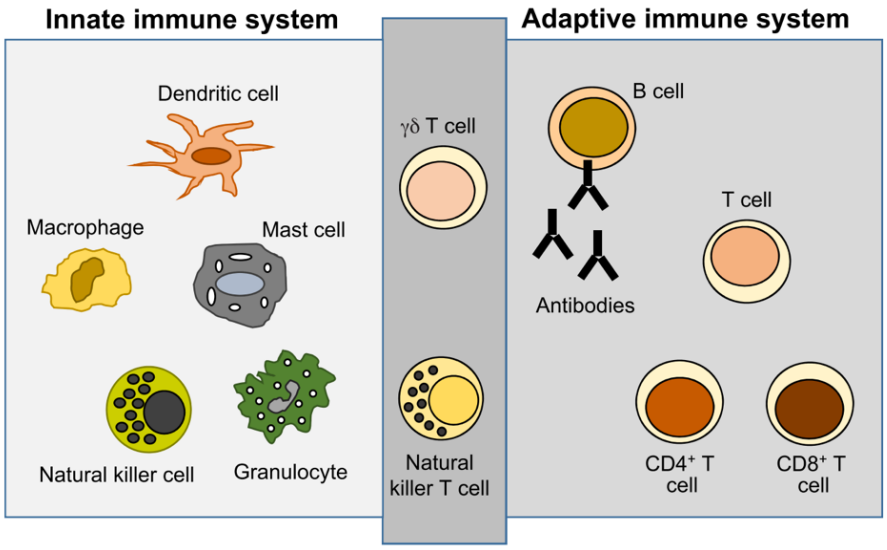


Figure 4. Overview of the humoral versus cellular response, adopted from Bárcena & Blanco, 2013.

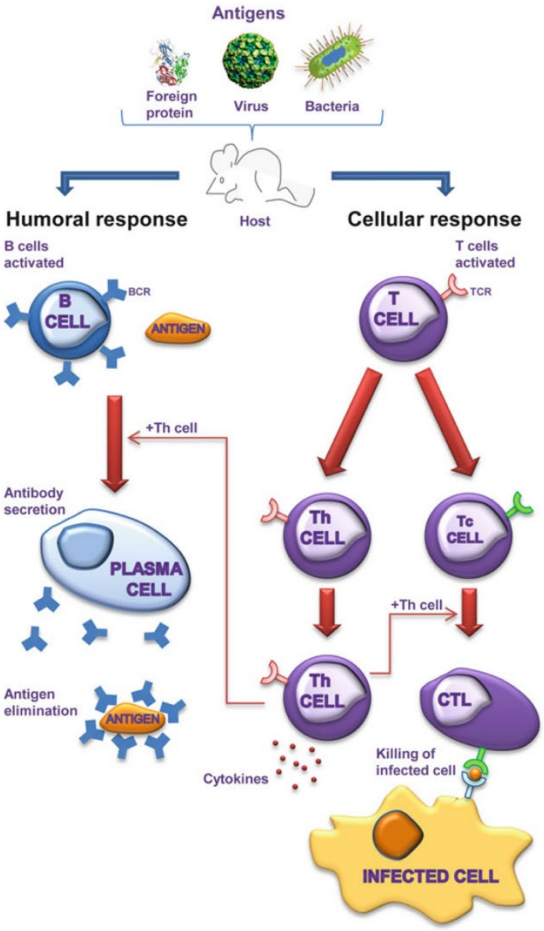
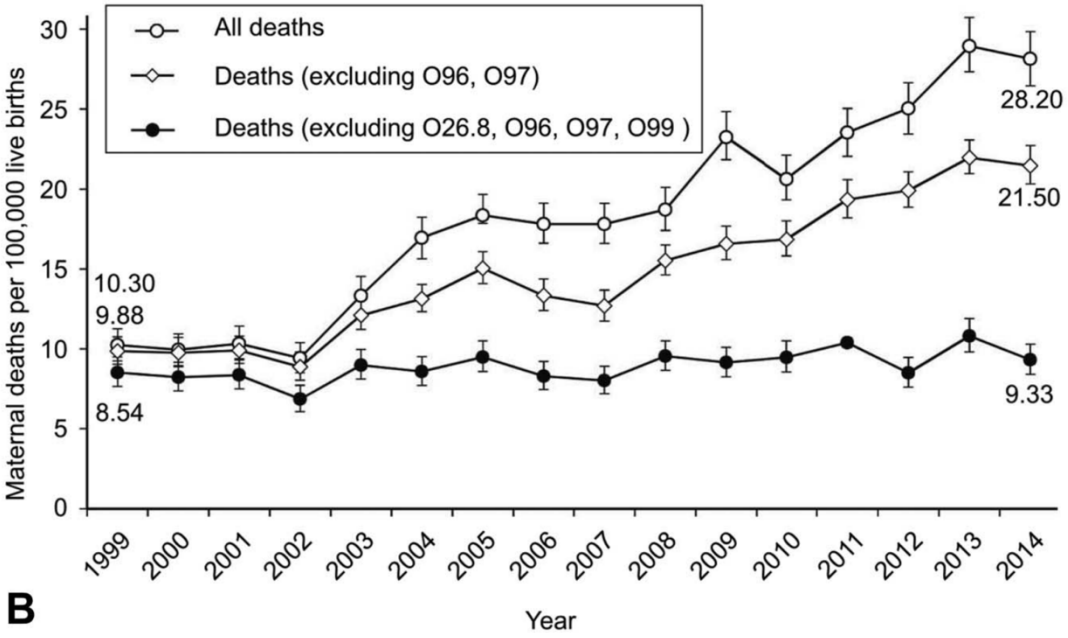


Figure 5. Maternal mortality rates in the USA from 1999 to 2014, adapted from Joseph et al, 2021.



II. The effects of time since delivery on female immune status in two ecologically distinct populations

This chapter is co-authored with Amy Boddy, Aaron Blackwell, Hillard Kaplan, Ben Trumble, Jonathan Steiglitz, and Michael Gurven and utilizes data collected by the Tsimane Health and Life History Project. The author of this dissertation proposed all hypotheses tested herein. The analyses, writing, and figures contained in this chapter are the work of the author of this dissertation.

A. Introduction

Few studies have characterized maternal immune function in the postpartum period, with most research effort focused on infant outcomes. Furthermore, current research on maternal postpartum infectious disease burden is complicated by predominance of HIV infection in represented study populations. Following a reliable trend of increased viral susceptibility during pregnancy, risk of HIV acquisition (and subsequent opportunistic infections) is highest during pregnancy and therefore postpartum morbidity due to HIV complications may not reflect immune changes that occur after delivery in the absence of this specific pathogen (Gray et al., 2005). One consistent finding that holds true for populations with and without high background prevalence of HIV is that bacterial agents are the most common proximate cause of postpartum infection (Chaim and Burstein, 2003; Mason and Aronoff, 2012; Gundersen et al., 2018). For example, endometritis is one of the leading causes of postpartum maternal infection and is primarily caused by group B streptococcus or staphylococcus bacteria. Conversely, there is evidence of enhanced postpartum clearance of viral and parasitic pathogens. Women chronically infected with hepatitis C before pregnancy exhibit marked postpartum decline in viral load, due to emergence of newly calibrated CD4+ T cell response (Coss et al., 2020). Women with chronic high-risk HPV also exhibit enhanced viral

clearance after delivery (Nobbenhuis et al., 2002). In areas where malaria is endemic, rapid postpartum clearance of malarial parasites instantiated during pregnancy has been reported (Boel et al., 2012). Taken together, these patterns indicate that the postpartum period is a unique and dynamic immunological phase – discrete from gestation, labor, and delivery.

To date, however, a paucity of data precludes a clear understanding of how time-since-delivery impacts maternal immune status. Is delivery followed by rapid and linear return to pre-pregnancy immune baselines or is recovery non-linear? At what point are cycling baselines re-established? Do these patterns vary across human populations experiencing differential pathogen burden? Does breastfeeding behavior impact postpartum immune status, particularly in populations with access to breastfeeding alternatives? In this study, we aim to address these questions by utilizing data from the Tsimane Health and Life History Project (THLHP) and the National Health and Nutritional Exam Survey (NHANES) to estimate and compare the population-specific effects of month since delivery on total and differential leukocyte count, neutrophil-lymphocyte ratio (NLR), and C-reactive protein (CRP) among the Tsimane, a natural-fertility subsistence population inhabiting the Amazonian River basin, and women in the USA (Figure 1). We also leverage variation in breastfeeding presence/absence among USA mothers to model the effects of breastfeeding absence on immune outcomes compared to Tsimane women, who exhibit nearly ubiquitous rates of on-demand breastfeeding (Veile et al., 2014).

1. Predictions

While pregnancy is associated with elevated susceptibility to viral and parasitic infections, disease risk patterns among postpartum women indicate enhanced viral and

parasite clearance. These patterns suggest that delivery not only alleviates the need for fetal tolerance but also prompts compensatory immunological defense against pathogens (e.g., large multicellular parasites, viral invaders) that may have been tolerated alongside the fetus during pregnancy. From this perspective, the immunological changes reliably induced by gestation (e.g., increased reliance on non-specific defense mechanisms, downregulation of cytotoxic lymphocytes) should be reversed in the months following delivery, rather than simply returning to cycling baselines. It is also well-established that breastfeeding behavior profoundly impacts maternal physiology during the postpartum period, with regular breastfeeding slowing the return to ovarian cycling (Neville and Neifert, 1983) and more thoroughly reversing pregnancy-induced metabolic shifts (Stuebe and Rich-Edwards, 2009), indicating that breastfeeding may mediate the effects of time since delivery on maternal immune recovery. Based on this framework, we predict that (1) month since delivery will exert equal but opposite population-specific effects on leukocyte differential, NLR, and CRP compared to the effects of month of gestation and (2) breastfeeding absence among USA women will be associated with reduced magnitude of postpartum effect and potentially earlier return to population-specific cycling baselines.

2. The Bolivian Amazon and the United States

The Tsimane are a natural fertility subsistence population of roughly 15,000 people inhabiting the Bolivian Amazonian River basin, an environment rich in pathogen biodiversity. Despite increasing access to treatments (primarily via annual medical visits by the Tsimane Health and Life History Project and the establishment of a hospital near the city of San Borja), infectious disease remains the primary cause of morbidity and mortality

(Kaplan et al., 2015; Gurven et al., 2019). Conversely, allergies, atopy, autoimmune disease, and atherosclerosis are rare (Gurven et al., 2007a, 2008). In previous decades, roughly 14% of infants died in their first year of life (Gurven et al., 2007a), though this rate has dropped to ~4.6% as of 2015 (Kaplan et al., 2015). Despite increased access to treatment, most Tsimane individuals are chronically infected with at least one species of helminth and over a third are infected with giardia (Martin et al., 2013). Compared to Western clinical standards, Tsimane adults exhibit comparatively higher erythrocyte sedimentation rate (a marker of chronic inflammation), greater total leukocyte, neutrophil, lymphocyte, eosinophil, B cell, and natural killer cell counts, and higher antibody levels and lower basophil and monocyte counts (Blackwell et al., 2016b). Even compared to the Shuar, another Amazonian subsistence population, the Tsimane exhibit elevated IgE, a marker of parasite response (Blackwell et al., 2011). Conversely, comparatively lower basophil and monocyte counts observed among the Tsimane are most likely due to recruitment of these cell types into localized tissue during infection. Such chronic immune activation is linked to reduced investment in growth (Foster et al., 2005; Blackwell et al., 2017), elevated resting metabolic rate (Gurven et al., 2016), and relatively dampened hormone production (Trumble et al., 2016). The Tsimane are also a natural fertility population without regular access to contraception or breastfeeding alternatives. Consequently, Tsimane women have an average of 9 live births over the reproductive lifespan (McAllister et al., 2012) and nearly all Tsimane mothers practice on-demand breastfeeding, with a mean weaning age of 19 months (Veile et al., 2014). There are costs to such high reproductive output among the Tsimane, considering a large proportion of

deaths occur in childbirth and Tsimane female mortality exceeds male mortality during the reproductive years (Gurven et al., 2007b).

On average, ecological conditions in the United States offer a stark contrast to those experienced by the Tsimane. The US has seen public health measures such as widespread vaccination, access to clean drinking water, and general sanitation efforts massively reduce infectious disease mortality rates across all age groups over the past century (Armstrong et al., 1999; Roush, 2007). In 1900, the top three leading causes of death in the USA were pneumonia, tuberculosis, and diarrhea/enteritis. By 1997, the top three causes of mortality had shifted to heart disease, cancer, and stroke. According to 2018 data from the Centers for Disease Control (CDC), average life expectancy in the USA is now approximately 78.7 years (representing more than a 60% increase in lifespan since 1900), infant mortality rate is approximately 5.7 deaths per 1,000 live births, and maternal mortality rate is ~17.4 per 100,000 live births (Joseph et al., 2021). While curbed pathogen exposure results in comparatively lower baseline immune activation than the Tsimane, conditions such as adiposity and smoking promote inflammatory activation similar to that observed among populations with high microbial exposure (Blackwell et al., 2016a). The USA is a mixed fertility population, with widespread access to birth control and breastfeeding alternatives. In 2020, the average fertility rate was 1.78 births per woman – much lower average parity than contemporary natural fertility populations such as the Hadza (Jones et al., 1992), Tsimane (McAllister et al., 2012), and Shuar (Jokisch and McSweeney, 2011). Over the past several decades, public health efforts have focused on increasing breastfeeding rates to meet current World Health Organization goals, with largely favorable outcomes. According to data from

the CDC, breastfeeding initiation and continuation have improved markedly across all metrics and demographic groups, though there is still widespread variation.

3. Immunological baselines and trimester-based effects vary between Tsimane and USA women

Previous research has shown that Tsimane and USA women exhibit divergent non-pregnant immune baselines and response patterns during pregnancy (Hové et al., 2020). Non-pregnant, non-lactating Tsimane women possess higher baseline neutrophil, eosinophil, total lymphocyte, and total leukocyte counts than their peers in the USA (Hové et al., 2020). This pattern of elevated baseline immune measures among Tsimane women holds, even in broader comparisons. For example, baseline eosinophil count among Tsimane women is higher than median values reported among non-pregnant regularly cycling women in Portugal (Belo et al., 2005) and Morocco (Bakrim et al., 2018), as well as general reference values for women in Rwanda, Uganda, Kenya, Zambia (Karita et al., 2009), and South Africa (Smit et al., 2019). Reflecting such elevated baselines, Tsimane women possess higher total leukocyte count than reported in comparable sample populations from Nigeria (Ifeanyi et al., 2014), China (Shen et al., 2010), Malawi (Mandala et al., 2017), Portugal (Belo et al., 2005), Libya (Elsayed Azab, 2017), Germany (Kühnert et al., 1998), Morocco (Bakrim et al., 2018), and rural India (Purohit et al., 2015). These patterns clearly indicate that marked eosinophilia is an especially robust driver of high leukocyte count among the Tsimane, highlighting the outsized role of extracellular parasites in shaping baseline immune status. Among both Tsimane and USA women, pregnancy is marked by elevated neutrophil count, reduced lymphocyte count, higher NLR, and increased C-reactive protein concentration – patterns that have been reported in numerous other populations (Belo et al., 2005; Purohit et al., 2015;

Elsayed Azab, 2017; Bakrim et al., 2018). In most populations (including the USA), the increase in neutrophil count during pregnancy is steep enough to substantially drive up total leukocyte count (Akingbola et al., 2006; Purohit et al., 2015; Genetu et al., 2017; Sanci et al., 2017; Gebreweld et al., 2018; Mba et al., 2019). Pregnant Tsimane women, on the other hand, retain comparatively high lymphocyte and eosinophil counts, fewer neutrophils and monocytes, and therefore possess relatively stable total leukocyte count across all three trimesters (Hové et al., 2020). Tsimane women also appear to undergo comparatively less inflammatory activation during pregnancy, reflected by attenuated increase in C-reactive protein concentration (Hové et al., 2020). Such patterns among the Tsimane may be due to stronger constraints imposed by infection risk, competing demands on energy distribution, and/or better immune calibration (via exposure to helminths) attenuating inflammatory response to fetal antigens during pregnancy.

B. Materials and Methods

Both THLHP and NHANES datasets were limited to regularly cycling, pregnant, and postpartum females between the ages of 18 and 45 with recorded white blood cell differential and/or C-reactive protein concentration. Table 1 contains descriptive statistics for all measures and for both populations.

1. Tsimane Health and Life History Project (THLHP)

THLHP data, which were collected between 2004 and 2014 by the Tsimane Health and Life History Project (http://tsimane.anth.ucsb.edu/index.html) (Blackwell et al., 2011, 2015, 2016b; Martin et al., 2013; Gurven et al., 2017), were mixed cross-sectional and

longitudinal. All data used in this study were collected with the approval of the Gran Consejo Tsimane (the governing body overseeing Tsimane affairs) and by institutional review boards at the University of California, Santa Barbara (UCSB) and the University of New Mexico (UNM). Informed consent was obtained first during a community-wide meeting open to all Tsimane residents and then at the individual level before each medical visit and interview. When applicable, medical services were provided to participants following initial medical visit. For a comprehensive overview of the THLHP, see Gurven et al (Gurven et al., 2017).

For this study, inclusion in THLHP cycling group was limited to individuals who had reported regular menstrual cycles and were not pregnant at time of exam. Additionally, individuals who had a recorded time since last delivery that fell above the 75% population quantile (35.33 months) were excluded from the cycling group to avoid selection bias. Pregnancy status was determined during medical visits based on date of last menses, with urinary pregnancy tests administered by the physician when pregnancy was suspected. Pregnancies were cross validated against subsequent annual demographic and census interviews that ascertained children's birth dates, permitting detection of pregnancies occurring between medical visits and pregnancies that went undocumented during previous physician examinations (Blackwell et al., 2015). Venous blood drawn into heparinized vacutainers was analyzed immediately after collection. Total leukocyte counts were determined with a QBC Autoread Plus dry hematology system (QBC Diagnostics), and relative fractions of neutrophils, eosinophils, lymphocytes, basophils, and monocytes were determined manually by microscopy with a hemocytometer. Blood drawn into serum vacutainers was allowed to clot and then centrifuged to separate serum, which was frozen in liquid nitrogen and then transported on dry ice for analysis at the University of California,

Santa Barbara (UCSB). Serum samples were then analyzed to determine CRP concentration at the UCSB Human Biodemography lab via enzyme immunoassay (ELISA) (Brindle et al., 2010), using a protocol validated against the same University of Washington lab responsible for NHANES data analyses (Blackwell et al., 2016b).

2. National Health and Nutrition Examination Survey (NHANES)

NHANES data (https://www.cdc.gov/nchs/nhanes/index.htm), collected between 2003 and 2016, were exclusively cross-sectional. CRP was measured using nephelometry, while total and differential leukocyte counts were measured using the Coulter method. Individuals were included in the NHANES cycling group if they were not currently pregnant and had reported a regular menstrual cycle either at time of exam or within the preceding two months. No exclusions were based on medical diagnoses. In the NHANES dataset, pregnancy was determined by participant report or urine test at the time of exam. Breastfeeding status in the NHANES sample was based on self-reported presence or absence of any current breastfeeding. For complete descriptive statistics on the NHANES breastfeeding subsample, see Table 2.

3. Statistical Analyses

To model the effects of month since delivery and draw direct comparisons to the effects of month of pregnancy, we created a dummy variable called reproductive month (RM) that included data from cycling, pregnant, and postpartum individuals, with zero centered on estimated time of delivery. Regularly cycling women were assigned an RM value of -10, while ARM values -9 through -1 months corresponded to months 1 through 9 of pregnancy and ARM values 1-20 corresponded to months 1-20 of the postpartum period. We employed fully Bayesian multilevel models using the *brms* package (Bürkner, 2017) to estimate the

population-specific effects of RM on white blood cell differential (i.e., total leukocyte, neutrophil, lymphocyte, eosinophil, monocyte, and basophil count), NLR, and C-reactive protein concentration. Since we predicted non-linear effects for all outcome variables, we used non-parametric regression models with RM as the smooth term. The *brms* package utilizes conservative default spline parameters (e.g., knots) based on those used in the *mgcv* package, which penalize for overfitting, and weakly informative priors scaled to the data (Wood et al., 2016; Bürkner, 2017). We therefore used the default smoothing parameters to fit all final models.

Since outcome variable distributions differed, each was modeled separately. THLHP and NHANES total leukocyte, neutrophil, lymphocyte count, NLR, and C-reactive protein concentration data were all log-transformed and modeled using gaussian distributions. Zero-inflated models were used to estimate raw THLHP monocyte count and THLHP and NHANES basophil count. Both raw THLHP and NHANES eosinophil counts were modeled using a negative binomial distribution. NHANES monocyte count was modeled using non-log-transformed data and gaussian distribution. While NHANES did not report date of exam, the THLHP dataset included this information, which was used to check for any effects of seasonality in this population. None were found and therefore season was not included in any of the THLHP-specific models. All models accounted for the fixed-effects of BMI, age, and parity (i.e., number of previous live births), with predicted values standardized by population-pooled age and BMI and population-specific median parity. Since the THLHP dataset included repeat samples, all THLHP-specific models also included the group-level effects of participant ID number. When sampling from the posterior distribution simulated by each model, we limited our draws to 1-16 months since delivery, since expanding posterior

sampling to a wider range of values would have likely introduced bias by effectively eliminating individuals with shorter interbirth intervals (especially in the THLHP dataset).

To assess the effects of breastfeeding presence/absence of postpartum immune recovery in the USA, we ran separate models for each outcome measure with breastfeeding status added as an interaction term. To ensure matching anchor points at estimated time of delivery, we duplicated the entire NHANES sample of cycling and pregnant individuals, giving every individual in the first duplicated sample a dummy breastfeeding value of "Yes" and all individuals in the second duplicated sample a dummy value of "No". Using these merged duplicated samples, we modeled the full effects of RM by breastfeeding status, anchoring each spline by the full set of cycling and pregnant individuals, and limited our posterior sampling to months 1 through 16 since delivery. As stated above, z-scored BMI co-varied substantially by month of pregnancy and the same standardizing method was used to extract breastfeeding specific predicted values.

C. Results

1. Cycling baselines differ between Tsimane and USA women

Among non-pregnant regularly cycling females, Tsimane women exhibited higher C-reactive protein concentration (Δ 1.48 mg/L; 95% CI = 0.94, 2.28) and median total white blood cell count (Δ 2233 cells/uL; 95% CI = 2027,2539), due to greater neutrophil (Δ 658 cells/uL; 95% CI = 470, 830), eosinophil (Δ 1274 cells/uL; 95% CI = 1189, 1371), and total lymphocyte count (Δ 647 cells/uL; 95% CI = 571, 731). Conversely, cycling Tsimane females exhibited lower neutrophil-to-lymphocyte ratio (Δ -0.22; 95% CI = -0.30,-0.15),

monocyte (Δ -471 cells/uL; 95% CI = -480, -460), and basophil (Δ -40 cells/uL; 95% CI = -43, -39) count (Table 3, Figure 2).

2. For majority of immune biomarkers, most extreme shifts occur during pregnancy

Pregnancy was marked by population-specific peaks in neutrophil count (and monocyte count among USA women), nadirs in total lymphocyte count (and eosinophil count among Tsimane women), and consequent peaks in neutrophil-to-lymphocyte ratio (Table 4, Figure 2). Among women in the USA, expansion of neutrophil and monocyte cell populations during the first several months of gestation corresponded to robust leukocytosis, with predicted median total white blood cell count reaching a high of 9510 cells/μL (95% CI = 9162, 9859) among women in month six of pregnancy. Among the Tsimane, however, total white blood cell count was elevated, regardless of gestation month, with little added effect of pregnancy. In both populations, NLR was highest among women in month six of pregnancy, but the peak among USA women was 1.34 (95% CI = 1.06, 1.62) higher than that exhibited in Tsimane women. Effects of gestational month of CRP also diverged sharply between the Tsimane and the USA. In the USA, early pregnancy was marked by significant stepwise increase in CRP concentration, hitting a high of 4.38 mg/L (95% CI = 3.70, 5.37) among

women in month 5 of gestation, while estimated median CRP among pregnancy Tsimane women remained near cycling baseline for all nine months of pregnancy.

3. Largest divergence in total leukocyte count between populations occurs during the postpartum period

Among Tsimane women, eosinophil and total lymphocyte count peaked after delivery, reaching highs of 1842 (95% CI = 1621, 2054) and 2951 (95% CI = 2814, 3082) cells/μL at months 3 and 4, respectively. Consequently, median total leukocyte count also peaked after delivery, cresting at 9885 cells/μL (95% CI = 9476, 10259) among mothers in the third month since delivery. Months 4-7 since delivery were negatively associated with estimated median eosinophil count, after which eosinophil counts remained comparable to cycling baselines regardless of time since delivery. Conversely, postpartum recovery had a highly non-linear effect on total lymphocyte count, which fell to below cycling baseline again among women months 11 and 12 postpartum and then rose again to cycling baselines among women in months 13-16 postpartum. Months 1-6 since delivery had a slight negative effect on neutrophil count, which returned to cycling baseline among women 6 months postpartum and remained stable thereafter. As a result, NLR overlapped with cycling baselines by month 2 post-delivery. Month of delivery had no significant effect on CRP, which remained at cycling baselines among all postpartum Tsimane women, regardless of time since delivery. Among postpartum USA women, the first several months following delivery exerted were associated with reduced neutrophil and monocyte count, resulting in nadirs of 3613 (95% CI = 3362, 3834) and 482 (95% CI = 457, 507) cells/μL at months 4 and 6, respectively, while total lymphocyte count overlapped with cycling baselines by the second month postpartum.

Consequently, delivery was followed by reduced NLR, which dipped to 1.51 (95% CI = 1.19, 1.89) at month 4 postpartum. The overall effect of month since delivery on total leukocyte count varied substantially between USA and Tsimane women, with estimated median total leukocyte count diverging by a maximum of 2307 cells/μL (95% CI = 2702, 3669) among USA and Tsimane women in month 3 postpartum. Among USA women, CRP remained elevated above baseline for the first three months following delivery but overlapped cycling baselines by month 4 and remained stable throughout the postpartum period (Table 5).

4. Breastfeeding presence/absence in USA is not a robust indicator of effects of month since delivery

Predicted values for white blood cell differential and C-reactive protein among breastfeeding and non-breastfeeding USA mothers are shown in Figure 3 and provided in Table 6. The effects of reported presence/absence of any breastfeeding on peripheral immune status were slight, with overlapping confidence intervals for predicted median values at each month postpartum and for each immune marker. Such within-population variation was far smaller than the differences between populations described above.

D. Discussion

Our results clearly indicate that the postpartum period (especially the first year) is a unique immunological period, distinct from both pregnancy and regular cycling. We found that month since delivery generally exerts opposing (but not always equal) effects on maternal immune function compared to those induced during gestation. We also report strong evidence that, in addition to shaping immune baselines and response patterns during

pregnancy, ecological conditions (e.g., pathogen composition and burden) strongly mediate the effects of month since delivery on immune status.

We found that the largest divergence in the number of circulating leukocytes between Tsimane and USA women during any reproductive phase occurred in the first several months of the postpartum period, wherein total leukocyte counts were elevated among Tsimane women and reduced among USA women. Increased leukocyte count among the Tsimane was primarily driven by elevated total lymphocyte and eosinophil counts, whereas decreased leukocyte count among USA women was due to reduced monocyte and neutrophil count (reflected in an acute post-delivery dip in NLR). These differences may stem, in part, from divergent pathogen clearance requirements present after delivery. Among the Tsimane, particularly high exposure to a particularly wide breadth of viruses and helminth species potentially privileged by the maternal immune system during pregnancy (Blackwell et al., 2015) may necessitate a particularly strong uptick of lymphocyte and eosinophil production after delivery. Future research parsing out postpartum shifts in specific lymphocyte subsets among Tsimane women may be especially useful in determining host-pathogen interactions after delivery, considering evidence of highly non-linear recovery pattern in total lymphocyte count. Conversely, the overall reduction in total leukocyte count among postpartum USA women due to lowered neutrophil and monocyte counts may provide insight into the preponderance of bacterial infections reported among postpartum women in previous studies. It is possible that postpartum drops in neutrophil and monocyte production create increased susceptibility to bacterial infections in this population. On the other hand, bacterial infections instantiated around the time of delivery (due to trauma from vaginal delivery or C-section) could cause increased neutrophil and monocyte recruitment into tissues. Future investigation

of such causal pathways is key to enhancing understanding of how maternal immune recovery corresponds to actual disease risk.

As shown in Figure 2 Panel B, inflammatory elevation among pregnant women is far more robust among USA women. Contrary to expectations, however, month since delivery did not exert opposing effects on CRP in either population. Rather, our results indicated a relatively quick and linear return to cycling baselines in both populations. The effects of month since delivery on NLR were similarly muted, with relatively minor reductions in NLR among postpartum USA women in the first six months following delivery and almost immediate return to cycling baselines among Tsimane women. Future studies incorporating a broader range of immune markers (e.g., antibody levels) are likely to provide further insight into determinants of acute inflammation among postpartum mothers.

The estimated effects of breastfeeding presence/absence effects on postpartum recovery among USA women were smaller than expected. These results should not be taken as incontrovertible evidence that breastfeeding has no effect – rather, our findings suggest that reported presence/absence of any breastfeeding does not robustly predict variation in postpartum immune recovery. Future research carefully considering the intricacies of breastfeeding behavior (e.g., exclusive on-demand skin-to-skin breastfeeding, degree of supplementation) may unearth effects that we were unable to parse out in this study, a topic explicitly addressed in Chapter IV of this dissertation. Delivery mode (e.g., vaginal delivery versus C-section) is another key element we were unable to account for in our analyses. Given the myriad physiological processes that may be dysregulated preceding and/or following C-sections (both scheduled and emergency), it is likely that within-population variation in delivery mode produces diverging postpartum recovery patterns – especially in

the first several months following parturition. Further investigation of how C-sections affect immune recovery after delivery is critical, considering that this clinical intervention is becoming increasingly common across the globe (Kabakian-Khasholian et al., 2007; Long et al., 2015; Wyatt et al., 2021), negatively affects breastfeeding success (Hobbs et al., 2016), and substantially elevates postpartum infection risk (Mackeen et al., 2015). Future research incorporating matching hormone and immune data would be invaluable for determining the potential role that within and across-population variation in hormonal production may play in producing different postpartum immune trajectories. Lastly, future studies would greatly benefit from incorporating immune measures taken at time of labor and delivery and drawing comparisons to measures taken before and after parturition.

In sum, this study provides evidence that immune modulation required during reproductive effort does not end with labor and delivery. Rather, the effects of time since delivery on female immune status are often the opposite of those induced by pregnancy and many immune markers do not reach cycling baselines for numerous months following birth. Such data suggest that current definitions of the postpartum period, such as the widely used 42-day window, may obscure the unique immunological shifts that occur in the months following parturition (Høj, 2003). Lastly, our results provide evidence for the existence of population-specific reactions shaped by contemporary ecological conditions and those experienced throughout development (e.g., pathogen exposure). Moving forward, we hope that this study provides a springboard for continuing research of the postpartum period as an integrated yet discrete immunological context.

Chapter II: Figures and Tables

Figure 1. General description of immunological biomarkers used in this study.

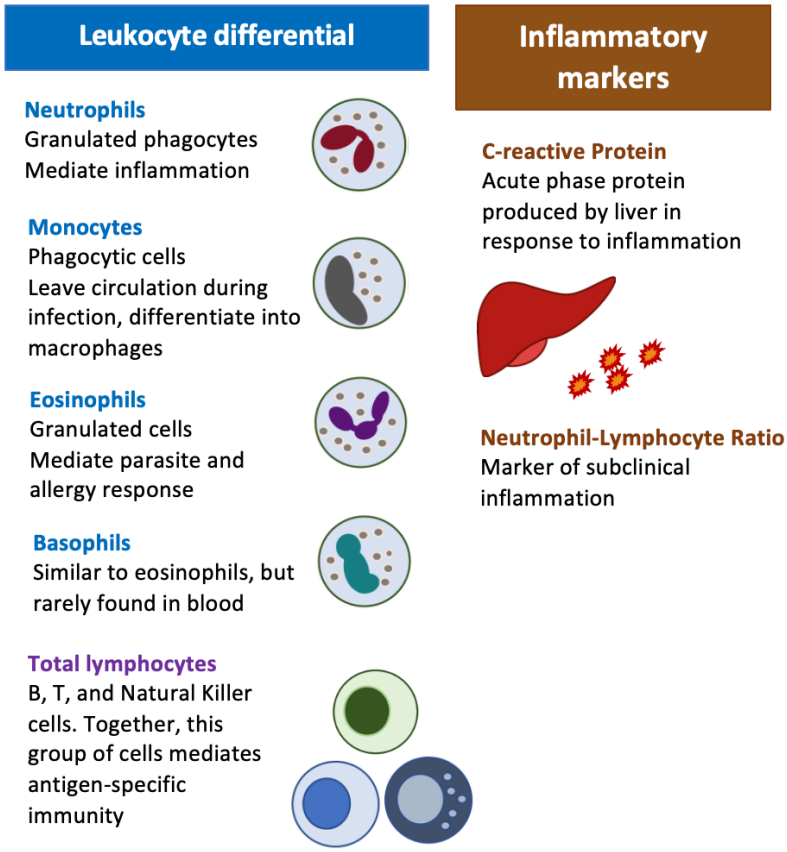


Figure 2. Model-estimated values for leukocyte differential (Panel A) and inflammatory markers CRP and NLR (Panel B) by population and reproductive month (RM), standardized by pooled median age and BMI and population-specific median parity. Dots indicate estimated median values, while error bars represent estimated 95% credible intervals. Hollow points correspond to maximum/minimum predicted median values. Color panels indicate reproductive phase. Solid horizontal lines correspond to model-estimated population-specific cycling "baselines".

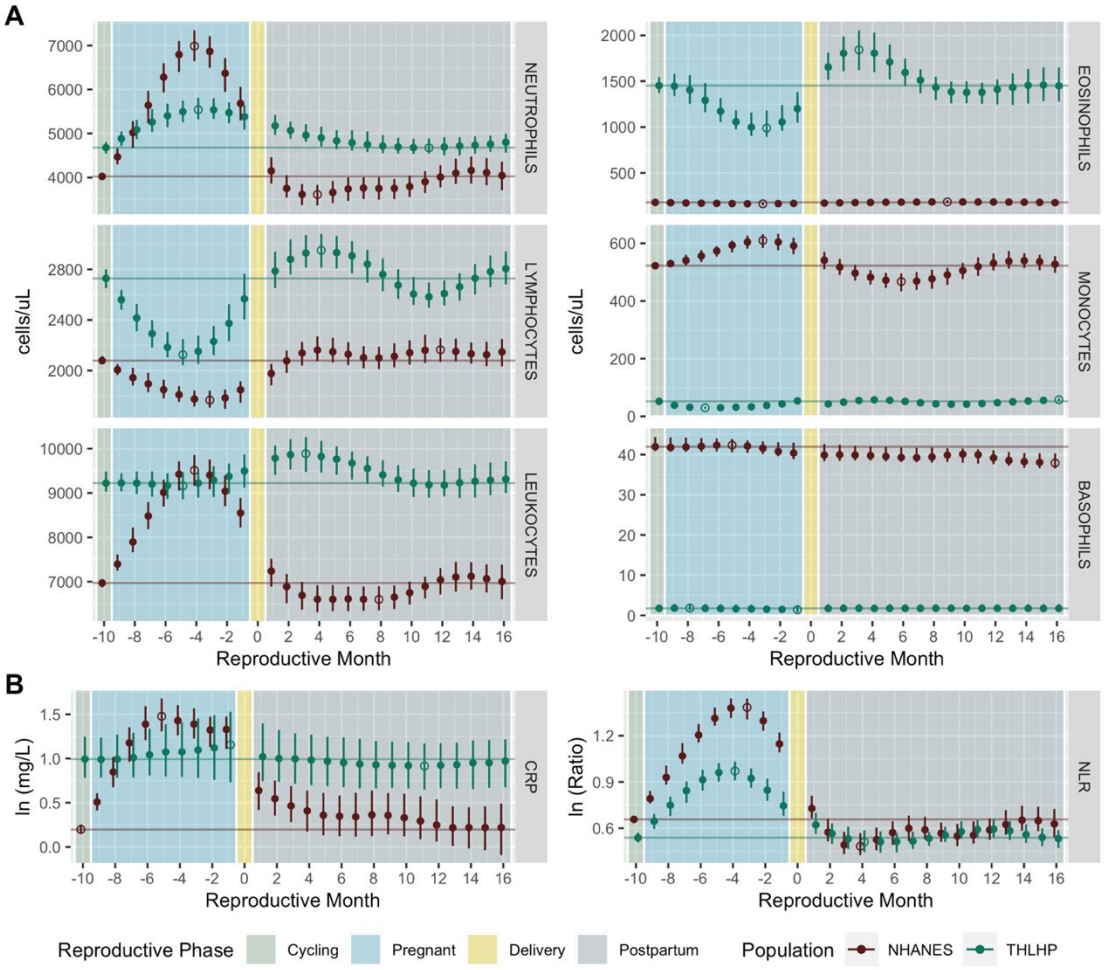


Figure 3. Model-estimated values for leukocyte differential (Panel A) and inflammatory markers CRP and NLR (Panel B) by breastfeeding status and month since delivery in NHANES dataset only, standardized by median age, BMI, and parity. Dots indicate estimated median values, while error bars represent estimated 95% credible intervals.

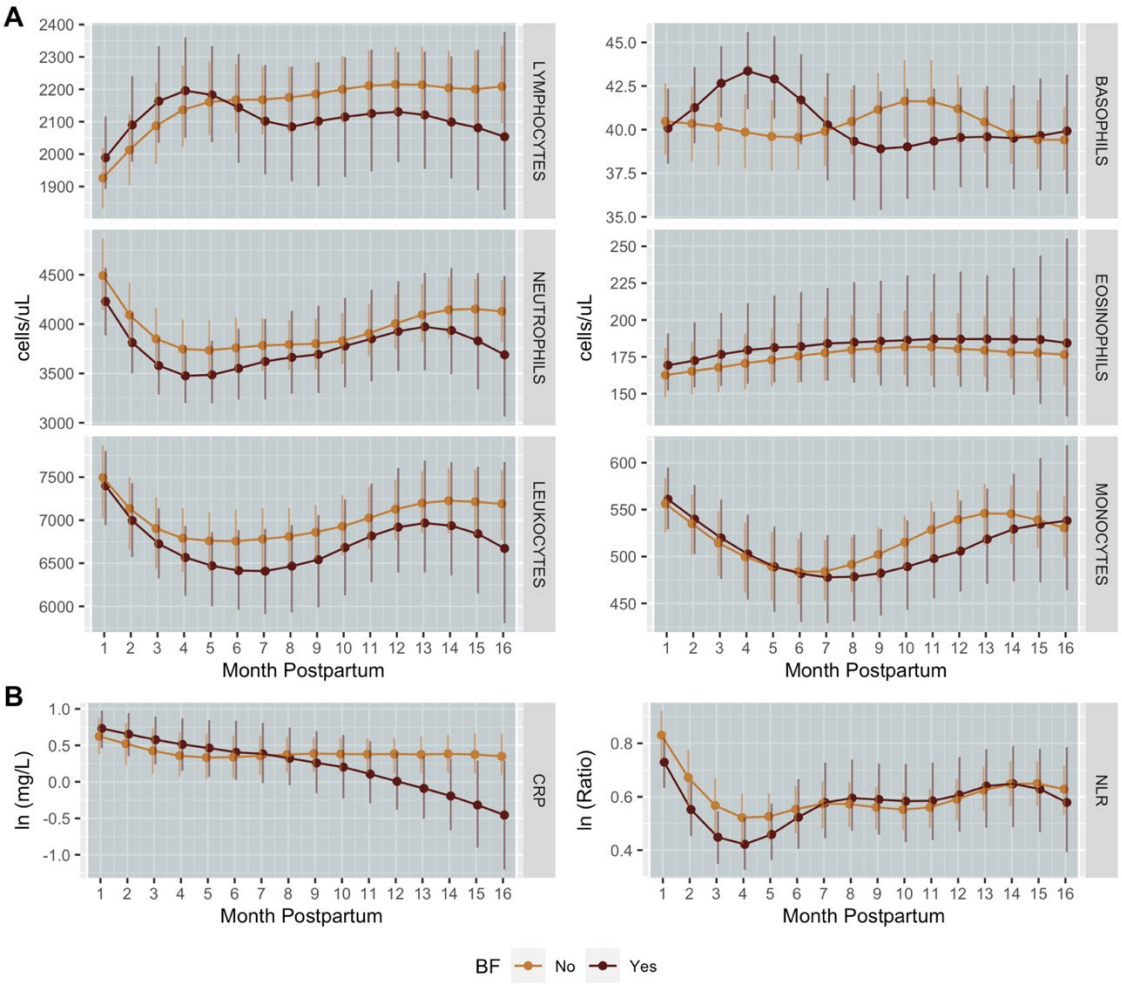


Table 1. Descriptive statistics by population and reproductive phase (Cycling = CYC, Pregnant = PREG, Postpartum = POSTPAR). N = total number of observations. RN = number of individuals with multiple observations. Age/BMI/Parity = median value (min-max). NP = absolute number and percent of nulliparous (for cycling group) / primiparous (for pregnant and postpartum groups) individuals in each sample.

Measure	Population	Phase	N	RN	Age	BMI	Parity	NP/PP
WBC DIF	NHANES	CYC	1183	0	25 (18-45)	25.21 (15.68-56.7)	0 (0-7)	133 (11.24%)
		PREG	295	0	27 (18-41)	28.78 (17.4-67.34)	1 (0-7)	141 (47.8%)
		POSTPAR	426	0	29 (20-44)	26.9 (15.64-73.43)	2 (1-9)	143 (33.57%)
	THLHP	CYC	508	115	35 (18-45)	23.71 (16.53-36.53)	7 (0-15)	26 (5.118%)
		PREG	255	29	32 (18-45)	24.06 (17.5-37.25)	6 (0-13)	19 (7.451%)
		POSTPAR	550	109	33 (18-45)	24 (16.53-36.53)	6 (0-16)	27 (4.909%)
CRP	NHANES	CYC	1175	0	25 (18-45)	25.21 (15.68-56.7)	0 (0-8)	134 (11.4%)
		PREG	276	0	27 (18-43)	28.63 (17.41-67.34)	1 (0-7)	136 (49.28%)
		POSTPAR	218	0	28 (20-41)	26.68 (15.64-73.43)	2 (1-6)	69 (31.65%)
	THLHP	CYC	116	7	40 (20-45)	24.03 (16.53-36.53)	9 (0-14)	1 (0.8621%)
		PREG	29	0	38 (19-44)	24.44 (20.6-32.05)	8 (1-13)	3 (10.34%)
		POSTPAR	105	6	39 (20-45)	24.42 (16.53-36.53)	9 (1-16)	1 (0.9524%)

Table 2. Descriptive statistics for NHANES-specific breastfeeding data by month postpartum, breastfeeding status, and measure type. N = total number of observations. RN = number of individuals with multiple observations. BF = Yes, currently breastfeeding / No, not currently breastfeeding. Age/BMI/parity = median value (min – max).

Month	BF	N		RN		AGE		BMI		PARITY	
		CRP	WBC	CRP	WBC	CRP	WBC	CRP	WBC	CRP	WBC
1	No	7	15	0	0	30 (20-40)	30 (20-40)	28.25 (22.15-41.09)	28.25 (22.15-41.09)	2 (1-4)	2 (1-7)
	Yes	7	12	0	0	24 (20-35)	25 (20-35)	36.05 (28.57-47.35)	30.48 (22-47.35)	2 (2-6)	2 (1-6)
2	No	9	14	0	0	29 (21-39)	30 (21-39)	25.86 (15.8-36.13)	26.05 (15.8-36.13)	2 (1-5)	2 (1-5)
	Yes	8	17	0	0	25 (22-28)	26 (20-35)	34.62 (20.41-39.25)	26.94 (20.41-39.25)	2 (1-5)	2 (1-6)
3	No	8	14	0	0	28 (24-41)	31 (24-41)	25.06 (18.97-40.51)	26.15 (18.97-40.51)	2.5 (1-5)	2 (1-5)
	Yes	4	12	0	0	24 (20-31)	25 (20-36)	25.74 (23.46-42.48)	25.74 (17.1-44.6)	1.5 (1-3)	2 (1-4)
4	No	4	9	0	0	30 (23-39)	33 (23-39)	25.22 (21.23-40.15)	24.8 (21.23-40.15)	2 (1-3)	2 (1-3)
	Yes	7	13	0	0	24 (20-33)	24 (20-44)	25.13 (20.63-31.28)	25.9 (20.63-45.1)	3 (1-5)	2 (1-5)
5	No	3	11	0	0	27 (22-31)	31 (22-37)	23.88 (21.33-36.67)	21.7 (18.9-36.67)	2 (2-4)	2 (1-4)
	Yes	6	13	0	0	26 (20-32)	29 (20-37)	28.12 (22.26-31.32)	29.58 (22.26-43.6)	1.5 (1-3)	2 (1-4)
6	No	3	6	0	0	29 (29-37)	30 (29-37)	25.52 (21.9-31.7)	21.35 (19.2-31.7)	3 (1-3)	2.5 (1-3)
	Yes	7	19	0	0	25 (23-34)	25 (20-36)	26.12 (18.58-40.6)	25.99 (18.58-45.9)	2 (1-4)	2 (1-4)
7	No	2	4	0	0	34 (30-38)	32 (23-38)	20.39 (17.9-22.88)	22.19 (17.9-37.6)	3.5 (3-4)	2.5 (1-4)
	Yes	5	14	0	0	28 (23-40)	24 (22-40)	31.24 (23.42-37.37)	31.22 (22.1-41)	3 (1-5)	1.5 (1-5)
8	No	4	7	0	0	26 (23-32)	29 (23-41)	28.74 (21.73-40.2)	31.82 (21.73-50.5)	1.5 (1-4)	2 (1-4)
	Yes	11	17	0	0	31 (20-32)	31 (20-41)	29.28 (22.33-48.45)	28.6 (19.3-48.45)	2 (1-4)	2 (1-4)
9	No	NA	2	NA	0	NA	24 (23-25)	NA	23.45 (23-23.9)	NA	2 (1-3)
	Yes	11	25	0	0	31 (20-41)	31 (20-42)	26.49 (20.45-51.3)	26.76 (20.45-51.3)	2 (1-3)	2 (1-9)
10	No	2	6	0	0	30 (26-33)	30 (23-41)	25.78 (25.68-25.88)	25.78 (23.7-39.2)	2 (2-2)	2 (1-4)
	Yes	17	27	0	0	24 (20-36)	24 (20-37)	32.02 (17.4-73.43)	30.7 (17.4-73.43)	2 (1-5)	2 (1-5)
11	No	2	5	0	0	29 (26-32)	30 (26-38)	29.72 (25.41-34.03)	25.41 (22.1-37.2)	3 (2-4)	4 (2-5)
	Yes	6	16	0	0	28 (20-31)	29 (20-44)	22.92 (18.53-39.8)	25.9 (18.53-39.8)	2 (1-4)	2 (1-8)
12	No	4	6	0	0	32 (23-35)	32 (23-35)	25.41 (19.44-27.24)	25.12 (19.44-27.24)	2.5 (2-4)	2 (1-4)
	Yes	13	19	0	0	27 (20-35)	27 (20-41)	25.29 (15.64-37.54)	25.29 (15.64-55)	1 (1-3)	1 (1-3)
13	No	2	2	0	0	32 (28-36)	32 (28-36)	31.82 (25.09-38.55)	31.82 (25.09-38.55)	2 (1-3)	2 (1-3)
	Yes	13	16	0	0	24 (21-35)	24 (21-35)	25.56 (17.54-32.79)	26.25 (17.54-45)	2 (1-3)	2 (1-3)
14	No	5	9	0	0	31 (29-35)	31 (28-40)	26.59 (23.38-27.76)	25.16 (19.9-29.2)	2 (1-3)	2 (1-3)
	Yes	6	14	0	0	32 (25-39)	30 (22-42)	28.07 (22.63-32.94)	28.64 (21.3-37.4)	2 (1-4)	2 (1-4)
15	No	1	2	0	0	35 (35-35)	37 (35-39)	21.02 (21.02-21.02)	23.61 (21.02-26.2)	1 (1-1)	1.5 (1-2)
	Yes	8	19	0	0	24 (20-37)	28 (20-38)	28.15 (20.79-34.94)	26 (18.6-40.2)	1.5 (1-4)	2 (1-4)
16	No	NA	1	NA	0	NA	41 (41-41)	NA	29.3 (29.3-29.3)	NA	1 (1-1)
	Yes	9	16	0	0	30 (23-37)	30 (21-37)	30.35 (20.47-39.47)	30.66 (20.47-39.7)	2 (1-4)	2 (1-5)

Table 3. Point estimates for each outcome variable among non-pregnant regularly cycling Tsimane (THLHP) and USA (NHANES) women and the estimated population difference (DELTA).

POP	WBC (cells/uL)	NEU (cells/uL)	LYM (cells/uL)	NLR (ratio)	EOS (cells/uL)	MON (cells/uL)	BAS (cells/uL)	CRP (mg/L)
THLHP	9222 (9029,9485)	4680 (4544,4803)	2728 (2653,2800)	1.71 (1.66,1.77)	1453 (1371,1545)	52 (47,57)	2 (1,3)	2.7 (2.19,3.49)
NHANES	6971 (6873,7062)	4022 (3944,4099)	2078 (2054,2114)	1.93 (1.89,1.97)	179 (172,187)	522 (514,531)	42 (41,44)	1.22 (1.17,1.29)
DELTA	2233 (2027,2539)	658 (470,830)	647 (571,731)	-0.22 (-0.3,-0.15)	1274 (1189,1371)	-471 (-480,-460)	-40 (-43,-39)	1.48 (0.94,2.28)

Table 4. Estimated median values (and 95% credible intervals) for each outcome variable for months 1-9 of gestation (Month Preg). Tsimane values = THLHP, USA values = NHANES, Tsimane delta from baseline = THLHP delta, USA delta from baseline = NHANES delta. Immune cell counts are in cells/uL, CRP is in mg/L.

Population	Month Preg	WBC	NEU	LYM	NLR	EOS	MON	BAS	CRP
THLHP	1	9224 (9035,9444)	4883 (4716,5047)	2560 (2481,2637)	1.91 (1.81,2)	1446 (1340,1580)	39 (34,43)	2 (1,3)	2.69 (2.19,3.44)
THLHP Δ		0 (-135,139)	210 (118,329)	-163 (-231,-105)	0.2 (0.11,0.26)	-19 (-114,95)	-13 (-16,-12)	0 (0,0)	0 (-0.18,0.27)
NHANES		7396 (7250,7612)	4468 (4299,4664)	2006 (1964,2051)	2.21 (2.14,2.32)	175 (166,184)	530 (518,543)	42 (41,44)	1.66 (1.51,1.84)
NHANES Δ		432 (306,607)	449 (265,571)	-75 (-118,-21)	0.28 (0.21,0.38)	-3 (-11,3)	7 (-1,20)	0 (0,0)	0.43 (0.29,0.62)
POP Δ		1825 (1537,2082)	423 (220,638)	553 (464,640)	-0.3 (-0.43,-0.17)	1269 (1161,1410)	-492 (-506,-480)	-40 (-42,-39)	1.05 (0.44,1.78)
THLHP	2	9222 (8978,9487)	5084 (4870,5310)	2416 (2311,2526)	2.12 (1.97,2.24)	1405 (1254,1567)	32 (28,36)	2 (1,3)	2.7 (2.19,3.56)
THLHP Δ		1 (-245,251)	401 (230,635)	-307 (-415,-212)	0.41 (0.25,0.53)	-74 (-221,118)	-20 (-23,-18)	0 (0,0)	0.03 (-0.24,0.57)
NHANES		7896 (7662,8220)	5020 (4669,5284)	1943 (1882,2021)	2.53 (2.42,2.74)	172 (160,183)	540 (523,560)	42 (41,44)	2.33 (1.96,2.77)
NHANES Δ		938 (687,1242)	981 (637,1223)	-140 (-207,-49)	0.6 (0.48,0.79)	-6 (-20,3)	18 (2,38)	0 (-1,0)	1.12 (0.76,1.55)
POP Δ		1311 (935,1736)	99 (-264,442)	468 (352,578)	-0.43 (-0.65,-0.25)	1235 (1083,1396)	-510 (-531,-490)	-40 (-42,-38)	0.47 (-0.37,1.26)
THLHP	3	9201 (8917,9489)	5260 (5034,5556)	2292 (2182,2397)	2.32 (2.15,2.47)	1292 (1162,1480)	30 (26,34)	2 (1,3)	2.75 (2.2,3.63)
THLHP Δ		-25 (-341,299)	580 (377,875)	-439 (-558,-321)	0.61 (0.41,0.76)	-157 (-323,26)	-22 (-25,-20)	0 (0,0)	0.11 (-0.2,0.77)
NHANES		8480 (8183,8795)	5643 (5228,5971)	1894 (1828,1982)	2.91 (2.77,3.17)	170 (154,180)	556 (535,576)	42 (41,44)	3.24 (2.62,3.87)
NHANES Δ		1495 (1180,1833)	1598 (1231,1910)	-191 (-253,-92)	0.98 (0.83,1.21)	-9 (-28,3)	32 (9,57)	0 (-1,1)	2.03 (1.36,2.61)

POP Δ		719 (287,1223)	-323 (-778,13)	395 (270,508)	-0.62 (-0.86,-0.4)	1131 (987,1314)	-526 (-549,-506)	-40 (-42,-39)	-0.32 (-1.32,0.59)
THLHP	4	9170 (8863,9446)	5404 (5142,5681)	2184 (2104,2304)	2.49 (2.33,2.66)	1172 (1053,1316)	30 (27,34)	2 (1,3)	2.84 (2.24,3.81)
THLHP Δ		-63 (-365,297)	707 (500,1025)	-539 (-630,-428)	0.78 (0.61,0.92)	-290 (-428,-140)	-22 (-25,-19)	0 (0,0)	0.15 (-0.16,0.86)
NHANES		9014 (8695,9298)	6281 (5931,6597)	1849 (1779,1927)	3.34 (3.18,3.59)	168 (150,180)	573 (553,590)	42 (41,44)	4.01 (3.35,4.92)
NHANES Δ		2053 (1755,2333)	2247 (1902,2575)	-228 (-293,-139)	1.41 (1.25,1.65)	-11 (-32,3)	51 (27,72)	0 (-1,1)	2.79 (2.1,3.67)
POP Δ		166 (-310,712)	-835 (-1315,-446)	335 (216,467)	-0.85 (-1.12,-0.63)	1006 (889,1153)	-543 (-562,-523)	-41 (-42,-39)	-1.05 (-2.28,-0.02)
THLHP	5	9160 (8855,9473)	5496 (5252,5749)	2125 (2042,2248)	2.61 (2.47,2.78)	1058 (977,1183)	32 (28,35)	2 (1,3)	2.93 (2.22,4)
THLHP Δ		-66 (-396,261)	800 (582,1109)	-591 (-703,-482)	0.91 (0.73,1.06)	-403 (-506,-262)	-20 (-23,-17)	0 (0,0)	0.19 (-0.11,0.96)
NHANES		9423 (9052,9713)	6794 (6397,7104)	1808 (1751,1877)	3.72 (3.54,3.98)	166 (148,180)	593 (568,609)	42 (41,44)	4.38 (3.7,5.37)
NHANES Δ		2463 (2118,2691)	2752 (2406,3077)	-268 (-335,-197)	1.8 (1.63,2.06)	-14 (-35,3)	68 (43,87)	0 (-1,2)	3.15 (2.44,4.13)
POP Δ		-247 (-641,307)	-1285 (-1683,-840)	316 (211,467)	-1.1 (-1.39,-0.9)	896 (812,1014)	-561 (-578,-537)	-41 (-42,-39)	-1.42 (-2.68,-0.1)
THLHP	6	9220 (8895,9518)	5541 (5321,5812)	2152 (2051,2276)	2.64 (2.49,2.81)	998 (904,1159)	33 (29,37)	2 (1,3)	2.93 (2.21,4.1)
THLHP Δ		-36 (-381,296)	847 (629,1148)	-582 (-692,-476)	0.92 (0.74,1.09)	-457 (-591,-293)	-19 (-22,-15)	0 (0,0)	0.22 (-0.15,1)
NHANES		9510 (9162,9859)	6987 (6645,7344)	1774 (1714,1842)	3.97 (3.73,4.23)	163 (146,181)	604 (581,626)	42 (40,44)	4.17 (3.54,4.97)
NHANES Δ		2552 (2248,2838)	2969 (2643,3330)	-307 (-369,-235)	2.05 (1.81,2.29)	-16 (-37,4)	82 (56,106)	0 (-1,1)	2.96 (2.26,3.74)
POP Δ		-321 (-746,193)	-1440 (-1842,-1018)	356 (257,521)	-1.34 (-1.62,-1.06)	839 (739,997)	-572 (-591,-547)	-40 (-42,-39)	-1.24 (-2.33,0.39)
THLHP	7	9287 (8913,9602)	5539 (5333,5798)	2230 (2122,2352)	2.52 (2.37,2.69)	989 (893,1180)	38 (33,42)	2 (1,2)	2.99 (2.18,4.28)

THLHP Δ		35 (-336,397)	848 (609,1133)	-498 (-611,-370)	0.8 (0.64,1)	-459 (-599,-277)	-14 (-18,-10)	0 (-1,0)	0.28 (-0.15,1.14)
NHANES		9404 (9086,9756)	6867 (6496,7214)	1766 (1706,1841)	3.99 (3.69,4.23)	163 (145,182)	610 (584,632)	42 (40,43)	4.01 (3.41,4.79)
NHANES Δ		2422 (2128,2720)	2844 (2541,3208)	-315 (-388,-238)	2.06 (1.74,2.33)	-16 (-36,4)	85 (57,110)	-1 (-1,0)	2.85 (2.18,3.59)
POP Δ		-135 (-601,384)	-1319 (-1743,-829)	462 (325,626)	-1.47 (-1.75,-1.14)	821 (729,1003)	-572 (-592,-546)	-40 (-42,-38)	-0.98 (-2.09,0.63)
THLHP		9374 (8986,9664)	5474 (5233,5709)	2373 (2232,2525)	2.33 (2.18,2.51)	1056 (958,1239)	43 (38,48)	1 (1,2)	3.07 (2.14,4.44)
THLHP Δ		157 (-251,488)	797 (546,1093)	-342 (-471,-194)	0.62 (0.45,0.79)	-391 (-532,-192)	-9 (-12,-5)	0 (-1,0)	0.35 (-0.16,1.39)
NHANES	8	9041 (8704,9394)	6369 (5948,6714)	1783 (1696,1849)	3.66 (3.43,3.89)	165 (147,182)	604 (575,633)	41 (40,43)	3.75 (3.1,4.36)
NHANES Δ		2074 (1750,2391)	2320 (1887,2698)	-290 (-400,-231)	1.72 (1.5,1.97)	-15 (-33,5)	81 (51,108)	-1 (-2,0)	2.52 (1.89,3.11)
POP Δ		360 (-91,801)	-908 (-1327,-348)	607 (432,780)	-1.33 (-1.6,-1.01)	896 (781,1075)	-562 (-587,-531)	-39 (-42,-38)	-0.67 (-1.71,0.88)
THLHP		9497 (9122,9868)	5382 (5092,5643)	2567 (2404,2765)	2.11 (1.97,2.31)	1199 (1048,1380)	54 (47,60)	1 (1,2)	3.17 (2.08,4.61)
THLHP Δ		236 (-167,614)	700 (403,1017)	-155 (-305,6)	0.39 (0.24,0.54)	-258 (-428,-81)	2 (-2,6)	0 (-1,0)	0.5 (-0.24,1.59)
NHANES	9	8549 (8223,8888)	5682 (5297,6065)	1847 (1745,1914)	3.15 (2.98,3.39)	168 (150,183)	591 (563,619)	40 (39,43)	3.78 (3.03,4.37)
NHANES Δ		1591 (1247,1872)	1653 (1259,2069)	-227 (-349,-160)	1.22 (1.03,1.45)	-11 (-30,8)	69 (39,97)	-2 (-3,-1)	2.55 (1.79,3.13)
POP Δ		941 (447,1378)	-321 (-751,203)	725 (560,943)	-1.04 (-1.31,-0.8)	1033 (874,1211)	-539 (-566,-508)	-39 (-42,-37)	-0.57 (-1.62,0.92)

Table 5. Estimated median values (and 95% credible intervals) for each outcome variable for months 1-16 since delivery (Month PP). Tsimane values = THLHP, USA values = NHANES, Tsimane delta from baseline = THLHP delta, USA delta from baseline = NHANES delta. Immune cell counts are in cells/uL, CRP is in mg/L.

Population	Month PP	WBC	NEU	LYM	NLR	EOS	MON	BAS	CRP
THLHP	1	9785 (9408,10068)	5176 (4968,5419)	2787 (2653,2940)	1.86 (1.76,2.01)	1654 (1512,1812)	43 (39,48)	2 (1,3)	2.77 (2.11,4.04)
THLHP Δ		553 (224,882)	505 (250,728)	58 (-79,204)	0.14 (0.02,0.26)	194 (40,344)	-9 (-10,-7)	0 (0,0)	0.08 (-0.24,0.94)
NHANES		7239 (6889,7517)	4150 (3860,4459)	1976 (1883,2052)	2.07 (1.94,2.25)	171 (156,187)	541 (510,570)	40 (38,43)	1.89 (1.52,2.33)
NHANES Δ		286 (-120,518)	129 (-138,423)	-106 (-192,-18)	0.14 (0.01,0.32)	-9 (-24,11)	19 (-10,50)	-2 (-4,-1)	0.68 (0.33,1.07)
POP Δ		2530 (2171,3024)	1058 (688,1358)	800 (661,1011)	-0.22 (-0.43,-0.01)	1484 (1345,1633)	-496 (-529,-466)	-38 (-41,-36)	0.88 (0.21,2.12)
THLHP	2	9862 (9528,10216)	5069 (4880,5267)	2880 (2759,3037)	1.76 (1.65,1.88)	1804 (1610,1988)	49 (45,55)	2 (1,3)	2.72 (2.09,3.76)
THLHP Δ		606 (269,994)	395 (172,584)	156 (13,284)	0.06 (-0.07,0.15)	346 (178,511)	-2 (-4,0)	0 (0,0)	0.06 (-0.26,0.72)
NHANES		6890 (6512,7171)	3751 (3542,4052)	2076 (1980,2158)	1.78 (1.67,1.94)	174 (156,192)	517 (490,546)	40 (38,42)	1.73 (1.43,2.12)
NHANES Δ		-68 (-484,171)	-266 (-466,35)	-3 (-88,68)	-0.15 (-0.27,0)	-6 (-23,13)	-6 (-31,24)	-2 (-3,-1)	0.5 (0.2,0.87)
POP Δ		2963 (2553,3522)	1317 (964,1550)	799 (664,990)	-0.03 (-0.22,0.18)	1631 (1440,1809)	-468 (-497,-442)	-38 (-41,-36)	1 (0.31,1.99)
THLHP	3	9885 (9476,10259)	4963 (4811,5211)	2929 (2814,3072)	1.7 (1.59,1.84)	1842 (1621,2054)	55 (49,62)	2 (1,3)	2.71 (2.04,3.61)
THLHP Δ		632 (292,1059)	304 (88,505)	201 (68,348)	-0.02 (-0.14,0.1)	385 (198,585)	3 (1,5)	0 (0,0)	0.04 (-0.29,0.55)
NHANES		6692 (6370,6997)	3615 (3372,3853)	2138 (2039,2226)	1.64 (1.54,1.78)	176 (159,196)	497 (470,525)	40 (39,42)	1.59 (1.32,1.99)
NHANES Δ		-270 (-622,-5)	-406 (-628,-174)	65 (-31,152)	-0.29 (-0.4,-0.15)	-3 (-20,15)	-27 (-51,1)	-2 (-3,-1)	0.37 (0.09,0.76)

POP Δ		3207 (2702,3669)	1371 (1035,1636)	800 (644,964)	0.05 (-0.12,0.22)	1671 (1450,1895)	-442 (-470,-418)	-38 (-40,-37)	1.13 (0.41,2.03)
THLHP	4	9823 (9461,10173)	4901 (4706,5157)	2951 (2814,3082)	1.67 (1.56,1.8)	1806 (1598,2031)	57 (52,64)	2 (1,3)	2.67 (2.01,3.56)
THLHP Δ		596 (265,1019)	235 (-32,441)	217 (97,353)	-0.04 (-0.17,0.06)	351 (164,538)	6 (4,8)	0 (0,0)	0.01 (-0.33,0.45)
NHANES		6604 (6312,6921)	3613 (3362,3834)	2161 (2075,2270)	1.62 (1.53,1.76)	178 (161,199)	482 (457,507)	40 (39,42)	1.51 (1.19,1.89)
NHANES Δ		-367 (-644,9)	-402 (-670,-205)	86 (-5,179)	-0.31 (-0.43,-0.17)	-1 (-17,17)	-41 (-67,-13)	-2 (-3,-1)	0.28 (-0.03,0.68)
POP Δ		3241 (2679,3671)	1275 (997,1601)	798 (618,958)	0.05 (-0.16,0.21)	1625 (1411,1855)	-424 (-449,-397)	-38 (-40,-37)	1.2 (0.45,1.99)
THLHP	5	9764 (9411,10051)	4834 (4644,5077)	2932 (2807,3064)	1.67 (1.55,1.79)	1710 (1519,1902)	56 (50,62)	2 (1,3)	2.64 (1.99,3.48)
THLHP Δ		492 (195,862)	175 (-60,359)	212 (93,321)	-0.05 (-0.17,0.06)	243 (85,442)	4 (2,7)	0 (0,0)	-0.05 (-0.37,0.38)
NHANES		6603 (6337,6923)	3659 (3418,3919)	2148 (2063,2260)	1.69 (1.58,1.83)	180 (163,201)	472 (445,497)	40 (38,42)	1.43 (1.14,1.84)
NHANES Δ		-382 (-627,-12)	-366 (-606,-81)	76 (-17,163)	-0.24 (-0.38,-0.1)	1 (-15,20)	-50 (-76,-24)	-3 (-3,-2)	0.23 (-0.09,0.63)
POP Δ		3182 (2690,3614)	1175 (805,1556)	779 (621,936)	-0.03 (-0.2,0.13)	1529 (1329,1728)	-416 (-441,-390)	-38 (-40,-36)	1.26 (0.52,1.97)
THLHP	6	9674 (9303,9934)	4791 (4609,5075)	2907 (2768,3023)	1.67 (1.56,1.77)	1595 (1447,1748)	52 (46,58)	2 (1,3)	2.59 (1.97,3.45)
THLHP Δ		429 (138,679)	118 (-120,381)	172 (53,289)	-0.06 (-0.15,0.06)	139 (-14,297)	0 (-2,3)	0 (0,0)	-0.08 (-0.42,0.37)
NHANES		6614 (6335,6888)	3740 (3466,4001)	2129 (2043,2224)	1.77 (1.63,1.91)	181 (167,201)	467 (434,495)	39 (38,42)	1.42 (1.13,1.79)
NHANES Δ		-347 (-648,-105)	-303 (-549,-6)	47 (-54,146)	-0.15 (-0.3,-0.01)	2 (-14,23)	-54 (-85,-26)	-3 (-3,-2)	0.2 (-0.1,0.62)
POP Δ		3087 (2521,3465)	1086 (699,1416)	776 (608,950)	-0.1 (-0.29,0.06)	1418 (1256,1570)	-414 (-445,-383)	-38 (-40,-36)	1.22 (0.37,1.96)
THLHP	7	9552 (9212,9823)	4749 (4576,5045)	2842 (2696,2955)	1.67 (1.59,1.78)	1511 (1369,1629)	47 (42,52)	2 (1,3)	2.55 (1.94,3.4)

THLHP Δ		312 (37,558)	83 (-142,333)	109 (-14,236)	-0.04 (-0.13,0.07)	32 (-122,169)	-5 (-7,-2)	0 (0,0)	-0.12 (-0.47,0.33)
NHANES		6603 (6343,6894)	3761 (3511,4004)	2102 (2017,2193)	1.82 (1.68,1.98)	182 (169,203)	469 (438,501)	39 (38,42)	1.41 (1.12,1.85)
NHANES Δ		-367 (-644,-79)	-255 (-514,-24)	23 (-65,122)	-0.11 (-0.26,0.05)	3 (-11,26)	-54 (-82,-23)	-3 (-4,-2)	0.2 (-0.12,0.64)
POP Δ		2964 (2446,3303)	1015 (623,1386)	742 (547,890)	-0.14 (-0.34,0.02)	1321 (1167,1450)	-421 (-454,-393)	-38 (-40,-36)	1.15 (0.36,1.93)
THLHP	8	9408 (9145,9739)	4714 (4550,4970)	2760 (2626,2887)	1.71 (1.62,1.78)	1433 (1295,1549)	44 (39,48)	2 (1,3)	2.53 (1.94,3.31)
THLHP Δ		204 (-90,422)	55 (-144,276)	34 (-92,151)	-0.01 (-0.11,0.08)	-30 (-172,87)	-8 (-11,-6)	0 (0,0)	-0.14 (-0.49,0.22)
NHANES		6600 (6354,6899)	3751 (3496,4004)	2100 (2010,2189)	1.8 (1.7,1.96)	183 (170,202)	477 (443,508)	39 (38,42)	1.44 (1.13,1.89)
NHANES Δ		-384 (-635,-76)	-266 (-517,-34)	17 (-72,121)	-0.13 (-0.25,0.04)	3 (-10,27)	-48 (-76,-13)	-3 (-4,-2)	0.21 (-0.07,0.64)
POP Δ		2819 (2346,3198)	993 (624,1302)	655 (486,796)	-0.09 (-0.28,0.04)	1247 (1115,1364)	-433 (-466,-399)	-38 (-40,-36)	1.07 (0.32,1.88)
THLHP	9	9295 (9025,9625)	4696 (4543,4904)	2675 (2566,2801)	1.74 (1.66,1.84)	1384 (1259,1497)	42 (38,47)	2 (1,3)	2.52 (1.95,3.28)
THLHP Δ		83 (-205,332)	20 (-141,214)	-47 (-178,67)	0.03 (-0.07,0.13)	-78 (-193,44)	-10 (-12,-8)	0 (0,0)	-0.16 (-0.51,0.15)
NHANES		6652 (6383,6913)	3753 (3511,3968)	2112 (2024,2216)	1.76 (1.67,1.89)	184 (170,204)	491 (456,515)	40 (38,42)	1.43 (1.15,1.84)
NHANES Δ		-340 (-589,-40)	-274 (-555,-35)	30 (-48,149)	-0.17 (-0.27,-0.02)	4 (-10,27)	-34 (-64,-6)	-3 (-3,-1)	0.22 (-0.08,0.64)
POP Δ		2658 (2272,3029)	960 (648,1254)	563 (411,706)	-0.01 (-0.17,0.11)	1195 (1074,1319)	-448 (-475,-412)	-38 (-40,-36)	1.02 (0.46,1.91)
THLHP	10	9220 (8912,9550)	4680 (4536,4882)	2605 (2519,2740)	1.78 (1.7,1.91)	1378 (1260,1498)	42 (38,47)	2 (1,3)	2.51 (1.93,3.27)
THLHP Δ		26 (-299,275)	-3 (-148,192)	-107 (-230,1)	0.07 (-0.03,0.17)	-90 (-187,27)	-9 (-12,-8)	0 (0,0)	-0.18 (-0.55,0.11)
NHANES		6751 (6485,7003)	3794 (3558,4039)	2140 (2047,2238)	1.73 (1.62,1.87)	183 (171,205)	505 (476,533)	40 (38,41)	1.39 (1.13,1.83)

NHANES Δ		-230 (-489,48)	-252 (-484,16)	63 (-27,161)	-0.2 (-0.29,-0.07)	5 (-9,26)	-20 (-51,12)	-3 (-4,-1)	0.17 (-0.13,0.6)
POP Δ		2474 (2134,2977)	891 (642,1236)	465 (301,649)	0.05 (-0.1,0.19)	1191 (1076,1322)	-463 (-490,-431)	-38 (-40,-36)	1.04 (0.41,1.96)
THLHP	11	9185 (8896,9517)	4674 (4545,4889)	2583 (2497,2697)	1.81 (1.72,1.92)	1379 (1254,1485)	45 (40,49)	2 (1,3)	2.5 (1.91,3.23)
THLHP Δ		-11 (-335,250)	-6 (-150,187)	-130 (-267,-35)	0.09 (-0.01,0.2)	-77 (-207,30)	-7 (-9,-6)	0 (0,0)	-0.17 (-0.56,0.08)
NHANES		6895 (6623,7111)	3903 (3644,4148)	2160 (2060,2283)	1.74 (1.65,1.9)	183 (169,204)	519 (487,551)	40 (38,41)	1.34 (1.09,1.77)
NHANES Δ		-79 (-343,150)	-145 (-372,160)	82 (-21,188)	-0.19 (-0.29,-0.04)	5 (-10,23)	-5 (-34,29)	-3 (-4,-1)	0.11 (-0.13,0.58)
POP Δ		2304 (1917,2808)	793 (561,1146)	427 (276,594)	0.07 (-0.1,0.2)	1197 (1074,1298)	-474 (-504,-445)	-38 (-40,-36)	1.11 (0.49,1.98)
THLHP	12	9176 (8926,9519)	4694 (4501,4917)	2609 (2515,2712)	1.81 (1.72,1.93)	1411 (1248,1520)	47 (42,52)	2 (1,3)	2.52 (1.94,3.23)
THLHP Δ		-36 (-326,245)	25 (-186,219)	-119 (-219,-10)	0.1 (0,0.21)	-54 (-212,75)	-5 (-6,-3)	0 (0,0)	-0.17 (-0.54,0.07)
NHANES		7041 (6712,7306)	4010 (3773,4281)	2164 (2067,2256)	1.8 (1.69,1.94)	183 (167,202)	531 (506,565)	39 (38,41)	1.28 (1.06,1.71)
NHANES Δ		67 (-258,380)	-12 (-250,249)	86 (-6,196)	-0.13 (-0.25,0.02)	5 (-12,24)	8 (-19,43)	-3 (-4,-2)	0.05 (-0.19,0.5)
POP Δ		2172 (1806,2627)	663 (432,1004)	456 (301,585)	0 (-0.17,0.15)	1225 (1067,1342)	-484 (-514,-456)	-37 (-39,-36)	1.2 (0.51,2)
THLHP	13	9230 (8885,9560)	4717 (4500,4916)	2662 (2553,2769)	1.79 (1.69,1.91)	1432 (1241,1586)	50 (45,56)	2 (1,3)	2.53 (1.93,3.25)
THLHP Δ		-9 (-328,292)	56 (-197,218)	-64 (-159,46)	0.07 (-0.02,0.2)	-32 (-191,142)	-2 (-4,0)	0 (0,0)	-0.14 (-0.51,0.11)
NHANES		7104 (6774,7444)	4100 (3854,4428)	2151 (2073,2253)	1.87 (1.75,2.05)	182 (165,201)	538 (512,573)	39 (37,41)	1.24 (1.01,1.58)
NHANES Δ		152 (-189,497)	82 (-140,412)	69 (-19,181)	-0.05 (-0.19,0.11)	3 (-14,20)	16 (-12,48)	-3 (-4,-3)	0.03 (-0.22,0.39)
POP Δ		2129 (1647,2524)	614 (297,915)	517 (378,665)	-0.1 (-0.28,0.11)	1245 (1063,1392)	-489 (-521,-466)	-37 (-39,-35)	1.27 (0.55,1.99)

THLHP	14	9265 (8897,9647)	4734 (4538,4936)	2730 (2601,2842)	1.75 (1.66,1.87)	1455 (1257,1633)	53 (48,59)	2 (1,3)	2.59 (1.92,3.33)
THLHP Δ		47 (-310,348)	77 (-148,259)	-6 (-101,109)	0.03 (-0.06,0.14)	-18 (-160,188)	1 (-1,3)	0 (0,0)	-0.11 (-0.48,0.23)
NHANES		7122 (6822,7439)	4159 (3861,4478)	2131 (2055,2222)	1.92 (1.79,2.11)	181 (163,198)	540 (514,565)	38 (37,40)	1.25 (0.98,1.57)
NHANES Δ		136 (-164,480)	139 (-141,455)	53 (-32,148)	-0.01 (-0.15,0.18)	2 (-17,21)	19 (-15,45)	-4 (-5,-3)	0.02 (-0.24,0.33)
POP Δ		2145 (1619,2542)	599 (236,927)	607 (444,738)	-0.16 (-0.39,0.03)	1273 (1088,1454)	-489 (-513,-460)	-37 (-39,-35)	1.31 (0.66,2.06)
THLHP	15	9288 (8950,9688)	4758 (4580,4952)	2782 (2660,2911)	1.72 (1.62,1.82)	1459 (1284,1650)	56 (50,61)	2 (1,3)	2.59 (1.95,3.36)
THLHP Δ		75 (-262,400)	81 (-127,289)	55 (-69,179)	0 (-0.11,0.09)	-10 (-147,208)	3 (1,6)	0 (0,0)	-0.06 (-0.48,0.25)
NHANES		7066 (6756,7393)	4111 (3822,4441)	2125 (2035,2227)	1.91 (1.79,2.1)	179 (161,196)	536 (506,560)	38 (37,40)	1.25 (0.96,1.58)
NHANES Δ		109 (-228,440)	77 (-196,409)	50 (-53,156)	-0.02 (-0.15,0.18)	0 (-20,21)	12 (-20,41)	-4 (-5,-3)	0.03 (-0.24,0.36)
POP Δ		2221 (1704,2660)	654 (279,1026)	654 (493,825)	-0.18 (-0.38,-0.01)	1281 (1105,1466)	-480 (-507,-452)	-36 (-39,-35)	1.34 (0.55,1.99)
THLHP	16	9312 (8999,9711)	4800 (4564,4997)	2806 (2681,2943)	1.7 (1.6,1.8)	1450 (1279,1650)	58 (52,63)	2 (1,3)	2.64 (1.96,3.38)
THLHP Δ		126 (-224,435)	109 (-128,341)	82 (-50,209)	-0.01 (-0.14,0.08)	-18 (-167,177)	6 (3,9)	0 (0,0)	-0.07 (-0.49,0.26)
NHANES		7004 (6603,7388)	4045 (3709,4360)	2147 (2033,2249)	1.87 (1.72,2.06)	176 (156,198)	528 (498,556)	38 (37,40)	1.25 (0.91,1.63)
NHANES Δ		26 (-334,470)	7 (-293,356)	64 (-46,172)	-0.06 (-0.21,0.14)	-3 (-24,23)	5 (-27,36)	-4 (-5,-3)	0.02 (-0.3,0.39)
POP Δ		2327 (1749,2783)	756 (317,1115)	662 (484,875)	-0.16 (-0.35,0.02)	1273 (1105,1451)	-468 (-502,-437)	-36 (-39,-34)	1.37 (0.57,2.23)

Table 6. Estimated median values (and 95% credible intervals) for each outcome variable by breastfeeding status (Yes/No) in the USA only, months 1-16 of the postpartum period (Month PP). DELTA = difference between Yes and No point estimates. Immune cell counts are in cells/uL, CRP is in mg/L.

Month PP	BF	WBC	NEU	LYM	NLR	EOS	MON	BAS	CRP
1	No	7495 (7115,7938)	4556 (4093,4913)	1950 (1859,2026)	2.28 (2.09,2.47)	168 (149,183)	552 (511,575)	41 (39,42)	2.46 (1.98,3.45)
	Yes	7375 (6873,7839)	4256 (3889,4596)	2000 (1887,2112)	2.06 (1.87,2.29)	175 (160,201)	549 (514,576)	40 (38,42)	2.65 (2.03,3.41)
	DELTA	145 (-542,830)	298 (-219,781)	-52 (-198,78)	0.21 (-0.04,0.44)	-9 (-34,17)	2 (-42,39)	0 (-1,2)	-0.2 (-1.08,0.76)
2	No	7134 (6717,7625)	4141 (3704,4477)	2041 (1920,2147)	1.94 (1.75,2.11)	171 (152,184)	526 (484,556)	41 (39,43)	2.2 (1.73,3)
	Yes	6992 (6464,7442)	3823 (3480,4134)	2093 (1976,2253)	1.72 (1.56,1.89)	180 (165,204)	528 (487,557)	41 (40,44)	2.47 (1.86,3.31)
	DELTA	173 (-561,843)	288 (-196,770)	-56 (-263,97)	0.22 (-0.01,0.46)	-11 (-34,13)	3 (-44,46)	-1 (-3,1)	-0.22 (-1.28,0.69)
3	No	6891 (6470,7321)	3850 (3557,4195)	2114 (1973,2244)	1.75 (1.57,1.91)	173 (156,186)	505 (462,537)	40 (39,42)	2.04 (1.58,2.73)
	Yes	6737 (6188,7125)	3598 (3289,3884)	2160 (2034,2339)	1.55 (1.42,1.69)	181 (165,204)	504 (458,537)	43 (41,46)	2.3 (1.73,3.14)
	DELTA	184 (-549,861)	264 (-218,713)	-64 (-320,141)	0.18 (0.01,0.44)	-12 (-39,12)	1 (-51,54)	-2 (-5,-1)	-0.38 (-1.35,0.64)
4	No	6766 (6386,7221)	3753 (3456,4039)	2166 (2024,2309)	1.69 (1.52,1.83)	174 (158,188)	486 (444,525)	40 (39,42)	1.91 (1.46,2.57)
	Yes	6596 (6062,6924)	3488 (3200,3797)	2197 (2084,2362)	1.52 (1.39,1.65)	184 (167,214)	487 (441,522)	44 (41,46)	2.21 (1.61,2.87)
	DELTA	234 (-394,819)	264 (-199,667)	-54 (-248,167)	0.16 (-0.02,0.38)	-10 (-42,14)	3 (-50,55)	-4 (-5,-1)	-0.4 (-1.29,0.62)
5	No	6742 (6329,7110)	3725 (3441,3992)	2190 (2047,2317)	1.7 (1.53,1.82)	177 (161,193)	480 (438,514)	40 (38,42)	1.84 (1.44,2.43)
	Yes	6473 (5951,6865)	3481 (3192,3824)	2194 (2060,2338)	1.58 (1.44,1.71)	185 (166,219)	474 (429,516)	43 (41,46)	2.12 (1.51,2.87)
	DELTA	291 (-310,900)	231 (-235,636)	-3 (-187,181)	0.1 (-0.07,0.33)	-10 (-43,16)	2 (-52,59)	-3 (-6,-1)	-0.29 (-1.26,0.6)

6	No	6708 (6365,7070)	3736 (3473,3972)	2183 (2071,2294)	1.74 (1.6,1.92)	180 (164,200)	474 (433,510)	40 (38,42)	1.8 (1.44,2.48)
	Yes	6407 (5928,6837)	3534 (3185,3938)	2150 (2013,2320)	1.69 (1.49,1.89)	186 (167,218)	467 (418,511)	42 (40,45)	2.08 (1.36,2.83)
	DELTA	371 (-230,995)	208 (-272,608)	21 (-173,193)	0.04 (-0.17,0.3)	-8 (-42,20)	3 (-48,61)	-2 (-5,0)	-0.15 (-1.16,0.76)
7	No	6725 (6385,7085)	3745 (3522,3989)	2170 (2059,2288)	1.77 (1.64,1.95)	184 (169,213)	477 (433,513)	40 (38,42)	1.86 (1.47,2.39)
	Yes	6353 (5908,6803)	3583 (3255,4115)	2138 (1935,2284)	1.79 (1.57,2.03)	190 (169,224)	464 (418,511)	40 (38,44)	1.97 (1.22,2.89)
	DELTA	391 (-263,977)	144 (-374,638)	24 (-149,266)	0 (-0.33,0.25)	-6 (-44,24)	9 (-40,61)	0 (-4,3)	0.02 (-1.01,0.9)
8	No	6780 (6402,7137)	3749 (3572,3980)	2173 (2071,2286)	1.76 (1.65,1.91)	186 (171,220)	484 (448,516)	41 (39,43)	1.97 (1.55,2.49)
	Yes	6386 (5898,6752)	3624 (3298,4163)	2128 (1899,2279)	1.81 (1.59,2.1)	190 (169,224)	466 (424,510)	39 (36,43)	1.82 (1.08,2.68)
	DELTA	414 (-258,1031)	153 (-367,579)	33 (-154,308)	-0.02 (-0.42,0.21)	-5 (-46,28)	15 (-30,70)	2 (-2,5)	0.13 (-0.86,1.01)
9	No	6835 (6481,7167)	3761 (3529,4066)	2182 (2086,2285)	1.74 (1.64,1.88)	188 (173,223)	494 (464,527)	41 (40,44)	1.96 (1.63,2.5)
	Yes	6482 (5888,6890)	3666 (3287,4177)	2123 (1900,2295)	1.8 (1.58,2.1)	192 (168,228)	472 (424,510)	39 (36,43)	1.7 (1,2.6)
	DELTA	410 (-240,1105)	135 (-424,534)	57 (-164,311)	-0.06 (-0.39,0.18)	-4 (-45,28)	25 (-28,76)	3 (-1,6)	0.3 (-0.62,1.1)
10	No	6908 (6548,7254)	3809 (3607,4197)	2202 (2097,2295)	1.72 (1.63,1.87)	189 (172,220)	507 (481,538)	42 (40,44)	1.98 (1.57,2.51)
	Yes	6569 (5919,7141)	3762 (3365,4235)	2147 (1953,2306)	1.8 (1.55,2.08)	190 (167,229)	479 (427,519)	39 (36,43)	1.56 (0.93,2.3)
	DELTA	319 (-246,993)	81 (-605,585)	53 (-142,288)	-0.07 (-0.36,0.22)	-2 (-49,29)	31 (-18,91)	3 (-1,6)	0.4 (-0.42,1.15)
11	No	6991 (6662,7349)	3909 (3685,4239)	2222 (2117,2303)	1.74 (1.62,1.89)	189 (170,219)	523 (493,553)	42 (40,44)	1.96 (1.5,2.52)
	Yes	6648 (6116,7360)	3835 (3459,4348)	2158 (1974,2347)	1.8 (1.57,2.05)	190 (167,232)	489 (435,528)	40 (37,43)	1.44 (0.94,2.01)

	DELTA	289 (-449,994)	87 (-481,589)	51 (-119,295)	-0.03 (-0.35,0.22)	-2 (-44,30)	35 (-9,96)	2 (-1,6)	0.49 (-0.19,1.28)
12	No	7074 (6731,7453)	4032 (3779,4354)	2232 (2127,2334)	1.8 (1.64,1.96)	189 (169,218)	532 (505,569)	41 (40,43)	1.97 (1.48,2.4)
	Yes	6759 (6258,7632)	3913 (3549,4398)	2168 (1996,2337)	1.83 (1.57,2.12)	189 (166,235)	499 (445,544)	40 (37,43)	1.35 (0.9,1.97)
	DELTA	328 (-519,915)	120 (-443,592)	57 (-126,275)	-0.02 (-0.32,0.23)	-2 (-47,30)	35 (-26,94)	2 (-1,4)	0.6 (-0.07,1.32)
13	No	7142 (6833,7539)	4142 (3806,4475)	2235 (2127,2328)	1.87 (1.7,2.05)	188 (170,215)	537 (512,572)	41 (39,42)	1.94 (1.49,2.44)
	Yes	6836 (6241,7681)	3965 (3551,4410)	2143 (1971,2332)	1.88 (1.63,2.2)	189 (164,236)	517 (444,571)	40 (37,42)	1.25 (0.87,1.91)
	DELTA	334 (-550,966)	170 (-525,629)	77 (-112,284)	0 (-0.31,0.27)	-4 (-46,33)	23 (-36,87)	1 (-1,3)	0.66 (-0.01,1.3)
14	No	7173 (6862,7613)	4168 (3818,4499)	2233 (2132,2326)	1.9 (1.77,2.11)	187 (168,211)	536 (511,569)	40 (38,42)	1.89 (1.49,2.36)
	Yes	6856 (6314,7646)	3937 (3423,4440)	2120 (1943,2325)	1.9 (1.65,2.27)	189 (160,233)	527 (467,593)	40 (37,43)	1.17 (0.72,1.79)
	DELTA	323 (-436,1019)	230 (-454,810)	96 (-118,291)	0 (-0.33,0.32)	-4 (-45,33)	16 (-57,81)	0 (-2,3)	0.75 (-0.13,1.29)
15	No	7178 (6877,7605)	4151 (3837,4468)	2237 (2119,2344)	1.9 (1.78,2.06)	186 (167,210)	529 (498,560)	40 (38,41)	1.92 (1.48,2.29)
	Yes	6732 (6154,7530)	3801 (3240,4403)	2107 (1919,2323)	1.89 (1.58,2.19)	191 (156,240)	538 (463,610)	40 (37,43)	1.06 (0.6,1.9)
	DELTA	440 (-337,1014)	375 (-323,878)	109 (-99,351)	0.02 (-0.38,0.39)	-7 (-58,33)	-2 (-88,68)	-1 (-4,3)	0.78 (-0.15,1.43)
16	No	7180 (6837,7555)	4081 (3788,4470)	2251 (2106,2367)	1.86 (1.75,2.02)	185 (166,211)	519 (483,551)	40 (38,42)	1.87 (1.4,2.29)
	Yes	6615 (5930,7600)	3641 (3120,4325)	2118 (1865,2347)	1.8 (1.48,2.13)	192 (149,249)	551 (456,638)	41 (37,44)	1.03 (0.48,2.16)
	DELTA	603 (-598,1275)	486 (-314,1048)	126 (-109,468)	0.06 (-0.28,0.5)	-7 (-66,43)	-23 (-119,68)	-1 (-5,2)	0.78 (-0.33,1.55)

III. Investigating the effects of ecological context and female reproductive phase on sexual dimorphism in immune status

This chapter is co-authored with Amy Boddy, Aaron Blackwell, Hillard Kaplan, Ben Trumble, Jonathan Steiglitz, and Michael Gurven and utilizes data collected by the Tsimane Health and Life History Project. The author of this dissertation proposed all hypotheses tested herein. The analyses, writing, and figures contained in this chapter are the work of the author of this dissertation.

A. Introduction

Stronger immune response and lower infectious disease burden have been observed in females compared to males across numerous taxa (Zuk et al., 2004; McKean and Nunney, 2005; Nunn et al., 2009; Foo et al., 2017), although effect size varies substantially based on phylogeny (Kelly et al., 2018) and species-specific life history traits (KLEIN and NELSON, 1999; Fuxjager et al., 2011). Among mammals, in particular, there is consistent male bias in parasite burden (Poulin, 1996; Moore and Wilson, 2002). There is also evidence that males in mammalian species are more susceptible to a wide breadth of viral infections, although such findings may be skewed by predominance of data from murine models (Klein and Huber, 2010). In humans, sexual dimorphism in immune function and disease burden emerges after puberty (Klein and Flanagan, 2016), with reproductive-aged females generally mounting stronger cellular and antibody responses to many viral and bacterial pathogens (Fish, 2008; Takahashi et al., 2020b) and vaccination (MITCHELL, 1999; Klein et al., 2010, 2015; Voigt et al., 2019), and exhibiting greater neutrophil and monocyte counts (Bain, 1996), higher neutrophil-lymphocyte ratio (Chen et al., 2016), enhanced neutrophil and monocyte activation during immune challenges (García-Durán et al., 1999; Carlisle et al., 2021), higher CD4:CD8 (Amadori et al., 1995), and elevated T cell cytotoxicity (Hewagama et al., 2009).

While variation in exposure risk may contribute to divergent disease burden between

males and females (Zuk and McKean, 1996), differences in baseline immune status indicate underlying physiological mechanisms of dimorphism. There is strong evidence that sex-specific hormone production after sexual maturity drives a substantial portion of dimorphism in baseline immune function, given the dose-dependent stimulatory effects of estradiol and progesterone on female immune function and the generally suppressive effects of testosterone on male immunity (Koçar et al., 2001; Gayen et al., 2016; Taneja, 2018). In industrialized populations, at least, these immunological differences correspond to dimorphism in disease risk: women benefit from comparatively reduced infectious disease burden and lower risk of non-reproductive cancers but make up approximately 80% of autoimmune disease diagnoses (Whitacre, 2001) and suffer disproportionately from allergy and atopy (Laffont et al., 2017), indicating that there are both benefits and costs to enhanced immune vigilance.

Evolutionarily novel environmental conditions common throughout post-industrialized human societies (e.g., reduced parity and breastfeeding, increased sedentism) may exacerbate evolved sex differences in immunity, given evidence that growing up in an industrialized context with reduced constraints on energy allocation promotes higher progesterone production in females (Núñez-de la Mora et al., 2007) and higher adult testosterone levels in males (Bribiescas, 1996). To date, however, few studies have investigated sex biases in immune function between populations experiencing divergent ecological conditions. Furthermore, even in otherwise well-studied populations, data on sex bias in immune function is skewed by disproportionate sampling of non-pregnant, non-lactating females –

despite established knowledge regarding the robust hormonal changes that occur during and after pregnancy (Neville and Neifert, 1983).

In this study, we utilize data from the Tsimane Health and Life History Project (THLHP) and the National Health and Nutritional Examination Survey (NHANES) to estimate the effects of sex on neutrophil-to-lymphocyte ratio (NLR), neutrophil, total lymphocyte, eosinophil, monocyte, and total leukocyte count among the Tsimane, a natural-fertility subsistence population inhabiting the Amazonian River basin, and a representative sample from the United States. We predict that (1) sexual dimorphism will be greatest among reproductive-aged individuals in both populations, with females exhibiting greater immune cell counts and higher NLR than their male peers, and (2) the degree of sexual dimorphism will be relatively attenuated among the Tsimane at all ages. Given sex-specific hormone production patterns that emerge after puberty, regularly cycling females should exhibit divergent baseline immune status compared to males. Estradiol and progesterone levels are acutely elevated above baseline during pregnancy (Neville and Neifert, 1983) and therefore immune changes during gestation should be a primary driver of sexual dimorphism *within* each population. Conversely, given the reversal of pregnancy-induced hormonal patterns, suppression of ovulation (Neville and Neifert, 1983) and immune shifts (described in Chapter II) that occur after delivery, postpartum females may exhibit the least amount of divergence from male counterparts. To test these predictions, we estimate and compare the effects of female reproductive state (i.e., cycling, pregnancy, postpartum) on degree of sexual dimorphism among USA and Tsimane individuals. Lastly, we investigate the age-dependent effects of sex on immune status among post-menopausal females and age-matched males. Given the drop-off in female hormone production after menopause and limited evidence that

sex biases are either attenuated or reversed in later ages (Chen et al., 2016), we expect that sexual dimorphism in immune status will be reduced among post-menopausal women and age-matched men, but that increased lifetime exposure to sex hormones in the USA will result in comparatively greater magnitude of enduring sex bias.

1. The Tsimane experience ecological conditions that may temper sex hormone production in both males and females

Hormone production in both males and females is sensitive to environmental inputs, reflecting trade-offs between reproductive effort and other energetically costly demands (e.g., growth). Energetic stress via infection, physical exertion, nutritional deficiency, or extended lactation suppresses ovarian function in females, resulting in lower progesterone and estradiol production (Jasienska and Ellison, 1998; Núñez-de la Mora et al., 2007; Valeggia and Ellison, 2009), and dampens testosterone production in males (Muehlenbein, 2006; Schroeder et al., 2021). Given the role of environmental inputs in shaping energy allocation, the Tsimane are likely to experience tight constraints on hormone production and correspondingly reduced risk of escalating downstream differences in immune function and disease morbidity following the onset of puberty.

As described in Chapter II, the Tsimane are a relatively small subsistence population inhabiting the Bolivian Amazonian River basin. Among the Tsimane, chronic exposure to diverse pathogens causes high infectious disease morbidity and mortality across all ages (Martin et al., 2013; Kaplan et al., 2015; Gurven et al., 2019), while incidence of allergies, atopy, autoimmune disease, obesity, and atherosclerosis is low (Gurven et al., 2007a, 2008; Kaplan et al., 2017). As a result of elevated pathogen burden, Tsimane individuals exhibit high levels of immune activation, including elevated erythrocyte sedimentation rate, greater

total leukocyte, neutrophil, lymphocyte, eosinophil, B cell, and natural killer cell counts, and higher antibody levels compared to Western clinical standards (Blackwell et al., 2011, 2016b), while basophil and monocyte counts are regularly lower among the Tsimane (likely due to increased recruitment into tissues during infection) (Blackwell et al., 2016b). Such high investment in immune function results in trade-offs with growth during development (Foster et al., 2005) and high resting metabolic rate during adulthood (Gurven et al., 2016), reflecting the substantial energetic demands of coping with pathogenic threats. Due to limited use of contraception and breastfeeding alternatives, Tsimane women have an average 9 live births over the reproductive lifespan (McAllister et al., 2012) and exhibit nearly ubiquitous rates of on-demand breastfeeding following parturition, with a mean infant weaning age of 27 months (Martin et al., 2016). Circulating testosterone production among Tsimane men has already been found to be substantially lower than in the USA, presumably as a result of sustained pathogen exposure and reduced energy budget (Trumble et al., 2012) as well as high levels of parental investment, which has been shown to reduce testosterone production (Gray et al., 2004; Muller et al., 2009; Alvarado et al., 2019). Sex hormone production in Tsimane women has not yet been as closely evaluated, but there is strong evidence from other populations that female sex hormone production is reduced when energetic stressors (e.g., increased physical activity, infection) are present (Jasienska and Ellison, 2004; Núñez-de la Mora et al., 2007).

By comparison, in the United States public health measures have substantially reduced infectious disease burden (Armstrong et al., 1999; Roush, 2007) while microbial deprivation, sedentism, altered diet, and other keystones of industrialized life have been linked to compromised immune calibration and the rise of chronic inflammatory disorders (e.g., atopy,

autoimmune disease) (Bloomfield et al., 2016). Obesity, which has reached epidemic proportions in the USA, also contributes to earlier age at menarche and elevated long-term production of estradiol in females (Emaus et al., 2008; Al-Awadhi et al., 2013) and delays male puberty (Kaplowitz, 1998), while access to birth control and breastfeeding alternatives has dramatically altered female reproductive strategies. According to data from the Centers for Disease Control (CDC), the average fertility rate among US women in 2020 was 1.78 births per woman and, as reported in Chapter II, few mothers in the USA sustain long-term, on-demand, at-the-nipple breastfeeding. Relatively high energetic balance among women in post-industrial populations also results in a faster return to cycling, even among mothers who exclusively breastfeed (Valeggia and Eellison, 2009).

B. Materials and Methods

For this study, both THLHP and NHANES datasets were limited to males and females with recorded white blood cell differential, age, and body mass index (BMI). No exclusions were based on medical diagnoses. To create functional age groups based on female reproductive status, we created a variable called Reproductive Category with three levels: "pre-pubertal", "reproductive", and "post-reproductive". Female individuals were assigned to one of these levels, based on reported reproductive status. Age-matched males were then assigned to each level, using propensity score matching via the *matchit* package in R (Ho et al., 2007). Within the "reproductive" group, women were further separated by reproductive phase (i.e., cycling, pregnant, or postpartum). Based on our findings from Chapter II, we limited our postpartum sample to women who were within a year of delivery, since our findings indicated that most immunological measures overlap with cycling baselines by 12 months after parturition. Table 1 contains descriptive statistics for all reproductive categories

across both datasets. Table 2 provides descriptive statistics by reproductive phase in both populations.

1. Tsimane Health and Life History Project (THLHP)

THLHP data, which were collected between 2004 and 2014 by the Tsimane Health and Life History Project (http://tsimane.anth.ucsb.edu/index.html) (Blackwell et al., 2011, 2015, 2016b; Martin et al., 2013; Gurven et al., 2017), were mixed cross-sectional and longitudinal. Approval by the Gran Consejo Tsimane and by institutional review boards at the University of California, Santa Barbara (UCSB) and the University of New Mexico (UNM) was obtained before any data were collected. Informed consent was obtained from participants during a community-wide meeting open to all Tsimane residents and again at the individual level before each medical visit and interview. In the case of minors, parental consent was given before data were collected. Total leukocyte count was obtained via venous blood draws and determined with a QBC Autoread Plus dry hematology system (QBC Diagnostics). Relative fractions of neutrophils, eosinophils, lymphocytes, basophils, and monocytes were then measured manually by microscopy with a hemocytometer. Females who had not yet reached menarche were assigned to the "pre-pubertal" group, those who were currently cycling, pregnant, or within the first 12 months of the postpartum period were assigned to the "reproductive" group, and those who were no longer regularly cycling due to menopause were placed in the "post-reproductive" group.

2. National Health and Nutrition Examination Survey (NHANES)

NHANES data (https://www.cdc.gov/nchs/nhanes/index.htm), collected between 2003 and 2016, were exclusively cross-sectional. Total and differential leukocyte counts were

measured using the Coulter method. Females who were under the age of 8 (for which reproductive data were redacted) or reported absence of menarche were assigned to the "pre-pubertal" group. Females ages 8 and above who were currently cycling, pregnant, or within the first 12 months of the postpartum period were assigned to the "reproductive" group, and females who were no longer regularly cycling due to menopause were placed in the "post-reproductive" group. Within the "reproductive" group, females who were not currently pregnant and had reported a regular menstrual cycle either at time of exam or within the preceding two months were binned as cycling, while those who self-reported being pregnant or had a positive urine test at the time of exam were categorized as currently pregnant. Those who were not currently pregnant but had given birth within the past 12 months were considered postpartum.

3. Statistical Analyses

All primary models were executed in R 4.1.2 (https://cran.r-project.org) using the *brms* package (Bürkner, 2017). We employed Bayesian multilevel models to estimate the population-specific age-dependent effects of sex on neutrophil-to-lymphocyte ratio (NLR), neutrophil, total lymphocyte, eosinophil, monocyte, and total leukocyte count within each reproductive category: "pre-pubertal", "reproductive", and "post-reproductive". Within the "reproductive" subset, we also modeled the population-specific age-dependent effects of reproductive phase (cycling, pregnant, postpartum). All models accounted for the population-level effects of body mass index (BMI). Due to repeat sampling, THLHP-specific models also accounted for the group-level effects of participant identification number. Since outcome variable distributions differed, each was modeled separately. THLHP and NHANES total leukocyte, neutrophil, lymphocyte count and NLR were all log-transformed and modeled

using gaussian distributions. Zero-inflated models were used to estimate raw THLHP monocyte count. Both raw THLHP and NHANES eosinophil counts were modeled using a negative binomial distribution. NHANES monocyte count was modeled using non-log-transformed data and gaussian distribution.

C. Results

1. Minimal pre-pubertal sexual dimorphism in immune cell counts, with exception of monocytes and eosinophils in USA

Within the pre-pubertal group, predicted total leukocyte counts did not vary substantially by sex in either population (Figure 1A, Table 3). In the USA, total leukocyte count among pre-pubertal females was only 0.23% (95% CI = -2.02%, 2.86%) lower than males, while leukocyte count among Tsimane females was 0.33% (95% CI = -4.37%, 1.91%) higher than age-matched males. As shown in Figure 2A, Figure 3A, and Figure 4A, there were similarly negligible sex differences in neutrophil count, total lymphocyte count, and NLR within the pre-pubertal group in both populations. Neutrophil counts among pre-pubertal USA and Tsimane females were higher than males by an estimated 1.27% (95% CI = -0.98%, 5.86%) and 3.17% (95% CI = -0.57%, 11.76%), respectively. As shown in Figure 2A, this very slight female bias in neutrophil count was age-dependent in both populations, with sex bias increasing across age. In the USA, total lymphocyte count among pre-pubertal females was 78.10 cells/uL (95% CI = 1.93, 198.10) and 2.71% (95% CI = 0.05%, 9.23%) higher than age-matched males. Sex bias in total lymphocyte count among the Tsimane was also small, but in the opposite direction: total lymphocyte count among pre-pubertal females was 2.39% (95% CI = -0.81%, 17.29%) lower than age-matched males. In both populations, these sex

differences were age-dependent, becoming more notable as age increased within the pre-pubertal group (Figure 3A). Sex differences in NLR within the pre-pubertal group were small in both the Tsimane and USA. In the USA, age-standardized median NLR among females was 4.11% (95% CI = -4.51%, 17.23%) higher than males; among the Tsimane, median NLR was 1.90% (95% CI = -16.28%, 46.21%) higher in females compared to age-matched males. While there were no substantial sex differences in eosinophils and monocytes among Tsimane individuals, pre-pubertal females in the USA exhibited 17.72% (95% CI = -0.30%, 38.93%) fewer eosinophils and 5.29% (95% CI = 2.21%, 11.21%) fewer monocytes than their age-matched male counterparts (Figure 5, Figure 6).

2. In the USA, post-pubertal sexual dimorphism in immune status varies by female reproductive status

For most immune measures, robust sex differences were observed among reproductive-age individuals in the USA. Compared to their age-matched male peers, reproductive-age females in the USA possessed higher neutrophil counts (Δ 16.49%; 95% CI = 5.74%, 24.48%), elevated NLR (Δ 18.46%; 95% CI = 5.83%, 25.33%), and greater total leukocyte counts (Δ 8.77%; 95% CI = 2.93%, 16.45%), but lower eosinophil (Δ -23.01%; 95% CI = -47.12%, -3.07%, and monocyte counts (Δ -5.58%; 95% CI = -15.46%, -0.07%). Conversely, age-standardized sex bias in total lymphocyte counts was marginal (Δ -0.55%; 95% CI = -8.31%, 3.78%). As shown in Figure 1A and Figure 2A, higher neutrophils and total leukocyte count among females with age, but lower levels with age among males resulted in much greater sex bias with age in neutrophil and total lymphocyte count.

When broken down by female reproductive phase, the effects of pregnancy on immune measures are profound. Neutrophils among cycling females were 6.86% (95% CI = 1.88%,

15.75%) higher than in males, while neutrophils among pregnant females were elevated above males by 39.15% (95% CI = 18.19%, 54.08%). Conversely, neutrophils among postpartum females in the USA were not substantially different from age-matched males (Δ -1.68%; 95% CI = -12.78%, 14.88%) (Figure 2B, Table 4). Similarly, total leukocyte count among cycling females was 3.97% (95% CI = 1.12%, 9.72%) higher than males, while pregnant females had counts that were 24.08% (95% CI = 9.43%, 37.74%) elevated above males. Conversely, total leukocyte counts among postpartum females and age-matched males were nearly identical (Δ -0.95%; 95% CI = -8.83%, 5.36%). Sex bias in NLR was also largest between pregnant females and age-matched males (Δ 43.52%; 95% CI = 24.27%, 54.33%) with no sizeable differences between males and cycling females (Δ 4.85%; 95% CI = -7.16%, 14.51%) or postpartum females (Δ -7.66%; 95% CI = -56.69%, 18.78%). While neutrophils, lymphocytes, and leukocytes were similar with age among males, cycling females, and postpartum females, age was positively associated with neutrophil and total leukocyte count and negatively associated with total lymphocyte count among pregnant females (Figure 1B, Figure 2B, Figure 3B). When broken down by reproductive phase, eosinophil counts were 17.88% (95% CI = 2.69%, 36.64%), 39.52% (95% CI = -5.31%, 121.49%,) and 14.17% (95% CI = -32.60%, 74.37%) higher in cycling, pregnant, and postpartum females compared to age-matched males, respectively (Table 5B, Table 4). Monocyte counts were 7.05% (95% CI = 0.79%, 20.82%) and 17.92% (95% CI = 2.81%, 52.42%) lower in cycling and postpartum females compared to age-matched males, respectively, while monocyte counts were 5.38% (95% CI = -1.61%, 17.52%) higher than males among pregnant females (Figure 6B, Table 4).

3. Among the Tsimane, comparatively attenuated effects of sex and age across most immune markers after onset of puberty

In contrast to the USA, total leukocyte counts among reproductive-age Tsimane females were 3.78% (95% CI = 0.31%, 12.92%) lower than age-matched males (Figure 1A, Table 3). Compared to their age-matched male peers, reproductive-age Tsimane females possessed marginally lower neutrophil counts (Δ -1.49%; 95% CI = -11.36%, 2.05%) and lower total lymphocyte counts (Δ -4.89%; 95% CI = -18.35%, -0.77%). Overall, NLR was slightly higher among reproductive-age females (Δ 7.88%; 95% CI = -19.07%, 29.58%). However, Figure 4A shows that this sex bias in NLR is highly dependent on age (due to positive association between age and NLR among males), with greater dimorphism present at younger ages. A similar pattern was observed for eosinophil and monocyte counts, wherein overall eosinophil and monocyte counts were slightly lower among females specifically due to high levels of dimorphism at younger ages while age had opposing effects on eosinophil and monocyte prevalence in males and females.

When broken down by female reproductive phase, neutrophil count among cycling females was 6.86% (95% CI = 1.88%, 15.75%) higher than males, while neutrophil count among pregnant females was elevated above males by 39.15% (95% CI = 18.19%, 54.08%). Conversely, neutrophil count among postpartum females was not substantially different from age-matched males (Δ -1.68%; 95% CI = -12.78%, 14.88%) (Figure 2B, Table 4). Similarly, total leukocyte count among cycling females was 3.97% (95% CI = 1.12%, 9.72%) higher than males, while pregnant females had counts that were 24.08% (95% CI = 9.43%, 37.74%) elevated above males. Conversely, total leukocyte counts among postpartum females and age-matched males were nearly identical (Δ -0.95%; 95% CI = -8.83%, 5.36%). Sex bias in

NLR was also largest between pregnant females and age-matched males (Δ 43.52%; 95% CI = 24.27%, 54.33%) with no sizeable differences between males and cycling females (Δ 4.85%; 95% CI = -7.16%, 14.51%) or postpartum females (Δ -7.66%; 95% CI = -56.69%, 18.78%). While age had little effect on neutrophil, total lymphocyte, or total leukocyte count among males, cycling females, or postpartum females, age had strong positive effects on neutrophil and total leukocyte count and strong negative effects on total lymphocyte count among pregnant females (Figure 1B, Figure 2B, Figure 3B). When broken down by reproductive phase, eosinophil counts were 17.88% (95% CI = 2.69%, 36.64%), 39.52% (95% CI = -5.31%, 121.49%,) and 14.17% (95% CI = -32.60%, 74.37%) higher in cycling, pregnant, and postpartum females compared to age-matched males, respectively (Table 5B, Table 4). Monocyte counts were 7.05% (95% CI = 0.79%, 20.82%) and 17.92% (95% CI = 2.81%, 52.42%) lower in cycling and postpartum females compared to age-matched males, respectively, while monocyte counts were 5.38% (95% CI = -1.61%, 17.52%) higher than males among pregnant females (Figure 6B, Table 4). As shown in Figure 6B, these effects varied by age.

4. Post-reproductive attenuation/reversal of sex biases observed in both USA and Tsimane

Within the post-reproductive group, sex biases in total leukocyte count were opposite to those observed in the reproductive group in the USA (Δ -2.59%; 95% CI = -6.30%, -0.34%) and largely absent among the Tsimane (Δ -0.29%; 95% CI = -9.55%, 5.14%) (Figure 1A, Table 3), a pattern driven largely by the same population-specific effects of sex on neutrophil count (Figure 2A, Table 3). Conversely, a post-reproductive reversal of sex bias in total lymphocyte count was observed in both populations. Within the post-reproductive group,

total lymphocyte count among post-reproductive females was 6.42% (95% CI = 0.87%, 14.93%) and 5.10% (95% CI = 1.07%, 11.44%) higher than age-matched males among Tsimane and USA individuals, respectively. Consequently, NLR among post-reproductive USA and Tsimane females was 16.54% (95% CI = 1.01%, 27.37%) and 16.31% (95% CI = -19.86%, 88.06%) lower than age-matched males, respectively. Within the USA, eosinophil and monocyte counts were 19.75% (95% CI = 5.98%, 34.36%) and 11.05% (95% CI = 6.94%, 14.73%) lower among post-reproductive females compared to age-matched males. Among the Tsimane, there were no differences in eosinophils between males and females in the post-reproductive group. Among the Tsimane, the estimated difference between monocyte count among all post-reproductive women compared to monocyte count among all age-matched males was minimal, with a wide credible interval (Δ -37.37%; 95% CI = -896.71%, -79.18%); as shown in Figure 6A, there were substantial of age on sex bias in monocyte count, with monocyte counts higher in females at younger ages and lower in females at older ages.

D. Discussion

As predicted, we found that, in both populations and for most immune biomarkers, there were minimal differences between pre-pubertal males and females who had not yet reached menarche – highlighting the roles that sexual maturation and differential sex hormone production play in producing phenotypic variation in immune status. Among reproductive-age individuals in the USA, we observed very strong female bias in neutrophil count, NLR, and total leukocyte count, and slight male bias in total lymphocyte count, increasing with age. In contrast, there was markedly less sexual dimorphism in neutrophil, NLR, and total leukocyte count among the Tsimane, with reproductive-age females possessing *lower*

neutrophil and total leukocyte counts than males at older ages. In the USA, sexual dimorphism in NLR, neutrophil, lymphocyte, and total leukocyte count was greatest between males and pregnant females and often insignificant between males and postpartum females. While the effects of female reproductive phase on sex bias were comparatively attenuated among the Tsimane, a similar pattern was observed for total lymphocytes (wherein male bias in total lymphocyte count was greatest when compared to pregnant females). Finally, we found that the effects of sex observed among reproductive-age individuals were reversed in the post-reproductive group in both populations, highlighting the cumulative effects of menopause and immune senescence. There were two unexpected deviations from these general trends: in the USA, both eosinophil and monocyte counts were higher in males than in females, regardless of reproductive category or age. Conversely, there was very little dimorphism in eosinophil count among Tsimane individuals by reproductive category or age, with a male bias only observed in reproductive-age individuals under the age of 30. Among the Tsimane, there was no sex bias in monocytes counts before puberty, with periods of both female and male bias in monocytes counts among reproductive and post-reproductive individuals due to opposing effects of age. In sum, these patterns suggest that the direction and timing of sex biases in eosinophil and monocyte count are especially variable across populations and may be primarily regulated by mechanisms other than hormonal differences.

Taken together, our findings provide strong evidence that the magnitude of sexual dimorphism in immune status is comparatively elevated in post-industrial populations. We also show that, within populations, pregnancy exacerbates most sex biases while the postpartum period is characterized by attenuated or ameliorated sexual dimorphism in immune profiles. In the USA, the effects of pregnancy were far more extreme than among

the Tsimane, echoing previous findings that neutrophil expansion, leukocytosis, and elevated NLR during pregnancy is particularly robust among USA women (Chapter II) (Hové et al., 2020). These patterns suggest that the comparatively attenuated degree of overall sexual dimorphism in immune outcomes among the Tsimane relates to (1) different immunological response to pregnancy and (2) a greater proportion of pregnant and postpartum/lactating women across the reproductive lifespan, due to higher parity. Further research is needed to elucidate potential mechanisms driving these observations. Do women in the USA and other post-industrial environments undergo larger spikes in estradiol and progesterone production during pregnancy compared to the Tsimane? If so, is this primarily due to fewer energetic constraints? Or are other mechanisms, such as less calibrated immunological response to fetal antigens, playing an underappreciated role?

We recommend that future research seeking to elucidate these pathways focus especially on the role of neutrophils. In addition to driving sexual dimorphism in total leukocyte count and NLR between pregnant females and age-matched males, neutrophils were the only immune cell type that we found to be substantially elevated among *cycling* females compared to age-matched males – a pattern we observed only in the USA. This suggests that neutrophils play an outsized role in shaping the enhanced responsivity, greater inflammatory capacity, and increased risk for autoimmune disease reported for women living in industrialized societies. Specifically, we recommend that future research focus on potential sex differences in neutrophil phenotype (e.g., estradiol hormone receptor density, oxidative bursts, release of extracellular traps) across ecologically diverse populations. As populations across the world continue to undergo many of the socio-ecological shifts often accompanying industrialization (e.g., reduced fertility, increased sedentism, reduced energetic constraints) a

better understanding of the consequences of such changes on immune function will become increasingly imperative.

Chapter III: Figures and Tables

Figure 1. Panel A = predicted total leukocyte count by age and sex by reproductive category (Prepubertal, Reproductive, and Post-reproductive) and population (THLHP, NHANES). B = predicted total leukocyte count by reproductive phase and population.

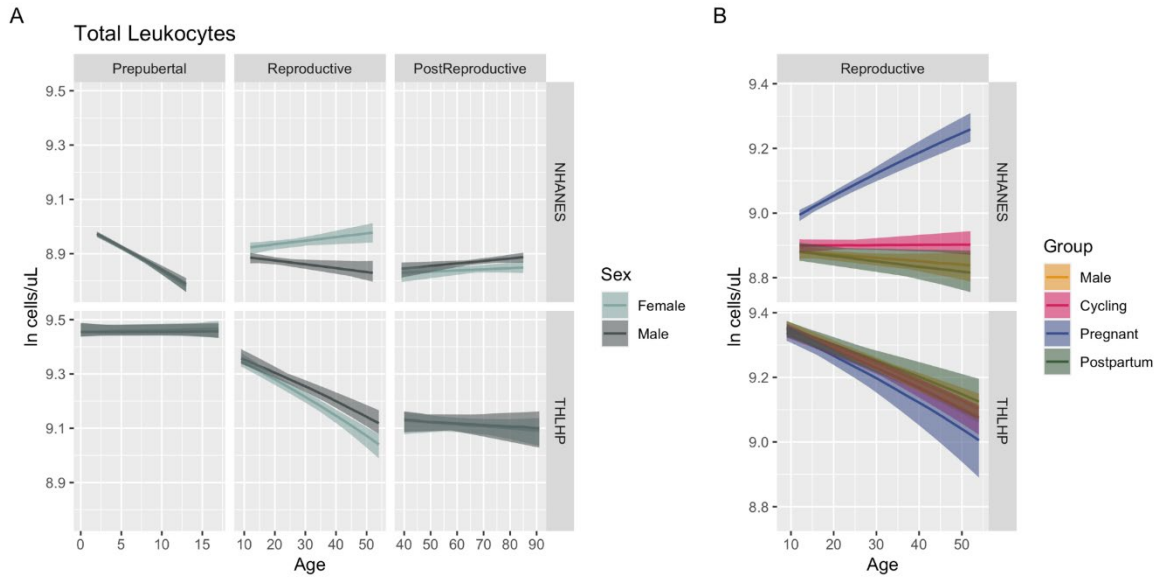


Figure 2. Panel A = predicted neutrophil count by age and sex by reproductive category (Prepubertal, Reproductive, and Post-reproductive) and population (THLHP, NHANES). B = predicted neutrophil count by reproductive phase and population.

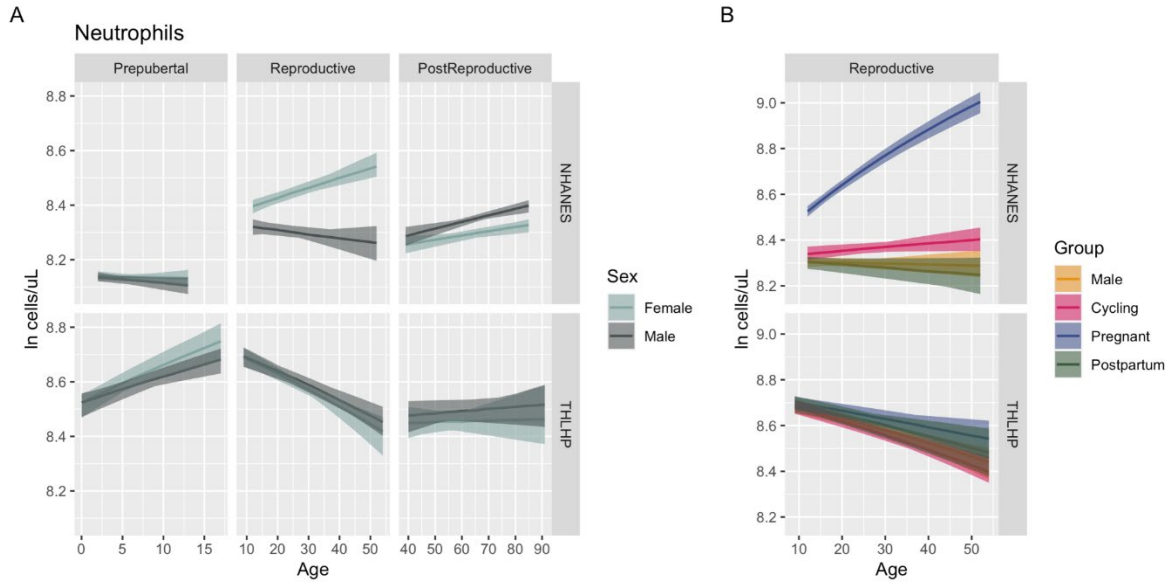


Figure 3. Panel A = predicted total lymphocyte count by age and sex by reproductive category (Prepubertal, Reproductive, and Post-reproductive) and population (THLHP, NHANES). B = predicted total lymphocyte count by reproductive phase and population.

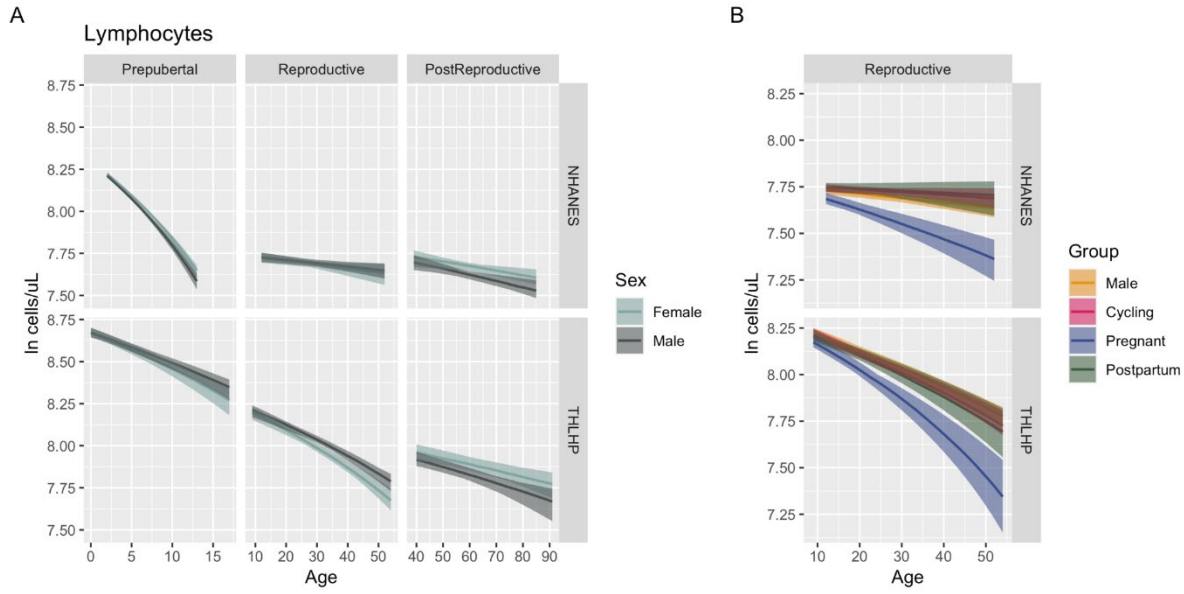


Figure 4. Panel A = predicted NLR by age and sex by reproductive category (Prepubertal, Reproductive, and Post-reproductive) and population (THLHP, NHANES). B = predicted NLR by reproductive phase and population.

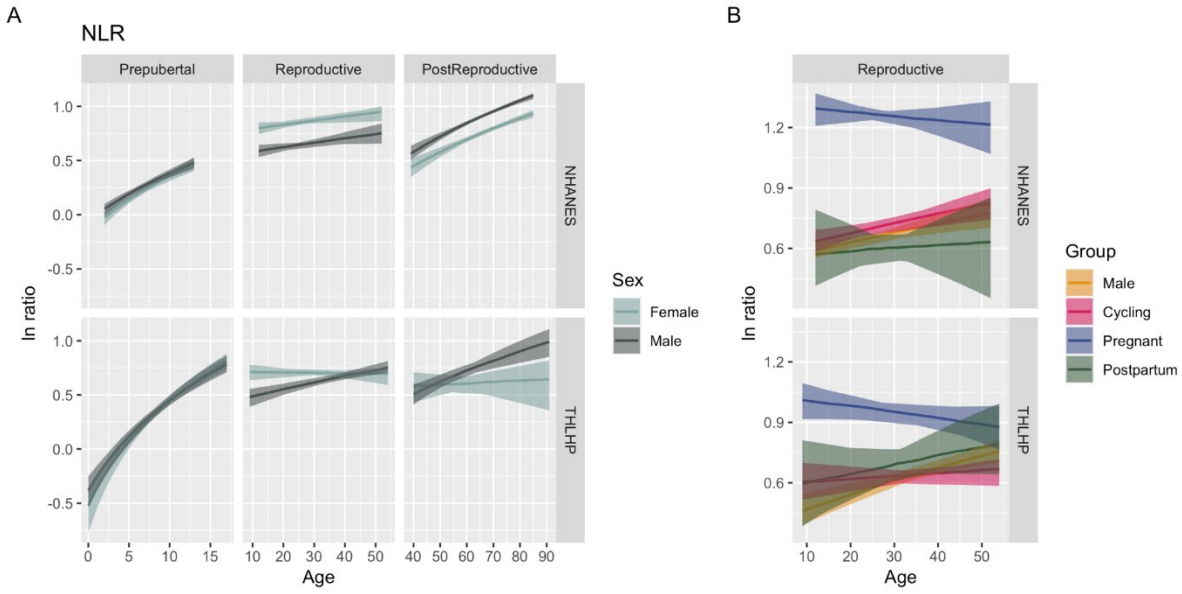


Figure 5. Panel A = predicted eosinophil count by age and sex by reproductive category (Prepubertal, Reproductive, and Post-reproductive) and population (THLHP, NHANES). B = predicted eosinophil count by reproductive phase and population.

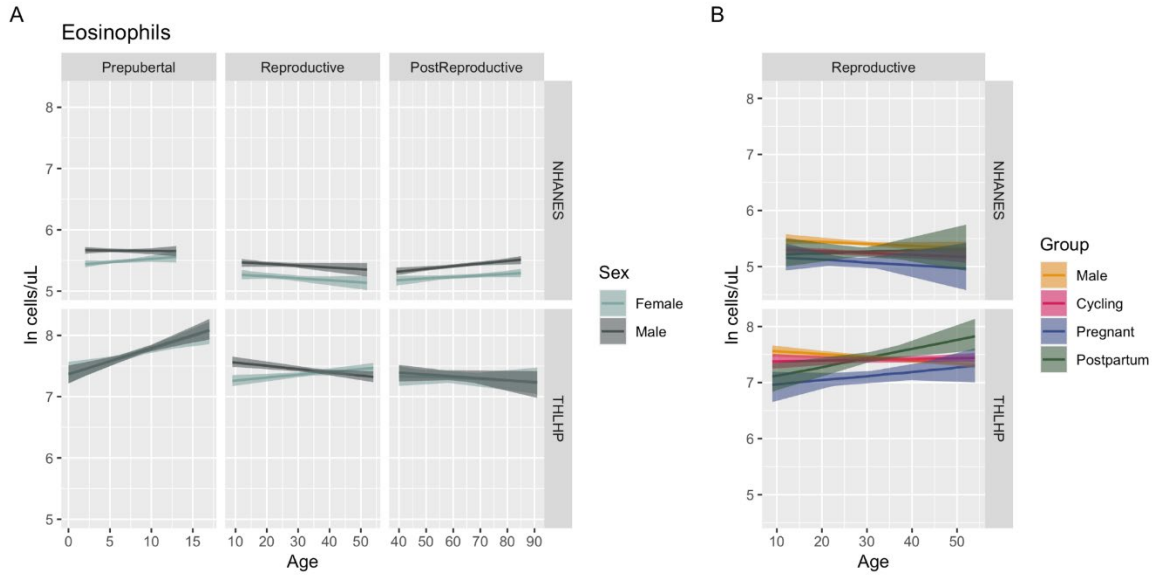


Figure 6. Panel A = predicted monocyte count by age and sex by reproductive category (Prepubertal, Reproductive, and Post-reproductive) and population (THLHP, NHANES). B = predicted monocyte count by reproductive phase and population.

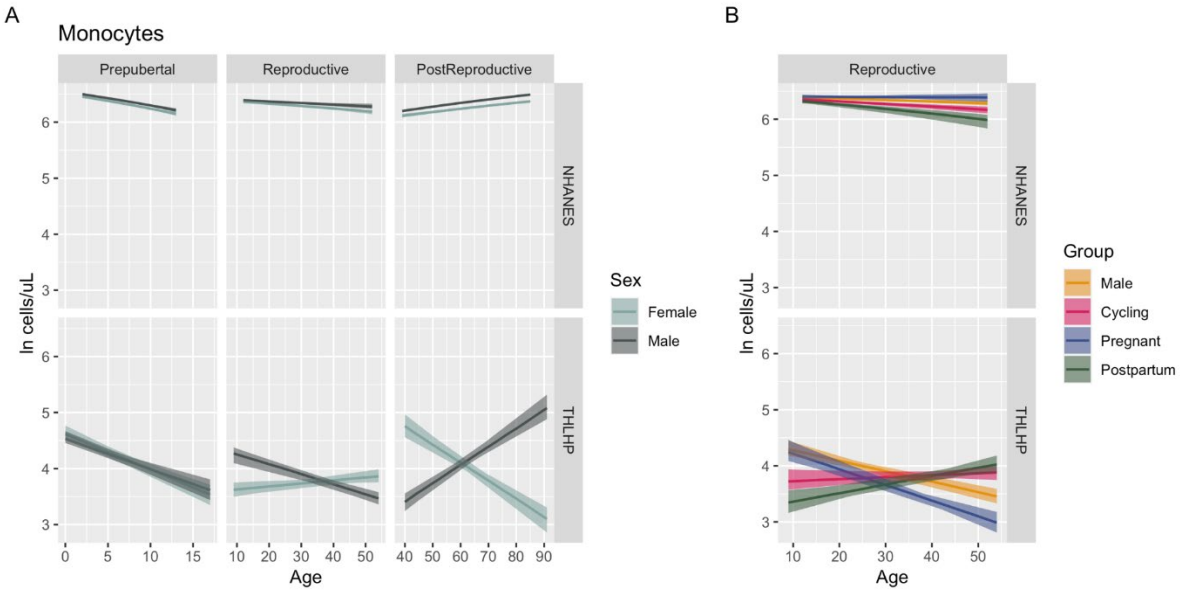


Table 2. Descriptive statistics by population, reproductive category, and sex. N = total sample size; RN = number of repeat samples; Age and BMI = median and range for participant age and body mass index.

Population	Reproductive Category	Sex	N	RN	Age	BMI
NHANES	Pre-pubertal	Female	5430	0	7.00 (2.00,13.00)	16.73 (11.74,46.10)
		Male	5430	0	7.00 (2.00,13.00)	16.73 (11.98,54.44)
	Reproductive	Female	2618	0	25.00 (12.00,52.00)	26.19 (14.09,80.60)
		Male	2618	0	25.00 (12.00,52.00)	25.76 (13.43,130.21)
	Post-reproductive	Female	6873	0	64.00 (39.00,85.00)	28.86 (13.18,84.40)
		Male	6873	0	64.00 (39.00,85.00)	28.00 (14.20,130.21)
THLHP	Pre-pubertal	Female	1310	295	7.00 (0.00,17.00)	16.43 (10.29,28.52)
		Male	1310	277	7.00 (0.00,17.00)	16.59 (10.30,24.68)
	Reproductive	Female	1968	877	37.00 (11.00,54.00)	23.61 (16.53,37.25)
		Male	1968	842	36.00 (9.00,54.00)	22.79 (12.88,31.55)
	Post-reproductive	Female	1154	768	59.00 (40.00,91.00)	22.89 (15.28,36.73)
		Male	1154	731	59.00 (40.00,90.00)	23.58 (17.22,31.59)

Table 2. Descriptive statistics by population and female reproductive phase. N = sample size; RN = number of repeat samples; Age and BMI = median and range for participant age and body mass index.

Population	Female Reproductive Phase	N	RN	Age	BMI
NHANES	Pre-menarche	5430	0	7.00 (2.00,13.00)	16.73 (11.74,46.10)
	Cycling	1492	0	19.00 (12.00,52.00)	24.69 (14.09,76.07)
	Pregnant	665	0	27.00 (15.00,41.00)	28.30 (15.81,80.60)
	Postpartum	461	0	28.00 (20.00,42.00)	27.40 (15.64,73.43)
	Menopause	6873	0	64.00 (39.00,85.00)	28.86 (13.18,84.40)
THLHP	Pre-menarche	1310	295	7.00 (0.00,17.00)	16.43 (10.29,28.52)
	Cycling	1441	600	39.00 (11.00,50.00)	23.47 (16.53,36.53)
	Pregnant	309	43	32.00 (13.00,54.00)	23.96 (17.50,37.25)
	Postpartum	218	11	31.00 (12.00,54.00)	23.80 (16.67,35.89)
	Menopause	1154	768	59.00 (40.00,91.00)	22.89 (15.28,36.73)

Table 3. Estimated differences between all age-matched males and females, standardized by reproductive category-specific median BMI (pooled across population and sex), separated by population and outcome variable. Absolute delta = raw difference between predicted values for males and females. % Delta = percent difference between predicted values for males and females.

Measure	Population	Reproductive Category	Absolute Delta (Female – Male)	% Delta (Female – Male)
WBC	NHANES	Pre-puberty	-16.83 (-182.64,143.18)	-0.23% (-2.86%,2.20%)
	THLHP		42.18 (-242.11,580.05)	0.33% (-1.91%,4.37%)
	NHANES	Reproductive	675.50 (218.22,1340.09)	8.77% (2.93%,16.45%)
	THLHP		-377.54 (-1089.96,-35.03)	-3.78% (-12.92%,-0.31%)
	NHANES	Post-Reproductive	-178.71 (-435.54,-23.57)	-2.59% (-6.30%,-0.34%)
	THLHP		-25.83 (-821.91,455.03)	-0.29% (-9.55%,5.14%)
NEU	NHANES	Pre-puberty	43.21 (-32.46,202.34)	1.27% (-0.98%,5.86%)
	THLHP		181.55 (-29.12,759.32)	3.17% (-0.57%,11.76%)
	NHANES	Reproductive	787.18 (254.39,1466.06)	16.49% (5.74%,28.48%)
	THLHP		-79.61 (-497.36,93.88)	-1.49% (-11.36%,2.05%)
	NHANES	Post-Reproductive	-208.76 (-392.91,-59.00)	-5.23% (-9.63%,-1.52%)
	THLHP		-191.78 (-600.00,164.00)	-4.09% (-13.88%,3.35%)
LYM	NHANES	Pre-puberty	78.10 (1.93,198.10)	2.71% (0.05%,9.23%)
	THLHP		-117.00 (-618.27,34.00)	-2.39% (-17.29%,0.81%)
	NHANES	Reproductive	-11.96 (-159.87,80.01)	-0.55% (-8.31%,3.78%)
	THLHP		-142.14 (-385.71,-28.10)	-4.89% (-18.35%,-0.77%)
	NHANES	Post-Reproductive	109.17 (23.89,238.63)	5.10% (1.07%,11.44%)
	THLHP		167.45 (23.93,354.63)	6.42% (0.87%,14.93%)
EOS	NHANES	Pre-puberty	-43.23 (-76.60,0.80)	-17.72% (-33.62%,0.30%)
	THLHP		-26.06 (-1092.85,538.46)	-1.20% (-38.93%,19.66%)
	NHANES	Reproductive	-42.06 (-75.17,-5.86)	-23.01% (-47.12%,-3.07%)
	THLHP		-133.67 (-729.91,438.22)	-8.46% (-55.59%,23.08%)
	NHANES	Post-Reproductive	-37.24 (-65.80,-11.13)	-19.75% (-34.36%,-5.98%)
	THLHP		-34.11 (-337.15,378.06)	-2.29% (-27.74%,23.68%)
MON	NHANES	Pre-puberty	-29.73 (-51.42,-12.81)	-5.29% (-11.21%,-2.21%)
	THLHP		0.41 (-8.48,24.91)	0.73% (-26.76%,21.18%)
	NHANES	Reproductive	-29.83 (-73.33,-0.44)	-5.58% (-15.46%,-0.07%)
	THLHP		-6.31 (-42.70,22.53)	-14.98% (-124.20%,41.79%)
	NHANES	Post-Reproductive	-57.50 (-86.28,-31.93)	-11.05% (-14.73%,-6.94%)
	THLHP		-18.99 (-182.70,110.70)	-37.37% (-896.71%,79.18%)
NLR	NHANES	Pre-puberty	-0.05 (-0.16,0.07)	-4.11% (-17.23%,4.51%)
	THLHP		-0.03 (-0.22,0.26)	-1.90% (-46.21%,16.28%)
	NHANES	Reproductive	0.44 (0.14,0.66)	18.46% (5.83%,25.33%)
	THLHP		0.16 (-0.34,0.63)	7.88% (-19.07%,29.58%)
	NHANES	Post-Reproductive	-0.34 (-0.57,-0.02)	-16.54% (-27.37%,-1.01%)
	THLHP		-0.31 (-1.26,0.39)	-16.31% (-88.06%,19.86%)

Table 4. Estimated differences between all age-matched males and females, standardized by reproductive category-specific median BMI (pooled across population and sex), separated by population and outcome variable. Absolute delta = raw difference between predicted values for males and females. % Delta = percent difference between predicted values for males and females.

Measure	Population	Cycling (Female – Male)		Pregnant (Female – Male)		Postpartum (Female – Male)	
		Absolute Delta	% Delta	Absolute Delta	% Delta	Absolute Delta	% Delta
WBC	NHANES	291.74 (81.87,732.68)	3.97% (1.12%,9.72%)	2230.78 (753.96,4035.07)	24.08% (9.43%,37.74%)	-66.48 (-570.86,371.29)	-0.95% (-8.83%,5.36%)
	THLHP	-130.59 (-682.76,313.14)	-1.27% (-8.07%,3.51%)	-463.87 (-1621.41,-53.19)	-4.76% (-22.34%,-0.47%)	72.85 (-523.38,1092.10)	0.69% (-6.03%,11.27%)
NEU	NHANES	295.51 (79.01,713.86)	6.86% (1.88%,15.75%)	2580.75 (916.98,4544.45)	39.15% (18.19%,54.08%)	-66.59 (-454.22,160.56)	-1.68% (-12.78%,4.07%)
	THLHP	-100.63 (-551.39,319.56)	-1.91% (-12.74%,6.76%)	246.97 (-16.05,1057.46)	4.45% (-0.34%,19.57%)	85.78 (-385.43,751.56)	1.56% (-8.86%,14.88%)
LYM	NHANES	66.60 (-15.94,225.48)	2.95% (-0.70%,9.81%)	-290.85 (-703.88,-60.97)	-15.48% (-49.58%,-2.78%)	84.85 (-69.37,363.68)	3.74% (-3.49%,15.22%)
	THLHP	-64.40 (-316.30,200.44)	-2.11% (-14.63%,8.16%)	-481.02 (-1132.28,-111.66)	-18.85% (-88.68%,-3.13%)	-96.06 (-484.82,207.67)	-3.20% (-24.34%,8.35%)
EOS	NHANES	-33.48 (-62.41,-4.97)	-17.88% (-36.64%,-2.69%)	-63.32 (-118.65,12.04)	-39.52% (-121.49%,5.31%)	-27.59 (-112.69,102.15)	-14.17% (-74.37%,32.60%)
	THLHP	-59.26 (-625.26,366.60)	-3.59% (-41.98%,20.04%)	-481.58 (-1202.06,560.42)	-39.18% (-141.22%,27.72%)	14.68 (-1090.30,1870.25)	0.84% (-105.52%,54.77%)
MON	NHANES	-36.93 (-93.74,-4.60)	-7.05% (-20.82%,-0.79%)	32.14 (-9.50,111.67)	5.38% (-1.61%,17.52%)	-85.62 (-179.43,-16.02)	-17.92% (-52.42%,-2.81%)
	THLHP	-3.65 (-43.04,22.21)	-8.14% (-107.05%,42.95%)	-10.64 (-17.89,17.15)	-29.30% (-95.58%,19.81%)	-8.80 (-57.58,34.78)	-21.89% (-226.16%,53.74%)
NLR	NHANES	0.10 (-0.15,0.36)	4.85% (-7.16%,14.51%)	1.53 (0.71,2.09)	43.52% (24.27%,54.33%)	-0.14 (-0.81,0.41)	-7.66% (-56.69%,18.78%)
	THLHP	0.03 (-0.36,0.39)	1.40% (-19.45%,20.20%)	0.72 (-0.01,1.47)	28.06% (-0.59%,49.11%)	0.14 (-0.27,0.60)	7.19% (-14.08%,26.20%)

IV. The relationship between infant feeding behavior and maternal inflammation status, physical health, and mental wellbeing

This chapter is co-authored with Aaron Blackwell, Melanie Martin, and Amy Boddy. The author of this dissertation proposed all hypotheses tested herein and collected all the data (with the help of Madison Hubble). The analyses, writing, and figures contained in this chapter are the work of the author of this dissertation.

A. Introduction

Among humans, the transition from pregnancy to the postpartum period is a time of substantial maternal recalibration, wherein the immunological requirements of fetal tolerance give way to a new suite of demands (e.g., heightened pathogen clearance, uterine involution) and extended immunological recovery. Throughout human evolution, selective pressures on postpartum maternal recovery and recalibration (and investment in future reproduction) have been balanced against concurrent infant needs, as human babies are highly dependent on maternal investment for survival. Breastfeeding, a highly conserved form of maternal investment, is a bi-directional exchange between mother and offspring, during which infants directly influence maternal hormone production (Matthiesen et al., 2001), metabolism (Butte and King, 2005b; Stuebe and Rich-Edwards, 2009), and sleep-waking patterns (Hunter et al., 2009) and mediate infant-to-mother cell trafficking (Dawe et al., 2007) and microbial exposure (Hassiotou et al., 2013; Breakey et al., 2015). This degree of access provides infants with passive immunity (Field, 2005), microbiome priming (Lönnerdal, 2003; Hurley and Theil, 2011; Al-Shehri et al., 2015), and tailored nutrition (Ballard and Morrow, 2013), thus enhancing offspring survival and boosting maternal fitness (Chen and Rogan, 2004). The well-documented benefits of breastfeeding for infant health outcomes, including reduced short-term morbidity and mortality and enhanced lifelong wellbeing (Chen and Rogan, 2004; Ip et al., 2007) provide the basis for current public health guidance on breastfeeding. While

the effects of lactation on maternal metabolic health and reproductive investment are well-documented, the potential benefits and costs of breastfeeding behavior on maternal immune recovery and perceived health have been relatively understudied.

From an evolutionary perspective, immunological recalibration needed after successful delivery evolved within the context of lactation and at-the-nipple breastfeeding. From this vantage point, at-the-nipple breastfeeding should confer substantial benefits on maternal immune recalibration and health while total absence or early cessation (in the absence of infant death) should be linked to deleterious outcomes due to evolutionary mismatch, just as it is for infants. There is some evidence that breastfeeding is associated with slower return to cycling immune baselines and reduced systemic inflammation (Groer et al., 2005; Kuzawa et al., 2013), but inconsistent sampling intervals and vague measures of breastfeeding behavior preclude a clear understanding of how breastfeeding and its intensity impact maternal immune and health status (as highlighted in Chapter II). Similarly, a history of breastfeeding has been linked to more long-term immunological benefits, such as reduced risk of breast cancer (Beral et al., 2002; Pavard and Metcalf, 2007), but potential immunological mechanisms underlying these relationships have not been fully elucidated.

Beyond benefits to maternal health, escalating energetic costs of sustained lactation may also result in maternal-offspring conflict, as interests over maternal resource allocation diverge over time (Trivers, 1974). From this perspective, mothers who sustain exclusive at-the-nipple breastfeeding for extended periods may experience diminishing returns on immune and health benefits due to depleted energetic reserves and increased fatigue. Moderating or supplementing at-the-nipple breastfeeding using other infant feeding methods may, to some degree and in a time-dependent manner, help mothers optimize their own

immune status and health. To date, however, a skewed focus on infant outcomes has impeded a firm understanding of how time since delivery may mediate any benefits of breastfeeding on maternal immune status and wellbeing – especially in populations where access to evolutionarily novel infant feeding strategies collide with infant-centered breastfeeding guidelines and mounting pressure to provide infants with exclusive access to breastmilk for six months.

To address these gaps in knowledge, this study uses a heterogenous sample of postpartum women in the USA to test the following hypotheses: (H1) at-the-nipple breastfeeding tempers morning peak in inflammatory salivary cytokines, reduces risk of physical illness, and curbs sickness behavior (i.e., depression); and (H2) these effects are strongest in the early postpartum period (i.e., first 70 days following delivery). Furthermore, we compare the effects of at-the-nipple breastfeeding to the effects of breastmilk expression/pumping to clearly parse out if, how, and when these infant feeding methods might differ in their impact on maternal outcomes.

1. Potential benefits and costs of breastfeeding

The initial phase of lactogenesis begins during pregnancy, but it is not until after delivery that progesterone levels fall precipitously and prolactin production ramps up, allowing for initial milk let-down. Sustained lactogenesis is then perpetuated by a positive feedback loop wherein infant suckling stimulates release of oxytocin and prolactin, hormones that control milk production and ejection and promote maternal-infant bonding (Neville and Neifert, 1983). Studies suggest that these hormones may also bolster immune competence while suppressing hyperinflammation (Hannah et al., 1996, 1997; Matthiesen et al., 2001; Neville, 2001; Yu-Lee, 2002; İşeri et al., 2005; Clodi et al., 2008; Deing et al., 2013; Costanza et al.,

2015), though their integrated role in postpartum immune function is not particularly well understood. Cortisol, the primary hormone of the hypothalamic-pituitary-adrenal (HPA) axis which coordinates the body's response to stress and regulates inflammation, also falls after delivery (ALLOLIO et al., 1990), with some evidence that initiation and continuation of breastfeeding further dampens cortisol production (at least immediately following breastfeeding bouts) (Mizuhata et al., 2020). In addition to inducing a potentially anti-inflammatory hormonal milieu, lactation also exerts strong effects on maternal metabolism. While pregnancy promotes transient maternal fat accumulation, elevated insulin resistance, and increased lipid and triglyceride levels, sustained lactation reverses these pregnancy-induced metabolic changes, curbing risk of developing type-2 diabetes (Stuebe, 2005) and limiting postpartum weight gain (Baker et al., 2008). Greater energetic throughput and tighter regulation of metabolic "resetting" induced by regular breastfeeding is likely to exert potent anti-inflammatory effects, given the immunological benefits associated with exercise (Nieman and Wentz, 2019), regulated caloric restriction (Almeneessier et al., 2019) and reduced adiposity (Bianchi, 2018).

Conversely, there are costs to prolonged, exclusive at-the-nipple breastfeeding. The relative energetic costs and hormonal effects of sustained, frequent breastfeeding also suppress ovarian function, resulting in sub-cycling levels of estradiol and progesterone and delayed resumption of regular cycling – a phenomenon called lactational amenorrhea (McNEILLY et al., 1994; Valeggia and Eellison, 2009). While lactational amenorrhea allows mothers to invest more in current offspring by delaying future reproduction, the accumulated energetic costs of long-term lactation can culminate in drained reserves and maternal depletion of key nutrients (Miller, 2010; Goetz and Valeggia, 2017), potentially decreasing

immune competence and enhancing susceptibility to infection. Furthermore, breastfeeding itself can be an appreciable source of direct exposure to foreign antigens. Infectious microbes (most commonly staphylococcus bacteria) can be transferred to mothers during nursing (Angelopoulou et al., 2018), where pathogens can infiltrate mammary tissue or reach the bloodstream (Geddes et al., 2012). The observation that actively nursing infants who are currently infected can elicit a rapid increase in the number of maternal immune cells transferred in breastmilk, even when the mother herself is asymptomatic, provides further evidence of infant-derived microbial transfer during breastfeeding bouts (Hassiotou et al., 2013). Rising costs of long-term on-demand breastfeeding, in tandem with infant growth and continued direct antigen exposure during nursing bouts, may result in dampened maternal immune competence, reduced inflammatory regulation, and sickness behavior, potentially favoring the adoption of supplementation or replacement behaviors to reoptimize maternal health and future reproductive effort. Evidence of such moderating behavior has been noted in many natural fertility populations (Sellen, 2001). Among the Tsimane, for example, nearly all mothers engage in sustained on-demand breastfeeding but a majority also report introduction of complimentary liquids and foods within the first five months, frequently citing a mix of perceived infant needs (e.g., increased hunger) and low breastmilk supply as the primary reasons for supplementation (Martin et al., 2016).

2. What counts as “exclusive breastfeeding” in the USA?

In contrast to natural fertility populations, mothers in the USA (and other industrializing/industrialized societies) are provided with unprecedented access to evolutionarily novel alternatives to at-the-nipple breastfeeding (e.g., electric breast pumps) while simultaneously encouraged to provide breastmilk, and only breastmilk, to infants six

months of age and younger. Current World Health Organization (WHO) guidelines recommend "exclusive breastfeeding for the [infant's] first 6 months of life" – without supplementation of any kind. Similarly, the Centers for Disease Control (CDC) recommend a minimum of six months exclusive breastfeeding, defined as a situation in which an infant is fed "only breast milk – no solids, water, or other liquids." In the USA, widescale implementation of these guidelines has shifted maternal behavior. In 2007, 74.8% of mothers reported having ever breastfed their infant. By 2018, this number had increased to 83.2%. In 2007, 41.5% of mothers reported "exclusive breastfeeding", compared to 57.6% in 2018. According to these metrics, breastfeeding initiation and continuation have improved markedly across all metrics and demographic groups. Reduced reliance on infant formula as a *replacement* for breastfeeding is likely to confer substantial benefits for mothers, especially in the early postpartum period, but rigid observance of these guidelines may also disrupt (or at least substantially reorganize) maternal optimizing strategies in the long-term (e.g., maintaining "exclusive breastfeeding" for longer than is optimal on an individual level in order to meet broader societal expectations).

Another key feature of current breastfeeding guidelines is that mothers who provide breastmilk via pumping, potentially without ever engaging in at-the-nipple breastfeeding, are binned as "exclusively breastfeeding" (hereafter referred to as "exclusive infant access to breastmilk"). A clear understanding of the potential consequences of this latter scenario is especially critical, considering a large majority of mothers in the USA already report using pumping as a replacement behavior for at-the-nipple breastfeeding, often beginning very early in the postpartum period (Rasmussen and Geraghty, 2011; Loewenberg Weisband et al., 2017). Pumping and at-the-nipple breastfeeding are likely to produce overlapping but

ultimately distinct effects on maternal outcomes. Pumping, especially using modern pumps with customizable settings, effectively mimics the mechanical aspects of infant suckling (Gardner et al., 2015), thus encouraging a similar positive feedback loop sustaining milk production while removing direct nipple contact between mother and infant. Reduced at the nipple contact might alleviate risk of direct microbial transfer from infant to mother, but breast pumps themselves can easily become contaminated, presenting an alternative source of pathogen exposure (Leiter et al., 2022). Pumping may be particularly useful in maintaining milk supply if the infant is born premature and/or has problems latching and more time is needed to successfully establish breastfeeding (Slusher et al., 2007; Meier et al., 2012), but early reliance on pumping may attenuate the predicted benefits of lactation on maternal physiology by disrupting the rhythmicity of milk production and ejection elicited by on-demand breastfeeding, increasing risk of milk stasis (a primary cause of breast inflammation) (Fetherston, 1998), and removing important signaling pathways between mother and infant (e.g., hormonal regulation, attachment, bonding). From this perspective, regular at-the-nipple breastfeeding is likely to confer enhanced benefits on mothers compared to pumping in the early postpartum period. On the other hand, pumping may provide mothers with an evolutionarily novel way to negotiate increasing parent-offspring conflict across the postpartum period. To date, however, such complexities in infant feeding behavior and their possible effects on maternal immune function, health, and wellbeing have been understudied.

3. Study objectives and predictions

In this study, we measured breastfeeding as the proportion of infant feeding bouts completed using at-the-nipple breastfeeding during a 24-hour collection period (% ATN breastfeeding) and pumping as the proportion of infant feeding bouts completed using non-

donated fresh or frozen expressed/pumped breastmilk (% pumping). We then estimated and compared the effects of % ATN breastfeeding and % pumping on postpartum depressive symptoms, perceived physical health, and evening-to-morning change in secretion rate of "pro-inflammatory" salivary cytokines IL-1ß, IL-6, IL-8, and TNF-α and C-reactive protein (CRP) (Table 1). We also separated our sample into two groups (early versus late postpartum period) and compared the time-dependent effects of % ATN breastfeeding and % pumping on each outcome measure.

We predicted that % ATN breastfeeding would be associated with fewer reported symptoms of physical illness and fewer symptoms of depression. Most salivary cytokines peak at time of waking (Izawa et al., 2013; Reinhardt et al., 2016; Sarkar et al., 2021; Wetterö et al., 2021), with adverse conditions (e.g., sleep deprivation, PTSD, sleep apnea, obesity, arthritis) commonly associated with an elevated morning peak in inflammatory cytokine levels (Irwin, 2006; Pervanidou et al., 2007; Hernández and Taylor, 2017). Based on this knowledge, we hypothesized that % ATN breastfeeding would have a negative effect on waking cytokine levels and therefore a negative effect on evening-to-morning change in IL-1ß, IL-6, IL-8, TNF-α, and C-reactive protein secretion rate. We also predicted that these effects of % ATN breastfeeding would be relatively attenuated among individuals in the later postpartum period, due to decreasing overlap between offspring and maternal optimums and accumulating costs of lactation. Lastly, we predicted that the effects of % ATN breastfeeding on maternal outcomes would be stronger than the effects of % pumping in the early postpartum period, but that the difference in these effects would be comparatively diminished in the later postpartum group.

B. Methods and Materials

1. Sample selection

Our sample was limited to postpartum women between the ages of 25 and 42 who lived in King County, WA, USA, had given birth within the last six months, and who indicated absence of previous cancer diagnosis, periodontal disease, and regular tobacco use/vaping. All participants (n=97) completed a single 24-hour collection period, beginning at 12 PM on day one and ending at 12 PM on day two. Because data for this study were collected during the height of the COVID-19 pandemic (September 29th, 2020 – July 28th, 2021), research protocols were specifically designed to reduce in-person contact. Collection kits were mailed to each participant before the start of the 24-hour collection period and all data were collected from home. Table 2 provides complete descriptive statistics.

2. Saliva samples

Each participant was asked to collect two passive drool saliva samples during the 24-hour period collection period. Participants were instructed to collect the first sample before going to bed on day one (S1) and the second at time of waking the following morning (S2). For each participant, 12:00:00 PM on day one of the collection period was assigned as the objective "start" time. The times at which the first and second samples (S1 and S2) were collected were calculated as the number of hours that had elapsed since the start time of 12:00:00 PM. For example, an individual who recorded a time of 9 PM for S1 and 6 AM for S2 would have sample times of 9 and 18 hours, respectively. To ensure sample integrity, participants were instructed to place saliva samples in a home freezer immediately after collection. At the end of the 24-hour collection period, saliva samples were then picked up by

research personnel (Carmen Hové or Madison Hubble) and transported on ice to the University of Washington CSDE laboratory, where samples were stored in a -80°C freezer until final processing. Three participants were unable to successfully complete this part of the study, resulting in 188 saliva samples from 94 participants. After data collection was complete, saliva samples were shipped over night on dry ice (in a single shipment) to Salimetrics, LLC (Carlsbad, CA) for batch analysis on August 17th, 2021. Samples were analyzed for IL-6, IL-8, TNF-α, and IL-1ß ("pro-inflammatory" cytokine panel) and C-reactive protein (CRP), with testing prioritized in this order. All tubes were weighed before analysis and all 5 assays were run in duplicate for each sample, with the mean for each used in all final analyses (CRP CV = 2.61%, IL6 CV = 5.00%, IL-1ß CV = 2.51%, IL-8 CV = 3.80%, TNF-α CV = 6.46%).

3. 24-hour infant feeding record

Over the course of the 24-hour collection period participants were asked to keep a simple infant feeding record noting the method(s) used during each infant feeding bout and the total number of feedings completed during the 24-hour timeframe. Possible infant feeding methods included use of (1) non-breastmilk liquids, (2) semi-solid/solid foods, (3) pumped/expressed breastmilk given to infant without storing, (4) participant's own previously refrigerated/frozen pumped/expressed breastmilk given to infant, (5) donated breastmilk, and (6) breastfeeding at the nipple. If participants also pumped/expressed breastmilk during the 24-hour collection period for later use, this was included as an additional feeding bout. When applicable, participants were able to indicate multiple methods used in a single feeding bout.

4. Questionnaire

At the end of the 24-hour collection period, participants completed an online Qualtrics questionnaire. To obtain matching longer-term data on infant feeding behavior, participants were asked to approximate the frequency with which they employed each infant feeding method listed above over the preceding two weeks, as well as how long after delivery each of the relevant methods were introduced. Perceived health and wellbeing was assessed using an expanded set of questions based on the Physical Health Questionnaire (Schat et al., 2005), designed to assess four distinct dimensions of somatic health (i.e., gastrointestinal problems, headaches, sleep disturbances, and respiratory illness) and symptom severity of preexisting conditions (e.g., arthritis, systemic lupus erythematosus), with added questions specific to common postpartum health problems (e.g., mastitis). Mental health status was assessed using the Edinburgh Postnatal Depression scale (Cox et al., 1987). In addition to these measures, relevant sociodemographic information (e.g., education attainment, income level, ethnicity), maternal and infant health history (e.g., delivery mode, pregnancy complications, parity, infant illness, presence/absence of menstruation resumption), non-breastfeeding mother-infant contact (e.g., co-sleeping practices), and self-reported impacts of the COVID-19 pandemic were collected.

5. Statistical Analyses

Explanatory variables. Breastfeeding proportion was calculated as the percentage of total infant feedings occurring via at-the-nipple breastfeeding during the 24-hour collection period (% ATN breastfeeding) while pumping proportion was calculated as the percentage of total infant feedings occurring via use of fresh or frozen expressed/pumped breastmilk from the mother over the 24-hour collection period (% pumping). Since individuals were able to

indicate multiple infant feeding methods for each feeding bout, these measures were not zero-sum indicators of either infant feeding method. To investigate potential time-dependent differences in the effects of infant feeding behavior on maternal health, we calculated the 50% quantile for days since delivery (70.0 days) and used this as a benchmark to split our sample into two groups: “early postpartum” and “late postpartum”. As defined here, the “early postpartum” period encompasses numerous key changes in maternal and fetal physiology, including uterine involution, transition from colostrum to mature breastmilk production, and infant latching (or lack thereof), fastest infant growth (Hui et al., 2010), and spans the period of time during which both maternal and infant mortality and morbidity risk is highest (Ronsmans and Graham, 2006; Gill et al., 2022).

Outcome variables. Composite physical health and mental wellbeing scores assessing current health status were calculated for each participant based on answers given to the modified perceived health questionnaire (PHQ score) (possible range = 0-16) and the Edinburgh postnatal depression scale (EPDS score) (possible range = 0-30), with higher values corresponding to more severe symptoms. Both EPDS and PHQ scores follow negative binomial distributions and were modeled as such. To account for the effects of salivary flow rate, absolute concentrations of all salivary biomarkers (IL-1ß, IL-6, IL-8, TNF-α, and CRP) were transformed into secretion rates before analysis. For each saliva sample, there was a recorded duration (in seconds) that it took for the participant to collect the sample as well as a corresponding weight (in grams) of the final sample. Since saliva is approximately 99% water, it is standard practice to use a 1:1 conversion rate for grams to milliliters (de Almeida et al., 2008). We therefore converted each sample weight to milliliters and the duration into minutes and calculated the flow rate (mL/min) for each sample. The final secretion rate of

each analyte for each sample was then calculated as the raw concentration (unit/mL) multiplied by the flow rate (mL/min). Finally, we used morning and evening salivary cytokine secretion rates to calculate the evening-to-morning change in secretion rate for each participant.

Exploratory/descriptive models. Using generalized linear models, we estimated the association between recorded infant feeding behavior during the 24-hour collection period and corresponding frequencies reported for the prior two weeks (collapsing all uses of pumped/expressed milk into a single category of "pumping"), thereby determining how strongly infant feeding behavior during the 24-hour collection period corresponded to longer-term behavior (Figure 1). We also used generalized linear models to estimate the effect of infant feeding method (% ATN breastfeeding, % pumping) and sample collection time (evening, morning) on salivary flow rate to check for potential confounds (e.g., altered hydration by time of day/infant feeding method) (Figure 2).

Primary models. All primary models were executed in R 4.1.2 (https://cran.r-project.org) using the *brms* package (Bürkner, 2017). Bayesian models were used to estimate the effects of % ATN breastfeeding on EPDS and PHQ score, accounting for maternal age, pre-pregnancy BMI, income, educational attainment, % pumping, delivery mode, and presence/absence of reported pregnancy complications, co-sleeping, night feeding, and alcohol consumption during the 24-hour collection period. To evaluate the effects of infant feeding behavior on evening-to-morning change in inflammatory cytokine secretion rates, we employed fully Bayesian models to estimate the effects of % ATN breastfeeding on evening-to-morning change in IL-1ß, IL-6, IL-8, TNF-α, and CRP secretion rate, accounting for the fixed effects of time between collections, maternal age, pre-pregnancy BMI, income,

educational attainment, % pumping, delivery mode, and presence/absence of reported pregnancy complications, co-sleeping, night feeding, and alcohol consumption during the 24-hour collection period. To investigate how time since delivery moderated the effects of breastfeeding proportion and category on maternal immune status and health outcomes, we employed the same model formulas as outlined above, this time adding categorical time since delivery ("early" versus "later "postpartum period) as an interaction term for both % ATN breastfeeding and % pumping. Predicted values by % ATN breastfeeding were standardized by mean values for all co-variates except % pumping, which was set to a zero value. Conversely, predicted values by % pumping were standardized by mean values for all co-variates except % ATN breastfeeding, which was set to zero.

C. Results

1. Most mothers employed a mixture of infant feeding methods

During the 24-hour collection period, only 11.5% (n=11) of participants in our sample reported a complete absence of breastfeeding, while 46.9% (n=45) indicated using a mix of breastfeeding and other infant feeding methods and 42.7% (n=41) individuals indicated exclusive at-the-nipple breastfeeding. When combined with reported long-term history of infant feeding behavior, only two participants in our sample had never breastfed their infant. Both these individuals reported reliance on pumped breastmilk, therefore no participants in our sample exhibited exclusive reliance on infant formula. Conversely, only 9.4% (n=9) of participants reported a history of using exclusive at-the-nipple breastfeeding with no supplementation or replacement at all. In sum, 83.3% of participants (n=80) reported either current or past use of expressed / pumped breastmilk, 32.3% (n=31) reported using non-

breastmilk liquids at some point, and 14.6% (n=14) indicated use of solid foods, while only 1 individual reported past use of donated breastmilk. In accordance with high rates of pumping and breastfeeding, 89.6% (n=86) of mothers reported current lactational amenorrhea. During the 24-hour hour collection period, nighttime feeding was reported by 85.4% of women (n=82) while only 23.7% (n=23) of mothers reported co-sleeping. As shown in Figure 1, infant feeding behavior reported during the 24-hour collection period was strongly associated with recalled behavior from the prior two weeks, indicating that data collected during the 24-hour sampling interval corresponded to broader temporal patterns of behavior. Complete descriptive statistics are provided in Table 2.

2. At-the-nipple breastfeeding associated with fewer symptoms of depression, while relationship between pumping and depression is mediated by time since delivery

Among all mothers in our sample, % ATN breastfeeding was negatively associated with EPDS score (negative logit ß = -0.0067; 95% CI = -0.0117, -0.0018) while there was only a very slight negative relationship between % pumping and EPDS score (negative logit ß = -0.0013, 95% CI = -0.0078, 0.0052) (Figure 3A, Table 3). In the early postpartum group, however, both % ATN breastfeeding (negative logit ß = -0.0088; 95% CI = -0.0168, -9e-04) and pumping (negative logit ß = -0.0078; 95% CI = -0.00172, 0.0020) were negatively correlated with EPDS score. While % ATN breastfeeding remained negatively associated with EPDS score among mothers in the late postpartum group (negative logit ß = -0.0050; 95% CI =-0.00116, 0.0015), pumping was positively correlated to EPDS score in this subset (negative logit ß = 0.0055; 95% CI = -0.0039, 0.0148).

3. Pumping associated with more symptoms of physical illness, regardless of time since delivery

As shown in Figure 2B and Table 3, % ATN breastfeeding was not strongly associated with PHQ score among all, early, or late postpartum women. Conversely, pumping was positively associated with PHQ score among all (negative logit ß = 0.0066; 95% CI = 0.0000,0.0130), albeit only slightly among mothers in the early (ß = 0.0078; 95% CI = -0.0018, 0.0176) and late (ß = 0.0058; 95% CI = -0.0033, 0.0148) postpartum period.

4. Both at-the-nipple breastfeeding and pumping correspond to reduced evening-to-morning rise in CRP

As shown in Figure 3 and Table 4, both % ATN breastfeeding (ß = -10.16 pg/mL/min/%; 95% CI = -14.88, -5.45) and % pumping (ß = -9.10 pg/mL/min/%; 95% CI = -14.85, -3.10) were negatively associated with evening-to-morning change in CRP secretion rate among all mothers in our sample. When broken down by time delivery, however, the relative strength of this relationship varied by infant feeding method. In the early postpartum group, % ATN breastfeeding had a comparatively stronger negative relationship with evening-to-morning change in CRP secretion rate (ß = -11.23 pg/mL/min/%; 95% CI = -19.06, -3.53) versus % pumping (ß = -5.23 pg/mL/min/%; 95% CI = -14.42, 3.58). In the late postpartum group, this pattern was reversed with % pumping having a more robust negative association with morning-to-evening change in CRP secretion rate (ß = -13.86 pg/mL/min/%; 95% CI = -22.10, -5.72) compared to % ATN breastfeeding (ß = -9.35 pg/mL/min/%; 95% CI = -15.49, -3.21).

5. Both at-the-nipple breastfeeding and pumping negatively associated with evening-to-morning change in IL-8

As shown in Figure 4A-C and Table 4, associations between % ATN breastfeeding and morning-to-evening change in IL-1ß, IL-6, and TNF-α secretion rate were minimal, regardless of time since delivery. Similarly, the associations between % pumping and morning-to-evening change IL-1ß and IL-6 secretion rate were relatively small and did not vary significantly by time since delivery. As shown in Figure 4D, both % ATN breastfeeding and pumping were negatively correlated with morning-to-evening change in IL-8 secretion rate, although this relationship was stronger for % pumping versus % ATN breastfeeding among all, early, and late postpartum mothers (Table 4).

D. Discussion

By investigating infant feeding behavior from the maternal perspective, our results illuminate trends more easily overlooked by infant-centered measures of breastfeeding success. In our sample of 97 mothers in the Seattle metro area, most mothers within the first six months of the postpartum period utilized at-the-nipple breastfeeding *and* other infant feeding methods, with pumping as the most common alternative method. Exclusive formula feeding was nonexistent while long-term exclusive breastfeeding (with no history of supplementation) was reported by less than 10% of participants. By quantifying these nuances in infant feeding behavior, we were able to more closely investigate the time-dependent relationships between reliance on at-the-nipple breastfeeding versus pumping and maternal health outcomes.

Our results indicate that more feeding bouts involving at-the-nipple breastfeeding corresponded to fewer symptoms of depression, especially among mothers in the early

postpartum period. While the association between pumping and depression was nearly identical to that of at-the-nipple breastfeeding among mothers in the early postpartum period, the effects of pumping showed a reverse relationship among mothers in the late postpartum period, with higher reliance on pumping associated with increased number of depressive symptoms. This pattern was the opposite of what we predicted, indicating that the protective effect of at-the-nipple breastfeeding might remain stable (at least within the first six months after delivery) while the benefits of pumping may be limited to the first several months after delivery. Contrary to expectations, we found minimal association between at-the-nipple breastfeeding and reported symptoms of physical illness but observed a positive relationship between pumping and physical symptoms, especially among mothers in the late postpartum period.

It is possible that a shared mechanism underlies the relationship between pumping and increased risk of depression and physical illness observed among mothers in the later postpartum period and/or these effects amplify each other (i.e., more physical symptoms increase risk of depression, depression increases feelings of physical illness). While it is tempting to interpret these results as evidence of fundamental differences between at-the-nipple breastfeeding and pumping (e.g., altered amplitude and frequency of oxytocin surges), we cannot rule out the possibility that individuals who are less prone to depression (via increased social support, etc.) may also have an easier time sustaining at-the-nipple breastfeeding through the first six months of the postpartum period. Given the well-documented effects of social support on both general depression risk (Werner-Seidler et al., 2017) and breastfeeding "success" (Raj and Plichta, 1998), it is likely that there are multiple pathways underpinning the association between breastfeeding, pumping, and depressive

symptoms. Future longitudinal research focused on the specific impacts of pumping versus at-the-nipple breastfeeding on maternal physiology (e.g., altered frequency of milk let-down, hormonal cycling) is warranted, especially given the increasing ubiquity of this infant feeding method (Rasmussen and Geraghty, 2011).

It is also worth noting that the overall variance in reported physical symptoms in our sample was much lower than expected and these results may be highly specific to our sample. Due to the COVID-19 pandemic, mothers in our study were subject to a stay-at-home order that was in effect throughout the entire study. The incidence of reported maternal, infant, and household member illnesses was very low, presumably because of drastic reductions in exposure to a variety of pathogens. Results generated within such an unusual context may underestimate the effects of infant feeding behavior on physical symptoms in populations experiencing regular exposure to a wider breadth of common infectious agents. Future research seeking to replicate these findings will provide greater confidence in the broad applicability of our findings.

Our results indicate that, even in the relative absence of pathogen exposure (due to the COVID-19 stay-at-home order in place throughout data collection), differences in infant feeding behavior correspond to variation in inflammatory status. Both at-the-nipple breastfeeding and pumping were associated with attenuated evening-to-morning rise in secretion rate of salivary C-reactive protein and IL-8. Taken together, these patterns provide evidence that both at-the-nipple breastfeeding and pumping may downregulate certain inflammatory processes, especially in the early postpartum period. Future studies investigating the effects of breastfeeding behavior on immunological responses to specific disease challenges during the postpartum period would be particularly useful in establishing a

broader understanding of these observed benefits. Continuing research on a broader array of immunological measures with different sampling methodology is also warranted. In particular, the time-dependent effects we report here would be best replicated using a longitudinal sampling method wherein direct comparisons could be made between individuals who maintain high degree of supplementation and/or replacement behavior across the entire first six months versus individuals who exhibit gradual attenuation in breastfeeding intensity over time. Due to methodological constraints, we were limited to the use of non-invasive sampling protocols and therefore future research including full blood draws and corresponding plasma and serum immunological phenotyping would be especially useful in elucidating the systemic effects of infant feeding behavior on maternal immune competence. Given robust prior knowledge that pregnancy induces changes in neutrophil prevalence (Hové et al., 2020) and our study's reported effects of breastfeeding on IL-8, a chemoattractant with high specificity for neutrophils, we recommend particular focus on this cell type in future research on postpartum maternal health.

In sum, these findings enhance understanding of breastfeeding as a dyadic rather than one-dimensional relationship, wherein the benefits, constraints and effects on maternal physiology are considered in addition to infant outcomes. This study provides evidence that current public health definitions of "exclusive breastfeeding" may, in some contexts, facilitate adoption of infant feeding behavior (e.g., exclusive reliance on pumping) that differ in effects on maternal postpartum biology and mental health relative to at-the-nipple breastfeeding. A clear understanding of how variation in infant feeding behavior impacts maternal outcomes is key, given that access to breastfeeding alternatives is rapidly spreading

across the globe. We hope that this study provides a useful initial foray into this critical area of research.

Chapter IV: Figures and Tables

Figure 6. Linear regression lines with corresponding raw data indicating the relationship between reported proportion of infant feed bouts (% IFB) occurring via breastfeeding, pumping, non-breastmilk liquids, and solid food during the 24-hour collection period (% IFB 24 hours) and over the preceding two weeks (% IFB 2 weeks).

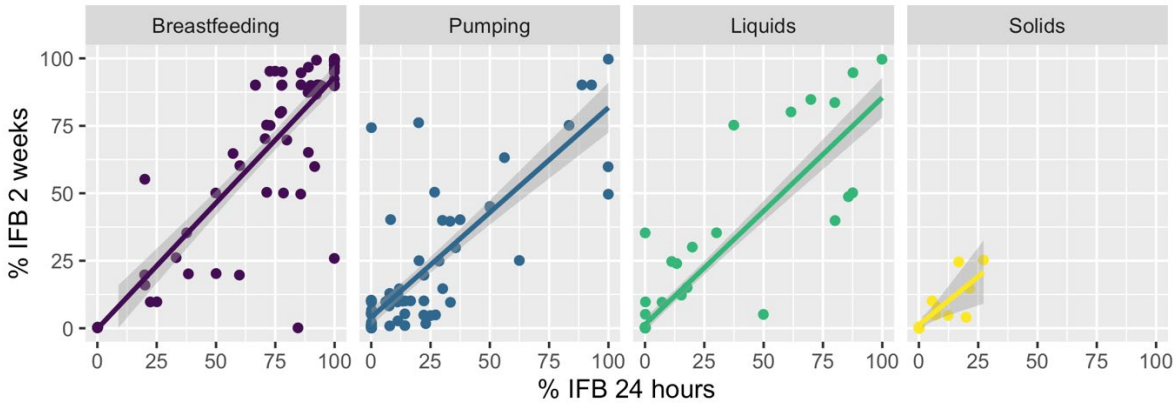


Figure 7. Linear regression lines plotted over raw values indicating the relationship between % ATN breastfeeding (Panel A) and % pumping (Panel B) on log salivary flow rate by sample collection time (1 = evening, 2 = morning).

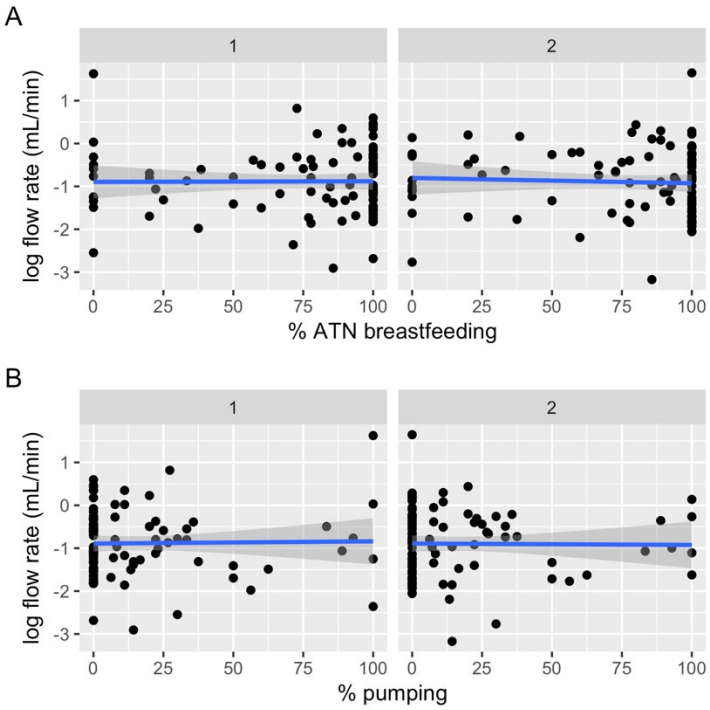


Figure 8. Predicted effects of % ATN breastfeeding and % pumping on EPDS score (Panel A) and PHQ score (Panel B) by time since delivery. IFM = infant feeding method. <70 DSD = less than 70 days since delivery. 70+ DSD = more than 70 days since delivery.

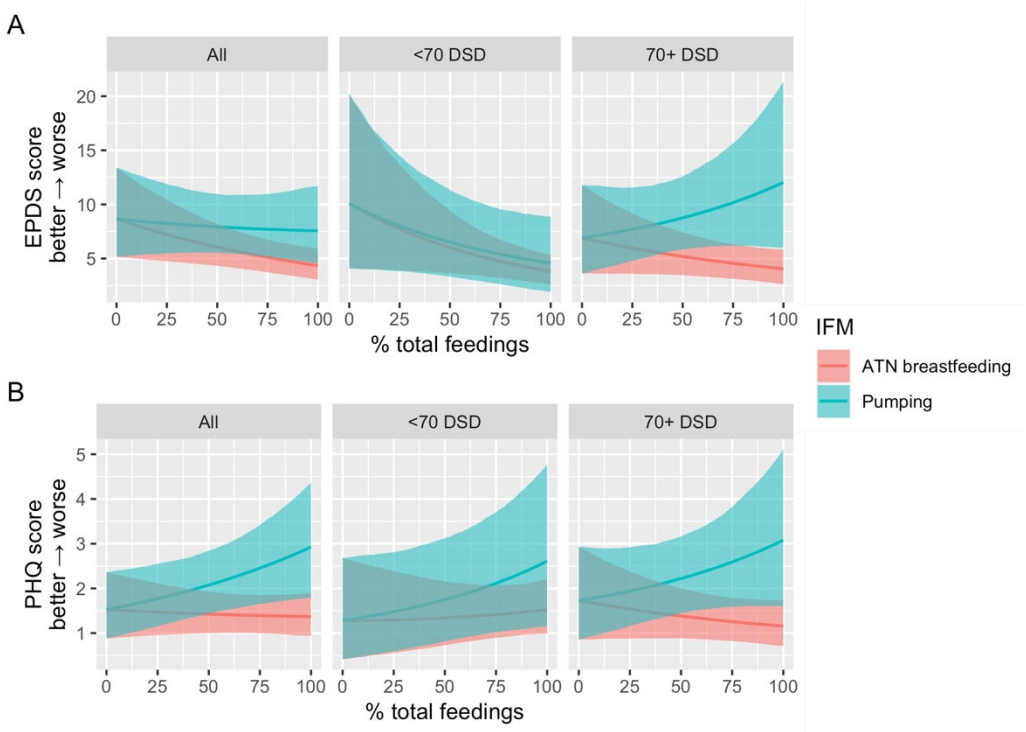


Figure 9. Predicted effects of % ATN breastfeeding and % pumping on evening-to-morning change in C-reactive protein secretion rate by time since delivery. <70 DSD = less than 70 days since delivery. 70+ DSD = more than 70 days since delivery. Horizonal black line = no difference between evening and morning secretion rate.

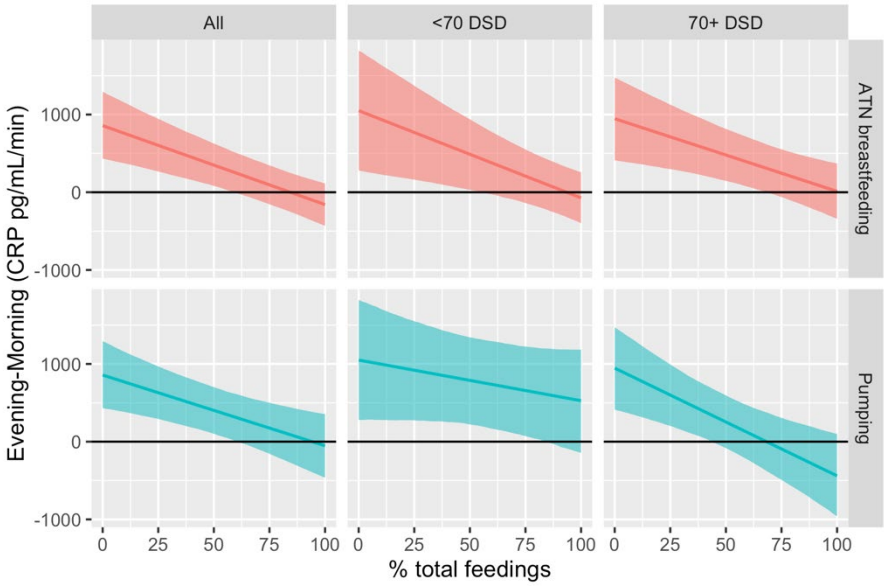


Figure 10. Predicted effects of % ATN breastfeeding and % pumping on evening-to-morning change in IL-1ß (Panel A), IL-6 (Panel B), TNF-α (Panel C), and IL-8 (Panel D) secretion rate by time since delivery. <70 DSD = less than 70 days since delivery. 70+ DSD = more than 70 days since delivery. Horizonal black line = no difference between evening and morning secretion rate.

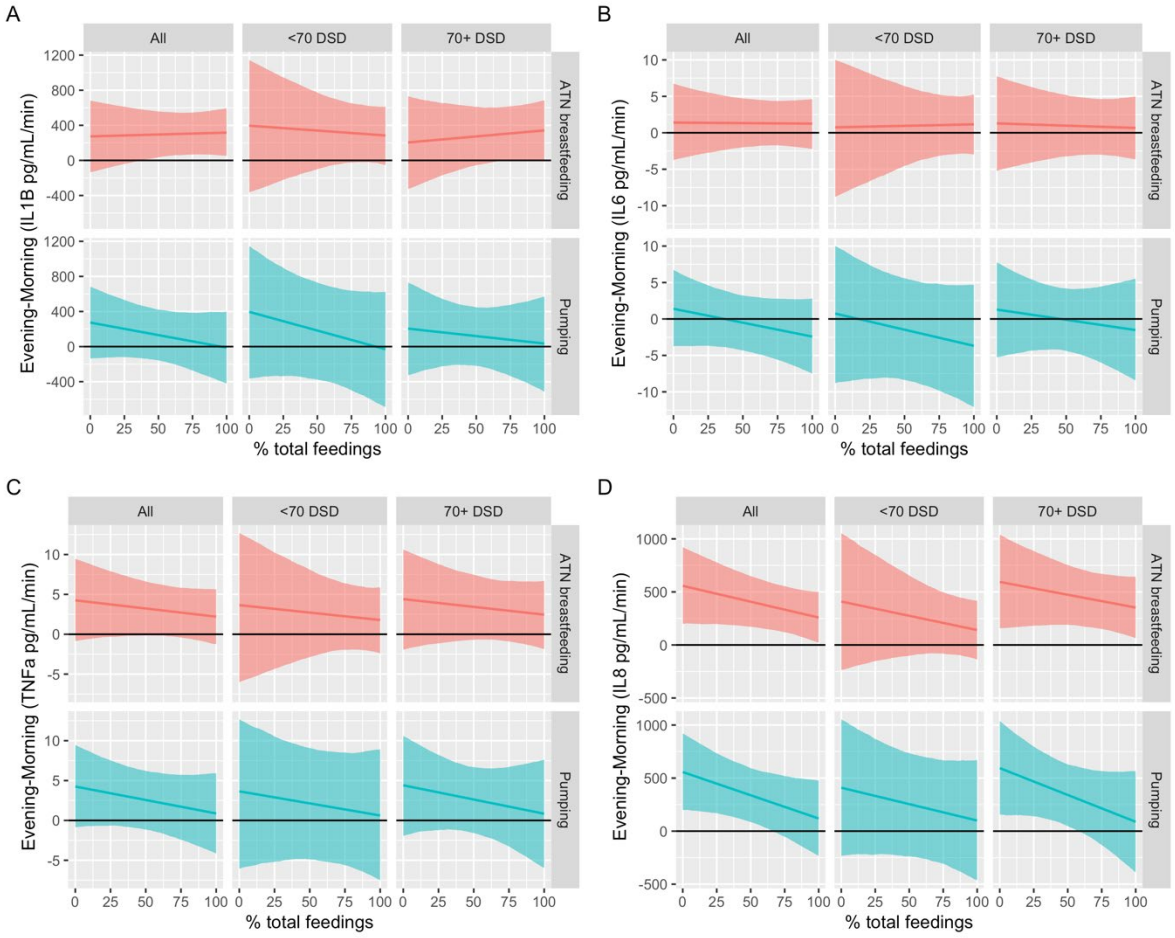


Table 3. Functional descriptions for each salivary immunological biomarker used in this study.

Measure		Function
	CRP	Acute phase protein synthesized by the liver in response to cytokine stimulation during an inflammatory event.
Cytokine panel	IL1ß	Pyrogenic cytokine secreted by monocytes and macrophages. Activated by exposure to essentially all microbial products via TLR ligands. Mediates inflammatory response and immune cell activity.
	IL6	Secreted by macrophages, osteoclasts, and smooth muscle cells. Inhibits effects of TNF-α. Mediator of acute phaser response. Stimulates acute phase protein synthesis, production of neutrophils, and B cell growth. Inhibits regulatory T cells.
	IL8	Chemokine produced by macrophages and epithelial cells. Exerts strong specificity for neutrophils, weak effects on other leukocytes. Stimulates phagocytosis by recruited immune cells.
	TNFα	Both a pyrogenic cytokine and an adipokine. Promotes insulin resistance. Produced by macrophages. Escalates inflammatory response.

Table 2. Descriptive statistics for study sample, separated out by health outcomes (EPDS and PHQ score) and salivary immune markers (CRP and cytokine panel). TSD = time since delivery, N = sample size, Age = maternal age, DSD = days since delivery (infant age), BMI = body mass index, Cycling = % of total sample reporting resumption of regular cycling; C-section = % of total sample reporting Cesarean section, Parity = number of live births, Comps = % of total sample reporting presence of at least one complication during most recent pregnancy.

Measure	TSD	N	Age	DSD	Pre-pregnancy BMI	Current BMI	Parity	Cycling	Infant sex (XX)	C-section	Comps
Health	**ALL**	**97**	**34 (25-42)**	**70 (8-212)**	**24.64 (18.38,45.49)**	**26.61 (19.30,48.92)**	**2 (1-5)**	**11.34%**	**42.27%**	**27.84%**	**45.36%**
	Early	49	34 (28-41)	44 (8-70)	24.56 (18.38,37.31)	26.81 (20.01,42.07)	2 (1-5)	0.00%	42.86%	30.61%	44.90%
	Late	47	34 (25-42)	132 (71-212)	25.35 (19.05,45.49)	25.95 (19.30,48.92)	1 (1-5)	23.40%	42.55%	25.53%	46.81%
Saliva	**ALL**	**94**	**34 (25-42)**	**70 (8-212)**	**24.80 (18.38,45.49)**	**26.62 (19.30,48.92)**	**2 (1-5)**	**11.70%**	**42.55%**	**27.66%**	**45.74%**
	Early	48	34 (28-41)	44 (8-70)	24.64 (18.38,37.31)	26.95 (20.01,42.07)	2 (1-5)	0.00%	41.67%	29.17%	43.75%
	Late	46	34 (25-42)	132 (71-212)	25.35 (19.05,45.49)	25.95 (19.30,48.92)	1 (1-5)	23.91%	43.48%	26.09%	47.83%

Table 3. Predicted point estimates at 0% (Y-intercept) and 100% ATN breastfeeding and pumping by measure (EPDS score, PHQ score) and time since delivery, as well as negative logit ß coefficients (since EPDS and PHQ were modeled using negative binomial distributions). IFM = infant feeding method. <70 DSD = less than 70 days since delivery. 70+ DSD = 70+ days since delivery.

Measure	Parameter	IFM	All	<70 DSD	70+ DSD
EPDS	Y-Intercept (0% Point Est)	--	8.58 (5.19,13.40)	9.94 (4.03,19.84)	7.00 (3.67,11.75)
	100% Point Est	ATN BF	4.33 (3.00, 5.97)	3.79 (2.56, 5.36)	4.04 (2.70, 5.83)
		Pumping	7.61 (4.57,11.92)	4.60 (1.96, 8.90)	12.14 (5.95,21.53)
	negative logit ß coefficient	ATN BF	-0.0067 (-0.0117,-0.0018)	-0.0088 (-0.0168,-9e-04)	-0.0050 (-0.0116,0.0015)
		Pumping	-0.0013 (-0.0078,0.0052)	-0.0078 (-0.0172,0.0020)	0.0055 (-0.0039,0.0148)
PHQ	Y-Intercept (0% Point Est)	--	1.51 (0.89, 2.34)	1.27 (0.42, 2.76)	1.73 (0.87, 2.87)
	100% Point Est	ATN BF	1.36 (0.92, 1.88)	1.52 (0.96, 2.21)	1.15 (0.69, 1.75)
		Pumping	2.93 (1.78, 4.31)	2.61 (1.15, 4.88)	3.06 (1.57, 5.06)
	negative logit ß coefficient	ATN BF	-9e-04 (-0.0062,0.0047)	0.0031 (-0.0061,0.0130)	-0.0037 (-0.0107,0.0035)
		Pumping	0.0066 (0.0000,0.0130)	0.0078 (-0.0018,0.0176)	0.0058 (-0.0033,0.0148)

Table 4. Predicted point estimates at 0% (Y-intercept) and 100% ATN breastfeeding and pumping by measure and time since delivery. IFM = infant feeding method. <70 DSD = less than 70 days since delivery. 70+ DSD = 70+ days since delivery.

Measure	Parameter	Unit	IFM	Time since delivery		
				All	<70 DSD	70+ DSD
CRP	Y-Intercept (0% Point Est)	pg/mL/min	--	857.36 (431.75,1292.77)	1050.11 (278.53,1822.27)	946.37 (411.24,1473.62)
	100% Point Est		ATN BF	-158.86 (-432.74, 112.73)	-72.83 (-398.09, 257.84)	11.59 (-343.00, 371.16)
			Pumping	-52.38 (-465.25, 355.82)	527.22 (-144.09,1185.11)	-440.05 (-961.90, 99.65)
	ß coefficient	pg/mL/min/%	ATN BF	-10.16 (-14.88,-5.45)	-11.23 (-19.06,-3.53)	-9.35 (-15.49,-3.21)
			Pumping	-9.10 (-14.85,-3.10)	-5.23 (-14.42,3.58)	-13.86 (-22.10,-5.72)
IL-1ß	Intercept (0% Point Est)	pg/mL/min	--	273.62 (-134.56, 682.29)	395.90 (-364.81,1146.82)	204.74 (-326.86, 733.15)
	100% Point Est		ATN BF	316.47 (51.75, 593.15)	284.34 (-52.66, 611.55)	341.88 (-5.36, 686.62)
			Pumping	-11.92 (-421.39, 397.67)	-30.56 (-689.20, 623.74)	34.54 (-514.85, 569.03)
	ß coefficient	pg/mL/min/%	ATN BF	0.43 (-4.27,5.02)	-1.12 (-8.61,6.25)	1.37 (-4.56,7.38)
			Pumping	-2.86 (-8.60,3.03)	-4.26 (-13.02,4.22)	-1.70 (-10.12,6.70)
IL-6	Intercept (0% Point Est)	pg/mL/min	--	1.40 (-3.75, 6.74)	0.74 (-8.80, 10.01)	1.29 (-5.23, 7.78)
	100% Point Est		ATN BF	1.26 (-2.22, 4.63)	1.16 (-2.95, 5.24)	0.66 (-3.64, 4.97)
			Pumping	-2.42 (-7.51, 2.80)	-3.68 (-12.08, 4.70)	-1.52 (-8.42, 5.54)
	ß coefficient	pg/mL/min/%	ATN BF	0.00 (-0.06,0.06)	0.00 (-0.09,0.10)	-0.01 (-0.08,0.07)
			Pumping	-0.04 (-0.11,0.03)	-0.04 (-0.15,0.06)	-0.03 (-0.13,0.08)
IL-8	Intercept (0% Point Est)	pg/mL/min	--	557.23 (199.47, 921.80)	409.37 (-236.32,1054.79)	594.10 (156.07,1039.05)
	100% Point Est		ATN BF	258.37 (21.17, 499.51)	140.54 (-137.14, 418.64)	352.52 (63.90, 644.14)
			Pumping	119.01 (-234.17, 477.12)	99.83 (-463.53, 668.15)	88.02 (-388.85, 565.76)
	ß coefficient	pg/mL/min/%	ATN BF	-2.99 (-6.92,0.85)	-2.69 (-9.33,3.69)	-2.42 (-7.37,2.49)
			Pumping	-4.38 (-9.35,0.60)	-3.10 (-10.42,4.14)	-5.06 (-12.20,2.31)
TNF-α	Intercept (0% Point Est)	pg/mL/min	--	4.25 (-0.84, 9.47)	3.65 (-6.05, 12.69)	4.41 (-1.93, 10.62)
	100% Point Est		ATN BF	2.21 (-1.25, 5.65)	1.79 (-2.42, 5.89)	2.46 (-1.87, 6.70)
			Pumping	0.87 (-4.16, 5.91)	0.62 (-7.51, 8.91)	0.84 (-5.99, 7.62)
	ß coefficient	pg/mL/min/%	ATN BF	-0.02 (-0.08,0.03)	-0.02 (-0.11,0.08)	-0.02 (-0.09,0.05)
			Pumping	-0.03 (-0.10,0.04)	-0.03 (-0.14,0.08)	-0.04 (-0.14,0.07)

V. Conclusion and future directions

In humans and other mammalian species, female reproduction is organized around successful gestation, an immunologically complex phenomenon that necessitates a fine balance between immune competence and fetal tolerance (Moffett and Loke, 2006; Kane et al., 2009; Erlebacher, 2013). The shifts in disease risk (e.g., increased susceptibility to viral pathogens, ameliorated symptoms of autoimmune disease) observed among pregnant women in well-studied populations have generated strong interest in elucidating the immunological mechanisms underpinning these changes. Data from an increasingly broad range of populations show that pregnancy is reliably marked by downregulated cellular immunity and parasite response (e.g., reduced lymphocyte and eosinophil counts) and increased reliance on humoral immunity and "non-specific" defense (e.g., neutrophil expansion, acute inflammation) (Belo et al., 2005; Purohit et al., 2015; Elsayed Azab, 2017; Bakrim et al., 2018), but that the magnitude of such shifts varies as a function of environment (Hové et al., 2020). To date, however, data on postpartum immunological recovery are comparatively scarce, especially outside of heavily industrialized countries, and even fewer studies have investigated the effects of breastfeeding/infant feeding behavior on postpartum immune trajectories. Consequently, much of the current knowledge of "normal" female immune function (and how it differs from male immune function across the lifespan) is limited to cycling and pregnant females within populations experiencing evolutionarily novel

conditions (e.g., energetic excess, reduced parity, microbial deprivation). This dissertation aims to address the gaps in knowledge.

In Chapter II, I utilize pre-existing data collected by the Tsimane Health and Life History Project (THLHP) and the National Health and Nutritional Survey (NHANES) to investigate how time since delivery impacts maternal immune status among the Tsimane, a natural fertility population inhabiting the Amazonian River basin, and women in the USA. I find that month since delivery exerts opposite effects on immune cell counts compared to gestation, producing a unique period of immune modulation. I also report that these postpartum effects vary between populations, with the greatest divergence in total immune cell count between Tsimane and USA women occurring at month three following parturition. Such differences in postpartum immune status appear to stem, in part, from different response patterns during pregnancy (Hové et al., 2020) and potentially divergent pathogen clearance requirements following delivery (Blackwell et al., 2015), illuminating the need for consideration of environmental inputs when determining "normal" patterns of postpartum recovery.

In Chapter III, I test the prediction that evolutionarily novel environmental conditions often characterizing post-industrial societies like the USA (e.g., lower fertility rates, sedentism) intensify evolved sex differences in immune function by increasing lifetime exposure to sex hormones in both sexes (Natri et al., 2019). Drawing on cross-population comparisons between the Tsimane and the USA, I provide strong evidence that the magnitude of sexual dimorphism in immune status is comparatively elevated in the USA. I also show that, within both populations, pregnancy produces and/or exacerbates most sex biases while sexual dimorphism is reduced or absent when females are in the postpartum

period. Taken together, these patterns suggest that the comparatively attenuated degree of overall sexual dimorphism in immune outcomes among the Tsimane relates to different immunological response to pregnancy and more time spent in the postpartum period across the reproductive lifespan (due to higher parity). Lastly, elevated neutrophil count among regularly cycling, non-pregnant, non-postpartum women in the USA compared to male counterparts indicates that this immune cell type may drive a large portion of the overarching sex biases in disease risk (e.g., female bias in autoimmune incidence, greater responsivity to myriad immune challenges) observed with the USA and other post-industrial countries.

In Chapter IV, I evaluate the impact of within-population variation in infant feeding behavior on maternal immune status, mental health, and reported physical health in a smaller but more targeted sample of mothers in the USA. I predicted that replacement or heavy supplementation of at-the-nipple breastfeeding would correspond to markers of heightened inflammation, more sickness behavior (e.g., depressive symptoms) and more symptoms of physical illness because of environmental mismatch. I also predicted that, due to increasing cost of lactation and risk of maternal-offspring conflict, exclusive at-the-nipple breastfeeding among mothers in the later postpartum period would be associated with diminishing returns on immune and health benefits. My results indicate that most mothers utilize both at-the-nipple breastfeeding and other infant feeding methods, especially pumping, highlighting the shifting landscape in infant feeding behavior (Rasmussen and Geraghty, 2011). I find that increased frequency of both at-the-nipple breastfeeding and pumping correspond to fewer indicators of postpartum depression in the early postpartum period, but that the relationship between pumping and depression is reversed among mothers in the late postpartum period. Furthermore, my findings show that heavy reliance on pumping is associated with increased

symptoms of physical illness, regardless of time since delivery, suggesting that pumping may be more physically taxing than at-the-nipple breastfeeding. Even in the relative absence of pathogen exposure, frequency of at-the-nipple breastfeeding and pumping both correspond to a more tempered evening-to-morning change in secretion rates of IL-8 and C-reactive protein (a maker of acute systemic inflammation), indicating similar anti-inflammatory effects of both infant feeding methods compared to other forms of supplementation.

Based on these findings, this dissertation provides a springboard for future research on immunological function across female reproduction and its consequences for health and wellbeing. Here, I highlight two primary avenues for future exploration: (A) broader sampling of the postpartum period across and within populations, and (B) broader and more specific measures of immune function, with specific focus on neutrophils as a potential driver of deleterious outcomes in females during and after pregnancy and overarching sex biases in disease risk often observed in post-industrial societies.

While drawing comparisons between the Tsimane and the USA provides a good starting point for contextualizing "normal" immune function during different phases of female reproduction (and the ensuing degree of dimorphism between males and females across the life course), future research on a wider breadth of populations is critical. Even the Tsimane and the Shuar, another non-industrialized Amazonian population, exhibit substantial differences in immune status (Blackwell et al., 2011), highlighting the fact that subsistence populations cannot be used interchangeably to provide context for data generated within post-industrial populations. As market integration, increasing urban population density, water sanitation, hospital access, shifts reproductive strategies, and other aspects of industrialization become increasingly shared norms across the globe (often in patchwork pattern), diverse combinations

of socioecological factors will extend the gradient of immunological variation – further moderated by underlying variability in endemic pathogen composition. This increasingly diverse array of socioecological conditions provides fertile ground for future research investigating the effects of local pathogen composition, reproductive strategies, hormone production, mode of delivery, and breastfeeding behavior on the immunology of female reproduction and concordant impacts on maternal health and wellbeing.

Furthermore, detailed investigation of immunological phenotypes and their relationship to specific disease outcomes is crucial. While beneficial for establishing broad patterns, use of basic immune cell counts, salivary cytokine profiles, and general markers of inflammatory status limited my ability to make any robust claims regarding immunological responsivity to different challenges (e.g., fetal antigens during pregnancy, breastfeeding bouts). Considering that results for all three chapters of this dissertation specifically pointed to neutrophils as a key immune component driving variation between and within populations, we strongly recommend that future studies focus on this immune cell type. Aside from increase prevalence, what role to neutrophils play in shaping the maternal immune response to fetal antigens during pregnancy? Much focus has been given to the role of eosinophils in parasitic infection and allergic response, but what effect does chronic helminth exposure (or lack thereof) have on neutrophil function? Continuing research into the wide-ranging functions of neutrophils has illuminated many unexpected roles that these cells play in generated responses to numerous immune challenges (Anthony et al., 2007; Hosoki et al., 2016; Gardinassi et al., 2017; Won et al., 2018), laying the groundwork for continuing research into how neutrophil phenotype is influenced by ecological conditions during development and consequent divergence in response patterns during each phase of female reproduction.

References

Adegnika AA, Agnandji ST, Chai SK, Ramharter M, Breitling L, Kendjo E, Issifou S, Yazdanbakhsh M, Kombila M, Kremsner PG. 2007. Increased prevalence of intestinal helminth infection during pregnancy in a Sub-Saharan African community. Wien Klin Wochenschr [Internet] 119:712–716. Available from: http://link.springer.com/10.1007/s00508-007-0907-z

Akingbola TS, Adewole IF, Adesina OA, Afolabi KA, Fehintola FA, Bamgboye EA, Aken'Ova YA, Shokunbi WA, Anwo JA, Nwegbu MM. 2006. Haematological profile of healthy pregnant women in Ibadan, South-western Nigeria. J Obstet Gynaecol (Lahore) 26:763–769.

Al-Awadhi N, Al-Kandari N, Al-Hasan T, Almurjan D, Ali S, Al-Taiar A. 2013. Age at menarche and its relationship to body mass index among adolescent girls in Kuwait. BMC Public Health 13.

Al-Shehri SS, Knox CL, Liley HG, Cowley DM, Wright JR, Henman MG, Hewavitharana AK, Charles BG, Shaw PN, Sweeney EL, Duley JA. 2015. Breastmilk-saliva interactions boost innate immunity by regulating the oral microbiome in early infancy. PLoS One 10:1–19.

ALLOLIO B, HOFFMANN J, LINTON EA, WINKELMANN W, KUSCHE M, SCHULTE HM. 1990. Diurnal Salivary Cortisol Patterns During Pregnancy and After Delivery: Relationship To Plasma Corticotrophin-Releasing-Hormone. Clin Endocrinol (Oxf) 33:279–289.

de Almeida PDV, Grégio AMT, Machado MAN, de Lima AAS, Azevedo LR. 2008. Saliva composition and functions: a comprehensive review. J Contemp Dent Pract [Internet] 9:72–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18335122

Almeneessier AS, BaHammam AA, Alzoghaibi M, Olaish AH, Nashwan SZ, BaHammam AS. 2019. The effects of diurnal intermittent fasting on proinflammatory cytokine levels while controlling for sleep/wake pattern, meal composition and energy expenditure. PLoS One [Internet] 14:e0226034. Available from: https://dx.plos.org/10.1371/journal.pone.0226034

Alvarado LC, Valeggia CR, Ellison PT, Lewarch CL, Muller MN. 2019. A Comparison of men's Life History, Aging, and Testosterone Levels among Datoga Pastoralists, Hadza Foragers, and Qom Transitional Foragers. Adapt Hum Behav Physiol [Internet] 5:251–273. Available from: http://link.springer.com/10.1007/s40750-019-00116-1

Amadori A, Zamarchi R, De Silvestro G, Forza G, Cavatton G, Danieli GA, Clementi M, Chieco-Bianchi L. 1995. Genetic control of the CD4/CD8 T-cell ratio in humans. Nat Med [Internet] 1:1279–1283. Available from: http://www.nature.com/articles/nm1295-1279

Angelopoulou A, Field D, Ryan CA, Stanton C, Hill C, Ross RP. 2018. The microbiology and treatment of human mastitis. Med Microbiol Immunol [Internet] 207:83–94. Available from: http://dx.doi.org/10.1007/s00430-017-0532-z

Anthony RM, Rutitzky LI, Urban Jr. JF, Stadecker MJ, Gause WC. 2007. Protective immune mechanisms in helminth infection. Nat Rev Immunol [Internet] 7:975–987. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18007680

Armstrong GL, Conn LA, Pinner RW. 1999. Trends in infectious disease mortality in the United States during the 20th century. J Am Med Assoc 281:61–66.

Bain BJ. 1996. Ethnic and sex differences in the total and differential white cell count and platelet count. J Clin Pathol [Internet] 49:664–666. Available from: http://jcp.bmj.com/cgi/doi/10.1136/jcp.49.8.664

Baker JL, Gamborg M, Heitmann BL, Lissner L, Sørensen TIA, Rasmussen KM. 2008. Breastfeeding reduces postpartum weight retention. Am J Clin Nutr 88:1543–1551.

Bakrim S, Motiaa Y, Ouarour A, Masrar A. 2018. Hematological parameters of the blood count in a healthy population of pregnant women in the northwest of Morocco (Tetouan-M'diq-Fnideq provinces). Pan Afr Med J 29:1–12.

Ballard O, Morrow AL. 2013. Human Milk Composition. Pediatr Clin North Am [Internet] 60:49–74. Available from: http://fn.bmj.com/cgi/doi/10.1136/archdischild-2015-308164%5Cnhttp://ajpregu.physiology.org/lookup/doi/10.1152/ajpregu.00504.2014%5Cnhttp://dx.plos.org/10.1371/journal.pone.0140587%5Cnhttp://www.nutritionj.com/content/12/1/103%5Cnhttp://www.ncbi.nlm.nih.g

Belo L, Santos-Silva A, Rocha S, Caslake M, Cooney J, Pereira-Leite L, Quintanilha A, Rebelo I. 2005. Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. Eur J Obstet Gynecol Reprod Biol [Internet] 123:46–51. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0301211505000849

Beral V, Bull D, Doll R, Peto R, Reeves G. 2002. Breast cancer and breastfeeding: Collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50 302 women with breast cancer and 96 973 women without the disease. Lancet 360:187–195.

Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. 1996. Male Fetal Progenitor Cells Persist in Maternal Blood for as Long as 27 Years Postpartum. Proc Natl Acad Sci 93:705–708.

Bianchi VE. 2018. Weight loss is a critical factor to reduce inflammation. Clin Nutr ESPEN [Internet] 28:21–35. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2405457718303954

Blackwell AD, Gurven MD, Sugiyama LS, Madimenos FC, Liebert MA, Martin MA, Kaplan HS, Snodgrass JJ. 2011. Evidence for a Peak Shift in a Humoral Response to Helminths: Age Profiles of IgE in the Shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. PLoS Negl Trop Dis [Internet] 5:e1218. Available from: https://dx.plos.org/10.1371/journal.pntd.0001218

Blackwell AD, Tamayo MA, Beheim B, Trumble BC, Stieglitz J, Hooper PL, Martin M, Kaplan H, Gurven M. 2015. Helminth infection, fecundity, and age of first pregnancy in women. Science (80-) 350:6–9.

Blackwell AD, Trumble BC, Maldonado Suarez I, Stieglitz J, Beheim B, Snodgrass JJ, Kaplan H, Gurven M. 2016a. Immune function in Amazonian horticulturalists. Ann Hum Biol 43:382–396.

Blackwell AD, Trumble BC, Maldonado Suarez I, Stieglitz J, Beheim B, Snodgrass JJ, Kaplan H, Gurven M. 2016b. Immune function in Amazonian horticulturalists. Ann Hum Biol [Internet] 43:382–396. Available from: http://www.tandfonline.com/doi/full/10.1080/03014460.2016.1189963

Blackwell AD, Urlacher SS, Beheim B, von Rueden C, Jaeggi A, Stieglitz J, Trumble BC, Gurven M, Kaplan H. 2017. Growth references for Tsimane forager-horticulturalists of the Bolivian Amazon. Am J Phys Anthropol [Internet] 162:441–461. Available from:

http://doi.wiley.com/10.1002/ajpa.23128
Bloomfield SF, Rook GAW, Scott EA, Shanahan F, Stanwell-Smith R, Turner P. 2016. Time to abandon the hygiene hypothesis: New perspectives on allergic disease, the human microbiome, infectious disease prevention and the role of targeted hygiene. Perspect Public Health 136:213–224.
Boddy AM, Fortunato A, Wilson Sayres M, Aktipis A. 2015. Fetal microchimerism and maternal health: A review and evolutionary analysis of cooperation and conflict beyond the womb. BioEssays [Internet] 37:1106–1118. Available from: http://doi.wiley.com/10.1002/bies.201500059
Boel ME, Rijken MJ, Brabin BJ, Nosten F, McGready R. 2012. The epidemiology of postpartum malaria: A systematic review. Malar J 11:1–7.
Breakey AA, Hinde K, Valeggia CR, Sinofsky A, Ellison PT. 2015. Illness in breastfeeding infants relates to concentration of lactoferrin and secretory Immunoglobulin A in mother's milk. Evol Med Public Heal 2015:21–31.
Bribiescas RG. 1996. Testosterone levels among Aché hunter-gatherer men. Hum Nat [Internet] 7:163–188. Available from: http://link.springer.com/10.1007/BF02692109
Brindle E, Fujita M, Shofer J, O'Connor KA. 2010. Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research. J Immunol Methods [Internet] 362:112–120. Available from: http://dx.doi.org/10.1016/j.jim.2010.09.014
Bürkner P-C. 2017. brms : An R Package for Bayesian Multilevel Models Using Stan. J Stat Softw 80:1–27.
Butte NF, King JC. 2005a. Energy requirements during pregnancy and lactation. Public Health Nutr [Internet] 8:1010–1027. Available from: http://www.journals.cambridge.org/abstract_S136898000500131X
Butte NF, King JC. 2005b. Energy requirements during pregnancy and lactation. Public Health Nutr 8:1010–1027.
Carlisle SM, Qin H, Hendrickson RC, Muwanguzi JE, Lefkowitz EJ, Kennedy RE, Yan Z, Yacoubian TA, Benveniste EN, West AB, Harms AS, Standaert DG. 2021. Sex-based differences in the activation of peripheral blood monocytes in early Parkinson disease. npj Park Dis [Internet] 7:36. Available from: http://dx.doi.org/10.1038/s41531-021-00180-z
Carter AM, Enders AC, Pijnenborg R. 2015. The role of invasive trophoblast in implantation and placentation of primates. Philos Trans R Soc B Biol Sci [Internet] 370:20140070. Available from: https://royalsocietypublishing.org/doi/10.1098/rstb.2014.0070
Chaim W, Burstein E. 2003. Postpartum infection treatments: a review. Expert Opin Pharmacother [Internet] 4:1297–1313. Available from: http://www.tandfonline.com/doi/full/10.1517/14656566.4.8.1297
Chen A, Rogan WJ. 2004. Breastfeeding and the Risk of Postneonatal Death in the United States. Pediatrics [Internet] 113:e435–e439. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.113.5.e435
Chen Y, Zhang Y, Zhao G, Chen C, Yang P, Ye S, Tan X. 2016. Difference in Leukocyte Composition between Women before and after Menopausal Age, and Distinct Sexual Dimorphism. PLoS One [Internet] 11:e0162953. Available from: http://dx.plos.org/10.1371/journal.pone.0162953
Chuong EB, Hannibal RL, Green SL, Baker JC. 2013. Evolutionary perspectives into

placental biology and disease. Appl Transl Genomics [Internet] 2:64–69. Available from: http://dx.doi.org/10.1016/j.atg.2013.07.001
Clodi M, Vila G, Geyeregger R, Riedl M, Stulnig TM, Struck J, Luger TA, Luger A. 2008. Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. Am J Physiol Metab [Internet] 295:E686–E691. Available from: https://www.physiology.org/doi/10.1152/ajpendo.90263.2008
Coss SL, Torres-Cornejo A, Prasad MR, Moore-Clingenpeel M, Grakoui A, Lauer GM, Walker CM, Honegger JR. 2020. CD4+ T cell restoration and control of hepatitis C virus replication after childbirth. J Clin Invest [Internet] 130:748–753. Available from: https://www.jci.org/articles/view/123623
Costanza M, Binart N, Steinman L, Pedotti R. 2015. Prolactin: A versatile regulator of inflammation and autoimmune pathology. Autoimmun Rev [Internet] 14:223–230. Available from: http://dx.doi.org/10.1016/j.autrev.2014.11.005
Cox JL, Holden JM, Sagovsky R. 1987. Detection of Postnatal Depression. Br J Psychiatry [Internet] 150:782–786. Available from: https://www.cambridge.org/core/product/identifier/S0007125000214712/type/journal_article
Dawe GS, Tan XW, Xiao ZC. 2007. Cell migration from baby to mother. Cell Adh Migr 1:19–27.
Deing V, Roggenkamp D, Kühnl J, Gruschka A, Stäb F, Wenck H, Bürkle A, Neufang G. 2013. Oxytocin modulates proliferation and stress responses of human skin cells: Implications for atopic dermatitis. Exp Dermatol 22:399–405.
Elsayed Azab A. 2017. Haematological Parameters in Pregnant Women Attended Antenatal Care at Sabratha Teaching Hospital in Northwest, Libya. Am J Lab Med [Internet] 2:60. Available from: http://www.sciencepublishinggroup.com/journal/paperinfo?journalid=235&doi=10.11648/j.ajlm.20170204.14
Emaus A, Espetvedt S, Veierød MB, Ballard-Barbash R, Furberg AS, Ellison PT, Jasienska G, Hjartåker A, Thune I. 2008. 17-Beta-Estradiol in Relation To Age At Menarche and Adult Obesity in Premenopausal Women. Hum Reprod 23:919–927.
Erlebacher A. 2013. Mechanisms of T cell tolerance towards the allogeneic fetus. Nat Rev Immunol [Internet] 13:23–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23237963
Fetherston C. 1998. Risk Factors for Lactation Mastitis. J Hum Lact [Internet] 14:101–109. Available from: http://journals.sagepub.com/doi/10.1177/089033449801400209
Field CJ. 2005. The immunological components of human milk and their effect on immune development in infants. J Nutr 135:1–4.
Fish EN. 2008. The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 8:737–744.
Foo YZ, Nakagawa S, Rhodes G, Simmons LW. 2017. The effects of sex hormones on immune function: a meta-analysis. Biol Rev [Internet] 92:551–571. Available from: http://doi.wiley.com/10.1111/brv.12243
Foster Z, Byron E, Reyes-García V, Huanca T, Vadez V, Apaza L, Pérez E, Tanner S, Gutierrez Y, Sandstrom B, Yakhedts A, Osborn C, Godoy RA, Leonard WR. 2005. Physical growth and nutritional status of Tsimane' Amerindian children of lowland Bolivia. Am J Phys Anthropol [Internet] 126:343–351. Available from:

http://doi.wiley.com/10.1002/ajpa.20098
Fuxjager MJ, Foufopoulos J, Diaz-Uriarte R, Marler CA. 2011. Functionally opposing effects of testosterone on two different types of parasite: implications for the immunocompetence handicap hypothesis. Funct Ecol [Internet] 25:132–138. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2435.2010.01784.x
García-Durán M, de Frutos T, Díaz-Recasens J, García-Gálvez G, Jiménez A, Montón M, Farré J, de Miguel LS, González-Fernández F, Arriero M del M, Rico L, García R, Casado S, López-Farré A. 1999. Estrogen Stimulates Neuronal Nitric Oxide Synthase Protein Expression in Human Neutrophils. Circ Res [Internet] 85:1020–1026. Available from: https://www.ahajournals.org/doi/10.1161/01.RES.85.11.1020
Gardinassi LG, DeSouza-Vieira TS, da Silva NO, Garcia GR, Borges VM, Campos RNS, de Almeida RP, de Miranda Santos IKF, Saraiva EM. 2017. Molecular signatures of neutrophil extracellular traps in human visceral leishmaniasis. Parasit Vectors [Internet] 10:285. Available from: http://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-017-2222-5
Gardner H, Kent JC, Lai CT, Mitoulas LR, Cregan MD, Hartmann PE, Geddes DT. 2015. Milk ejection patterns: An intra- individual comparison of breastfeeding and pumping. BMC Pregnancy Childbirth [Internet] 15:1–6. Available from: http://dx.doi.org/10.1186/s12884-015-0583-3
Garratt M, Gaillard JM, Brooks RC, Lemaître JF. 2013. Diversification of the eutherian placenta is associated with changes in the pace of life. Proc Natl Acad Sci U S A 110:7760–7765.
Gayen S, Maclary E, Hinten M, Kalantry S. 2016. Sex-specific silencing of X-linked genes by Xist RNA. Proc Natl Acad Sci [Internet] 113:E309–E318. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1515971113
Gebreweld A, Bekele D, Tsegaye A. 2018. Hematological profile of pregnant women at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. BMC Hematol [Internet] 18:15. Available from: https://bmchematol.biomedcentral.com/articles/10.1186/s12878-018-0111-6
Geddes DT, Aljazaf KM, Kent JC, Prime DK, Spatz DL, Garbin CP, Lai CT, Hartmann PE. 2012. Blood flow characteristics of the human lactating breast. J Hum Lact 28:145–152.
Genetu M, Damtie D, Workineh M, Mathewos Tebeje B, Enawgaw B, Deressa T. 2017. Immunological and hematological reference intervals among HIV-seronegative pregnant women in northwest Ethiopia. Int J Womens Health [Internet] Volume 9:145–150. Available from: https://www.dovepress.com/immunological-and-hematological-reference-intervals-among-hiv-seronega-peer-reviewed-article-IJWH
Gill CJ, Mwananyanda L, MacLeod WB, Kwenda G, Pieciak R, Mupila Z, Murphy C, Chikoti C, Forman L, Berklein F, Lapidot R, Chimoga C, Ngoma B, Larson A, Lungu J, Nakazwe R, Nzara D, Pemba L, Yankonde B, Chirwa A, Mwale M, Thea DM. 2022. Infant deaths from respiratory syncytial virus in Lusaka, Zambia from the ZPRIME study: a 3-year, systematic, post-mortem surveillance project. Lancet Glob Heal [Internet] 10:e269–e277. Available from: http://dx.doi.org/10.1016/S2214-109X(21)00518-0
Goetz TG, Valeggia C. 2017. The ecology of anemia: Anemia prevalence and correlated factors in adult indigenous women in Argentina. Am J Hum Biol [Internet] 29. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ajhb.22947

Gray PB, Chapman JF, Burnham TC, McIntyre MH, Lipson SF, Ellison PT. 2004. Human Male Pair Bonding and Testosterone. Hum Nat 15:119–131.
Gray RH, Li X, Kigozi G, Serwadda D, Brahmbhatt H, Wabwire-Mangen F, Nalugoda F, Kiddugavu M, Sewankambo N, Quinn TC, Reynolds SJ, Wawer MJ. 2005. Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study. Lancet [Internet] 366:1182–1188. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673605674818
Groer MW, Davis MW, Smith K, Casey K, Kramer V, Bukovsky E. 2005. Immunity, Inflammation and Infection in Post-partum Breast and Formula Feeders. Am J Reprod Immunol [Internet] 54:222–231. Available from: http://doi.wiley.com/10.1111/j.1600-0897.2005.00301.x
Gude NM, Roberts CT, Kalionis B, King RG. 2004. Growth and function of the normal human placenta. Thromb Res 114:397–407.
Gundersen TD, Krebs L, Loekkegaard ECL, Rasmussen SC, Glavind J, Clausen TD. 2018. Postpartum urinary tract infection by mode of delivery: a Danish nationwide cohort study. BMJ Open [Internet] 8:e018479. Available from: https://bmjopen.bmj.com/lookup/doi/10.1136/bmjopen-2017-018479
Gurven M, Kaplan H, Supa AZ. 2007a. Mortality experience of Tsimane Amerindians of Bolivia: Regional variation and temporal trends. Am J Hum Biol [Internet] 19:376–398. Available from: http://doi.wiley.com/10.1002/ajhb.20600
Gurven M, Kaplan H, Supa AZ. 2007b. Mortality experience of Tsimane Amerindians of Bolivia: Regional variation and temporal trends. Am J Hum Biol 19:376–398.
Gurven M, Kaplan H, Trumble B, Stieglitz J. 2019. The biodemography of human health in contemporary non-industrial populations: Insights from the Tsimane Health and Life History Project. In: Burger O, Lee R, Sear R, editors. Human Evolutionary Demography. . p 1–41.
Gurven M, Kaplan H, Winking J, Finch C, Crimmins EM. 2008. Aging and inflammation in two epidemiological worlds. J Gerontol A Biol Sci Med Sci 63:196–199.
Gurven M, Stieglitz J, Trumble B, Blackwell AD, Beheim B, Davis H, Hooper P, Kaplan H. 2017. The Tsimane Health and Life History Project: Integrating anthropology and biomedicine. Evol Anthropol 26:54–73.
Gurven MD, Trumble BC, Stieglitz J, Yetish G, Cummings D, Blackwell AD, Beheim B, Kaplan HS, Pontzer H. 2016. High resting metabolic rate among Amazonian forager-horticulturalists experiencing high pathogen burden. Am J Phys Anthropol [Internet] 161:414–425. Available from: http://doi.wiley.com/10.1002/ajpa.23040
Haig D. 1993. Genetic Conflicts in Human Pregnancy. Q Rev Biol [Internet] 68:495–532. Available from: https://www.journals.uchicago.edu/doi/10.1086/418300
Haig D. 2010. Transfers and transitions: Parent-offspring conflict, genomic imprinting, and the evolution of human life history. Proc Natl Acad Sci [Internet] 107:1731–1735. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0904111106
Haig D. 2014. Troubled sleep: Night waking, breastfeeding and parent-offspring conflict. Evol Med Public Heal [Internet] 2014:32–39. Available from: https://academic.oup.com/emph/article-lookup/doi/10.1093/emph/eou005
Hannah ME, Ohlsson A, Farine D, Hewson SA, Hodnett ED, Myhr TL, Wang EEL, Weston JA, Willan AR. 1996. Induction of Labor Compared with Expectant Management for Prelabor Rupture of the Membranes at Term. N Engl J Med [Internet] 334:1005–1010.

Available from: http://www.nejm.org/doi/abs/10.1056/NEJM199604183341601
Hannah ME, Ohlsson A, Wang EEL, Matlow A, Foster GA, Willan AR, Hodnett ED, Weston JA, Farine D, Seaward PGR. 1997. Maternal colonization with group B Streptococcus and prelabor rupture of membranes at term: The role of induction of labor. Am J Obstet Gynecol [Internet] 177:780–785. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002937897702680
Hassiotou F, Hepworth AR, Metzger P, Tat Lai C, Trengove N, Hartmann PE, Filgueira L. 2013. Maternal and infant infections stimulate a rapid leukocyte response in breastmilk. Clin Transl Immunol [Internet] 2:e3. Available from: http://dx.doi.org/10.1038/cti.2013.1
Hernández LM, Taylor MK. 2017. Diurnal Pattern of Salivary C-Reactive Protein and Associations with Biobehavioral Correlates in Military Men. Med Sci Sport Exerc 49:96.
Hewagama A, Patel D, Yarlagadda S, Strickland FM, Richardson BC. 2009. Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis. Genes Immun [Internet] 10:509–516. Available from: http://www.nature.com/articles/gene200912
Ho DE, Imai K, King G, Stuart EA. 2007. Matching as Nonparametric Preprocessing for Reducing Model Dependence in Parametric Causal Inference. Polit Anal [Internet] 15:199–236. Available from: https://www.cambridge.org/core/product/identifier/S1047198700006483/type/journal_article
Hobbs AJ, Mannion CA, McDonald SW, Brockway M, Tough SC. 2016. The impact of caesarean section on breastfeeding initiation, duration and difficulties in the first four months postpartum. BMC Pregnancy Childbirth [Internet] 16:1–9. Available from: http://dx.doi.org/10.1186/s12884-016-0876-1
Høj L. 2003. Maternal mortality: only 42 days? BJOG An Int J Obstet Gynaecol [Internet] 110:995–1000. Available from: http://doi.wiley.com/10.1016/S1470-0328(03)03907-7
Hollegaard B, Byars SG, Lykke J, Boomsma JJ. 2013. Parent-Offspring Conflict and the Persistence of Pregnancy-Induced Hypertension in Modern Humans. PLoS One 8.
Hosoki K, Itazawa T, Boldogh I, Sur S. 2016. Neutrophil recruitment by allergens contribute to allergic sensitization and allergic inflammation. Curr Opin Allergy Clin Immunol [Internet] 16:45–50. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00130832-201602000-00009
Hové C, Trumble BC, Anderson AS, Stieglitz J, Kaplan H, Gurven MD, Blackwell AD. 2020. Immune function during pregnancy varies between ecologically distinct populations. Evol Med Public Heal [Internet] 2020:114–128. Available from: https://academic.oup.com/emph/article/2020/1/114/5866980
Hui LL, Schooling CM, Wong MY, Ho LM, Lam TH, Leung GM. 2010. Infant Growth During the First Year of Life and Subsequent Hospitalization to 8 Years of Age. Epidemiology [Internet] 21:332–339. Available from: https://journals.lww.com/00001648-201005000-00008
Hunter LP, Rychnovsky JD, Yount SM. 2009. A selective review of maternal sleep characteristics in the postpartum period. JOGNN - J Obstet Gynecol Neonatal Nurs 38:60–68.

Hurley WL, Theil PK. 2011. Perspectives on immunoglobulins in colostrum and milk. Nutrients 3:442–474.
Ifeanyi OE, Ndubuisi OT, Obioma E, Leticia B, Chinedum Uche E. 2014. Haematological profile of pregnant women in Umuahia, Abia State, Nigeria. IntJCurrMicrobiolAppSci 3:713–718.
Ip S, Chung M, Raman G, Chew P, Magula N, Trikalinos T, Lau J, Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, Trikalinos T, Lau J. 2007. Breastfeeding and maternal and infant health outcomes in developed countries. Evid Technol Asses (Full Rep) 153:1–186.
Irwin MR. 2006. Sleep Deprivation and Activation of Morning Levels of Cellular and Genomic Markers of Inflammation. Arch Intern Med [Internet] 166:1756. Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.166.16.1756
İşeri SÖ, Şener G, Sağlam B, Gedik N, Ercan F, Yeğen BÇ. 2005. Oxytocin Protects Against Sepsis-Induced Multiple Organ Damage: Role of Neutrophils. J Surg Res [Internet] 126:73–81. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022480405000557
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 Behav Immun [Internet] 27:38–41. Available from: http://dx.doi.org/10.1016/j.bbi.2012.07.001
Jacobson DL, Gange SJ, Rose NR, Graham NMH. 1997. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clin Immunol Immunopathol 84:223–243.
Jaillon S, Berthenet K, Garlanda C. 2019. Sexual Dimorphism in Innate Immunity. Clin Rev Allergy Immunol [Internet] 56:308–321. Available from: http://link.springer.com/10.1007/s12016-017-8648-x
Jamieson DJ, Honein MA, Rasmussen SA, Williams JL, Swerdlow DL, Biggerstaff MS, Lindstrom S, Louie JK, Christ CM, Bohm SR, Fonseca VP, Ritger KA, Kuhles DJ, Eggers P, Bruce H, Davidson HA, Lutterloh E, Harris ML, Burke C, Cocoros N, Finelli L, MacFarlane KF, Shu B, Olsen SJ. 2009. H1N1 2009 influenza virus infection during pregnancy in the USA. Lancet 374:451–458.
Jasienska G, Ellison P. 2004. Energetic factors and seasonal changes in ovarian function in women from rural Poland. Am J Hum Biol 16:563–580.
Jasienska G, Ellison PT. 1998. Physical work causes suppression of ovarian function in women. ProcBiolSci [Internet] 265:1847–1851. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1689377/pdf/9802241.pdf
Jokisch BD, McSweeney K. 2011. Assessing the Potential of Indigenous-Run Demographic/Health Surveys: the 2005 Shuar Survey, Ecuador. Hum Ecol [Internet] 39:683–698. Available from: http://link.springer.com/10.1007/s10745-011-9419-6
Jones NGB, Smith LC, O'Connell JF, Hawkes K, Kamuzora CL. 1992. Demography of the Hadza, an increasing and high density population of savanna foragers. Am J Phys Anthropol [Internet] 89:159–181. Available from: http://doi.wiley.com/10.1002/ajpa.1330890204
Joseph KS, Boutin A, Lisonkova S, Muraca GM, Razaz N, John S, Mehrabadi A, Sabr Y, Ananth C V., Schisterman E. 2021. Maternal Mortality in the United States. Obstet Gynecol [Internet] 137:763–771. Available from: https://journals.lww.com/10.1097/AOG.0000000000004361

Kabakian-Khasholian T, Kaddour A, DeJong J, Shayboub R, Nassar A. 2007. The policy environment encouraging C-section in Lebanon. Health Policy (New York) [Internet] 83:37–49. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168851006002624
Kadel S, Kovats S. 2018. Sex Hormones Regulate Innate Immune Cells and Promote Sex Differences in Respiratory Virus Infection. Front Immunol [Internet] 9:1–15. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2018.01653/full
Kane N, Kelly R, Saunders PTK, Critchley HOD. 2009. Proliferation of Uterine Natural Killer Cells Is Induced by Human Chorionic Gonadotropin and Mediated via the Mannose Receptor. Endocrinology [Internet] 150:2882–2888. Available from: https://academic.oup.com/endo/article/150/6/2882/2456331
Kaplan H, Hooper PL, Stieglitz J, Gurven M. 2015. The Causal Relationship between Fertility and Infant Mortality. Popul Hum Sci:361–376.
Kaplan H, Thompson RC, Trumble BC, Wann LS, Allam AH, Beheim B, Frohlich B, Sutherland ML, Sutherland JD, Stieglitz J, Rodriguez DE, Michalik DE, Rowan CJ, Lombardi GP, Bedi R, Garcia AR, Min JK, Narula J, Finch CE, Gurven M, Thomas GS. 2017. Coronary atherosclerosis in indigenous South American Tsimane: a cross-sectional cohort study. Lancet [Internet] 389:1730–1739. Available from: http://dx.doi.org/10.1016/S0140-6736(17)30752-3
Kaplowitz P. 1998. Delayed puberty in obese boys: Comparison with constitutional delayed puberty and response to testosterone therapy. J Pediatr [Internet] 133:745–749. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022347698701441
Karita E, Ketter N, Price MA, Kayitenkore K, Kaleebu P, Nanvubya A, Anzala O, Jaoko W, Mutua G, Ruzagira E, Mulenga J, Sanders EJ, Mwangome M, Allen S, Bwanika A, Bahemuka U, Awuondo K, Omosa G, Farah B, Amornkul P, Birungi J, Yates S, Stoll-Johnson L, Gilmour J, Stevens G, Shutes E, Manigart O, Hughes P, Dally L, Scott J, Stevens W, Fast P, Kamali A. 2009. CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. PLoS One 4.
Kelly CD, Stoehr AM, Nunn C, Smyth KN, Prokop ZM. 2018. Sexual dimorphism in immunity across animals: a meta-analysis. Ecol Lett [Internet] 21:1885–1894. Available from: http://doi.wiley.com/10.1111/ele.13164
Khashan AS, Kenny LC, Laursen TM, Mahmood U, Mortensen PB, Henriksen TB, O'Donoghue K. 2011. Pregnancy and the risk of Autoimmune Disease. PLoS One 6.
Klein SL. 2004. Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite Immunol [Internet] 26:247–264. Available from: http://doi.wiley.com/10.1111/j.0141-9838.2004.00710.x
Klein SL, Flanagan KL. 2016. Sex differences in immune responses. Nat Rev Immunol [Internet] 16:626–638. Available from: http://www.nature.com/articles/nri.2016.90
Klein SL, Huber S. 2010. Sex Differences in Susceptibility to Viral Infection. In: Sex Hormones and Immunity to Infection. Berlin, Heidelberg: Springer Berlin Heidelberg. p 93–122. Available from: http://link.springer.com/10.1007/978-3-642-02155-8_4
Klein SL, Jedlicka A, Pekosz A. 2010. The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis [Internet] 10:338–349. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1473309910700499
Klein SL, Marriott I, Fish EN. 2015. Sex-based differences in immune function and responses to vaccination. Trans R Soc Trop Med Hyg [Internet] 109:9–15. Available

from: https://academic.oup.com/trstmh/article-lookup/doi/10.1093/trstmh/tru167
KLEIN SL, NELSON RJ. 1999. Social interactions unmask sex differences in humoral immunity in voles. Anim Behav [Internet] 57:603–610. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0003347298910387
Koçar IH, Yesilova Z, Özata M, Turan M, Sengül A, Özdemir IÇ. 2001. The effect of testosterone replacement treatment on immunological features of patients with Klinefelter's syndrome. Clin Exp Immunol [Internet] 121:448–452. Available from: https://academic.oup.com/cei/article/121/3/448/6461640
Kühnert M, Strohmeier R, Stegmüller M, Halberstadt E. 1998. Changes in lymphocyte subsets during normal pregnancy. Eur J Obstet Gynecol Reprod Biol 76:147–151.
Kuzawa CW, Adair LS, Borja J, Mcdade TW. 2013. C-reactive protein by pregnancy and lactational status among Filipino young adult women. Am J Hum Biol [Internet] 25:131–134. Available from: http://doi.wiley.com/10.1002/ajhb.22351
Laffont S, Blanquart E, Guéry J-C. 2017. Sex Differences in Asthma: A Key Role of Androgen-Signaling in Group 2 Innate Lymphoid Cells. Front Immunol [Internet] 8:1–7. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2017.01069/full
Leiter V, Agiliga A, Kennedy E, Mecham E. 2022. Pay at the pump?: Problems with electric breast pumps. Soc Sci Med [Internet] 292:114625. Available from: https://doi.org/10.1016/j.socscimed.2021.114625
Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS. 1997. Presence of fetal DNA in maternal plasma and serum. Lancet [Internet] 350:485–487. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673697021740
Lo YMD, Zhang J, Leung TN, Lau TK, Chang AMZ, Hjelm NM. 1999. Rapid Clearance of Fetal DNA from Maternal Plasma. Am J Hum Genet [Internet] 64:218–224. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0002929707616759
Loewenberg Weisband Y, Keim SA, Keder LM, Geraghty SR, Gallo MF. 2017. Early Breast Milk Pumping Intentions among Postpartum Women. Breastfeed Med 12:28–32.
Long Q, Kempas T, Madede T, Klemetti R, Hemminki E. 2015. Caesarean section rates in Mozambique. BMC Pregnancy Childbirth [Internet] 15:253. Available from: http://bmcpregnancychildbirth.biomedcentral.com/articles/10.1186/s12884-015-0686-x
Lönnerdal B. 2003. Nutritional and physiologic significance of human milk proteins. Am J Clin Nutr [Internet] 77:1537S-1543S. Available from: https://academic.oup.com/ajcn/article/77/6/1537S/4689886
Lorenz TK, Heiman JR, Demas GE. 2015. Sexual activity modulates shifts in TH1/TH2 cytokine profile across the menstrual cycle: An observational study. Fertil Steril 104:1513-1521.e4.
Lorenz TK, Heiman JR, Demas GE. 2018. Interactions Among Sexual Activity, Menstrual Cycle Phase, and Immune Function in Healthy Women. J Sex Res [Internet] 55:1087–1095. Available from: https://doi.org/10.1080/00224499.2017.1394961
Løvås K, Husebye ES. 2002. High prevalence and increasing incidence of Addison's disease in western Norway. Clin Endocrinol (Oxf) 56:787–791.
Lyall F. 2005. Priming and remodelling of human placental bed spiral arteries during pregnancy - A Review. Placenta 26.
Mackeen AD, Packard RE, Ota E, Speer L. 2015. Antibiotic regimens for postpartum endometritis. Cochrane database Syst Rev [Internet]:CD001067. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25922861

Mandala WL, Gondwe EN, Molyneux ME, MacLennan JM, MacLennan CA. 2017. Leukocyte counts and lymphocyte subsets in relation to pregnancy and HIV infection in Malawian women. Am J Reprod Immunol [Internet] 78. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/aji.12678

Martin M, Blackwell AD, Gurven M, Kaplan H. 2013. Make New Friends and Keep the Old? Parasite Coinfection and Comorbidity in Homo sapiens. In: Brinkworth JF, Pechenkina K, editors. Primates, Pathogens, and Evolution. New York, NY: Springer New York. p 363–387. Available from: http://link.springer.com/10.1007/978-1-4614-7181-3

Martin MA, Garcia G, Kaplan HS, Gurven MD. 2016. Conflict or congruence? Maternal and infant-centric factors associated with shorter exclusive breastfeeding durations among the Tsimane. Soc Sci Med [Internet] 170:9–17. Available from: http://dx.doi.org/10.1016/j.socscimed.2016.10.003

Mason KL, Aronoff DM. 2012. Postpartum Group A Streptococcus Sepsis and Maternal Immunology. Am J Reprod Immunol 67:91–100.

Matthiesen A-S, Ransjo-Arvidson A-B, Nissen E, Uvnas-Moberg K. 2001. Postpartum Maternal Oxytocin Release by Newborns: Effects of Infant Hand Massage and Sucking. Birth 28:13–19.

Mba CO, Jacob RB, Green MB, Zebedee LU. 2019. Hematological Profile of Pregnant Women in Port Harcourt, Nigeria. Int J Transl Med Res Public Heal 3:1–10.

McAllister L, Gurven M, Kaplan H, Stieglitz J. 2012. Why do women have more children than they want? Understanding differences in women's ideal and actual family size in a natural fertility population. Am J Hum Biol 24:786–799.

McKean KA, Nunney L. 2005. BATEMAN'S PRINCIPLE AND IMMUNITY: PHENOTYPICALLY PLASTIC REPRODUCTIVE STRATEGIES PREDICT CHANGES IN IMMUNOLOGICAL SEX DIFFERENCES. Evolution (N Y) [Internet] 59:1510. Available from: http://www.bioone.org/perlserv/?request=get-abstract&doi=10.1554%2F04-657

McNEILLY AS, TAY CCK, GLASIER A. 1994. Physiological Mechanisms Underlying Lactational Amenorrhea. Ann N Y Acad Sci [Internet] 709:145–155. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.1994.tb30394.x

Meier PP, Engstrom JL, Janes JE, Jegier BJ, Loera F. 2012. Breast pump suction patterns that mimic the human infant during breastfeeding: Greater milk output in less time spent pumping for breast pump-dependent mothers with premature infants. J Perinatol 32:103–110.

Miller EM. 2010. Maternal hemoglobin depletion in a settled Northern Kenyan pastoral population. Am J Hum Biol [Internet] 22:768–774. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ajhb.21078

MITCHELL LA. 1999. Sex differences in antibody- and cell-mediated immune response to rubella re-immunisation. J Med Microbiol [Internet] 48:1075–1080. Available from: https://www.microbiologyresearch.org/content/journal/jmm/10.1099/00222615-48-12-1075

Mizuhata K, Taniguchi H, Hikita N, Shimada M, Morokuma S. 2020. Effects of Breastfeeding on Stress Measured by Saliva Cortisol Level and Perceived Stress. Asian/Pacific Isl Nurs J 5:128–138.

Moffett A, Loke C. 2006. Immunology of placentation in eutherian mammals. Nat Rev Immunol [Internet] 6:584–594. Available from: https://doi.org/10.1038/nri1897

Molès J-P, Tuaillon E, Kankasa C, Bedin A-S, Nagot N, Marchant A, McDermid JM, Van de Perre P. 2018. Breastmilk cell trafficking induces microchimerism-mediated immune system maturation in the infant. Pediatr Allergy Immunol [Internet] 29:133–143. Available from: https://onlinelibrary.wiley.com/doi/10.1111/pai.12841

Monteiro C, Kasahara T, Sacramento PM, Dias A, Leite S, Silva VG, Gupta S, Agrawal A, Bento CAM. 2021. Human pregnancy levels of estrogen and progesterone contribute to humoral immunity by activating TFH/B cell axis. Eur J Immunol 51:167–179.

Moore SL, Wilson K. 2002. Parasites as a Viability Cost of Sexual Selection in Natural Populations of Mammals. Science (80-) [Internet] 297:2015–2018. Available from: https://www.science.org/doi/10.1126/science.1074196

Muehlenbein MP. 2006. Adaptive variation in testosterone levels in response to immune activation: empirical and theoretical perspectives. Soc Biol 53:13–23.

Muller MN, Marlowe FW, Bugumba R, Ellison PT, Muller MN, Marlowe FW, Bugumba R, Ellison PT. 2009. Testosterone and paternal care in East African foragers and pastoralists. Proc R Soc B 276:347–354.

Natri H, Garcia AR, Buetow KH, Trumble BC, Wilson MA. 2019. The Pregnancy Pickle: Evolved Immune Compensation Due to Pregnancy Underlies Sex Differences in Human Diseases. Trends Genet [Internet] 35:478–488. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168952519300794

Neville MC. 2001. Anatomy and Physiology of Lactation. Pediatr Clin North Am 48:13–34.

Neville MC, Neifert MR. 1983. Lactation. (Neville MC, Neifert MR, editors.). Boston, MA: Springer US. Available from: http://link.springer.com/10.1007/978-1-4613-3688-4

Nieman DC, Wentz LM. 2019. The compelling link between physical activity and the body's defense system. J Sport Heal Sci [Internet] 8:201–217. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2095254618301005

Nobbenhuis MAE, Helmerhorst TJM, van den Brule AJC, Rozendaal L, Bezemer PD, Voorhorst FJ, Meijer CJLM. 2002. High-risk human papillomavirus clearance in pregnant women: trends for lower clearance during pregnancy with a catch-up postpartum. Br J Cancer [Internet] 87:75–80. Available from: http://www.nature.com/articles/6600367

Núñez-de la Mora A, Chatterton RT, Choudhury OA, Napolitano DA, Bentley GR. 2007. Childhood Conditions Influence Adult Progesterone Levels. PLoS Med [Internet] 4:e167. Available from: https://dx.plos.org/10.1371/journal.pmed.0040167

Nunn CL, Lindenfors P, Pursall ER, Rolff J. 2009. On sexual dimorphism in immune function. Philos Trans R Soc B Biol Sci [Internet] 364:61–69. Available from: https://royalsocietypublishing.org/doi/10.1098/rstb.2008.0148

Ortuna E, Pierdominici M, Maselli A, Veroni C, Aloisi F, Shoenfeld Y. 2016. Sex-based differences in autoimmune diseases. Ann Ist Super Sanità [Internet] 52:205–212. Available from: https://academic.oup.com/trstmh/article-lookup/doi/10.1093/trstmh/tru167

Osol G, Mandala M. 2009. Maternal Uterine Vascular Remodeling During Pregnancy. Physiology [Internet] 24:58–71. Available from: http://www.physiology.org/doi/10.1152/physiol.00033.2008

Pavard S, Metcalf CJE. 2007. Negative Selection on BRCA1 Susceptibility Alleles Sheds Light on the Population Genetics of Late-Onset Diseases and Aging Theory. PLoS One [Internet] 2:e1206. Available from: https://dx.plos.org/10.1371/journal.pone.0001206

Pervanidou P, Kolaitis G, Charitaki S, Margeli A, Ferentinos S, Bakoula C, Lazaropoulou C, Papassotiriou I, Tsiantis J, Chrousos GP. 2007. Elevated morning serum interleukin (IL)-6 or evening salivary cortisol concentrations predict posttraumatic stress disorder in children and adolescents six months after a motor vehicle accident. Psychoneuroendocrinology 32:991–999.
Petroff MG. 2011. Review: Fetal antigens – Identity, origins, and influences on the maternal immune system. Placenta [Internet] 32:S176–S181. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0143400410005163
Phillippe M. 2015. Cell-free fetal DNA, telomeres, and the spontaneous onset of parturition. Reprod Sci 22:1186–1201.
Poulin R. 1996. Sexual Inequalities in Helminth Infections: A Cost of Being a Male? Am Nat [Internet] 147:287–295. Available from: https://www.journals.uchicago.edu/doi/10.1086/285851
Purohit G, Shah T, Harsoda JM, Purohit MG. 2015. Hematological profile of normal pregnant women in Western India. Sch J Appl Med Sci (SJAMS [Internet] 3:2195–2199. Available from: www.saspublisher.com
Raj VK, Plichta SB. 1998. The Role of Social Support in Breastfeeding Promotion: A Literature Review. J Hum Lact [Internet] 14:41–45. Available from: http://journals.sagepub.com/doi/10.1177/089033449801400114
Rasmussen KM, Geraghty SR. 2011. The Quiet Revolution: Breastfeeding Transformed With the Use of Breast Pumps. Am J Public Health [Internet] 101:1356–1359. Available from: http://ajph.aphapublications.org/doi/10.2105/AJPH.2011.300136
Reinhardt ÉL, Fernandes PACM, Markus RP, Fischer FM. 2016. Short sleep duration increases salivary IL-6 production. Chronobiol Int [Internet] 33:780–782. Available from: http://dx.doi.org/10.3109/07420528.2016.1167710
Robertson SA, Sharkey DJ. 2016. Seminal fluid and fertility in women. Fertil Steril [Internet] 106:511–519. Available from: http://dx.doi.org/10.1016/j.fertnstert.2016.07.1101
Ronsmans C, Graham WJ. 2006. Maternal mortality: who, when, where, and why. Lancet 368:1189–1200.
Rook G, Bäckhed F, Levin BR, McFall-Ngai MJ, McLean AR. 2017. Evolution, human-microbe interactions, and life history plasticity. Lancet [Internet] 390:521–530. Available from: http://dx.doi.org/10.1016/S0140-6736(17)30566-4
Roush SW. 2007. Historical Comparisons of Morbidity and Mortality for Vaccine-Preventable Diseases in the United States. JAMA [Internet] 298:2155. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.298.18.2155
Sanci M, Töz E, Ince O, Özcan A, Polater K, Inan AH, Beyan E, Akkaya E. 2017. Reference values for maternal total and differential leukocyte counts in different trimesters of pregnancy and the initial postpartum period in western Turkey. J Obstet Gynaecol (Lahore) [Internet] 37:571–575. Available from: https://www.tandfonline.com/doi/full/10.1080/01443615.2016.1268575
Sarkar A, Kuehl MN, Alman AC, Burkhardt BR. 2021. Linking the oral microbiome and salivary cytokine abundance to circadian oscillations. Sci Rep [Internet] 11:1–13. Available from: https://doi.org/10.1038/s41598-021-81420-3
Schat ACH, Kelloway EK, Desmarais S. 2005. The Physical Health Questionnaire (PHQ): Construct validation of a self-report scale of somatic symptoms. J Occup Health Psychol 10:363–381.

Schroeder M, Schaumburg B, Mueller Z, Parplys A, Jarczak D, Roedl K, Nierhaus A, de Heer G, Grensemann J, Schneider B, Stoll F, Bai T, Jacobsen H, Zickler M, Stanelle-Bertram S, Klaetschke K, Renné T, Meinhardt A, Aberle J, Hiller J, Peine S, Kreienbrock L, Klingel K, Kluge S, Gabriel G. 2021. High estradiol and low testosterone levels are associated with critical illness in male but not in female COVID-19 patients: a retrospective cohort study. Emerg Microbes Infect 10:1807–1818.

Schuberth HJ, Taylor U, Zerbe H, Waberski D, Hunter R, Rath D. 2008. Immunological responses to semen in the female genital tract. Theriogenology [Internet] 70:1174–1181. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0093691X08005207

Schumacher A, Costa S-D, Zenclussen AC. 2014. Endocrine Factors Modulating Immune Responses in Pregnancy. Front Immunol [Internet] 5:1–12. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2014.00196/abstract

Sellen DW. 2001. Comparison of infant feeding patterns reported for nonindustrial populations with current recommendations. J Nutr [Internet] 131:2707–2715. Available from: http://jn.nutrition.org/content/131/10/2707.short%5Cnpapers3://publication/uuid/DF0BFD22-8E9F-48AF-BC81-37390824077A

Shen C, Jiang YM, Shi H, Liu JH, Zhou WJ, Dai QK, Yang H. 2010. A prospective, sequential and longitudinal study of haematological profile during normal pregnancy in Chinese women. J Obstet Gynaecol (Lahore) 30:357–361.

Slusher T, Slusher IL, Biomdo M, Bode-Thomas F, Curtis BA, Meier P. 2007. Electric breast pump use increases maternal milk volume in African nurseries. J Trop Pediatr 53:125–130.

Smit FC, Davison GM, Hoffmann M, Erasmus RT, Davids S, Matsha TE. 2019. Full blood count and white cell differential count reference ranges obtained from a healthy urban South African population residing in the Western Cape of South Africa. Int J Lab Hematol 41:635–641.

Stuebe A, Rich-Edwards J. 2009. The Reset Hypothesis: Lactation and Maternal Metabolism. Am J Perinatol [Internet] 26:081–088. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0028-1103034

Stuebe AM. 2005. Duration of Lactation and Incidence of Type 2 Diabetes. Jama [Internet] 294:2601. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.294.20.2601

Takahashi A, Ohira H, Abe K, Zeniya M, Abe M, Arinaga-Hino T, Torimura T, Yoshizawa K, Takaki A, Kang JH, Suzuki Y, Nakamoto N, Inui A, Tanaka A, Takikawa H. 2020a. Increasing incidence of acute autoimmune hepatitis: a nationwide survey in Japan. Sci Rep [Internet] 10:1–9. Available from: https://doi.org/10.1038/s41598-020-71296-0

Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J, Silva J, Mao T, Oh JE, Tokuyama M, Lu P, Venkataraman A, Park A, Liu F, Meir A, Sun J, Wang EY, Casanovas-Massana A, Wyllie AL, Vogels CBF, Earnest R, Lapidus S, Ott IM, Moore AJ, Shaw A, Fournier JB, Odio CD, Farhadian S, Dela Cruz C, Grubaugh ND, Schulz WL, Ring AM, Ko AI, Omer SB, Iwasaki A. 2020b. Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature [Internet] 588:315–320. Available from: http://www.nature.com/articles/s41586-020-2700-3

Taneja V. 2018. Sex Hormones Determine Immune Response. Front Immunol [Internet] 9:1–5. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2018.01931/full

Thomas MR, Tutschek B, Frost A, Rodeck CH, Yazdani N, Craft I, Williamson R. 1995. The time of appearance and disappearance of fetal DNA from the maternal circulation. Prenat Diagn 15:641–646.
Thong YH, Steele RW, Vincent MM, Hensen SA, Bellanti JA. 1973. Impaired in Vitro Cell-Mediated Immunity to Rubella Virus during Pregnancy. N Engl J Med [Internet] 289:604–606. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM197309202891203
Trivers RL. 1974. Parent-Offspring Conflict. Am Zool 14:249–264.
Trumble BC, Blackwell AD, Stieglitz J, Thompson ME, Suarez IM, Kaplan H, Gurven M. 2016. Associations between male testosterone and immune function in a pathogenically stressed forager-horticultural population. Am J Phys Anthropol [Internet] 161:494–505. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0031938416312148
Trumble BC, Cummings D, von Rueden C, O'Connor K a., Smith EA, Gurven M, Kaplan H. 2012. Physical competition increases testosterone among Amazonian forager-horticulturalists: a test of the 'challenge hypothesis.' Proc R Soc B Biol Sci [Internet] 279:2907–2912. Available from: https://royalsocietypublishing.org/doi/10.1098/rspb.2012.0455
Valeggia C, Eellison P. 2009. Interactions between metabolic and reproductive functions in the resumption of postpartum fecundity. Am J Hum Biol 21:559–566.
Valeggia C, Ellison PT. 2009. Interactions between metabolic and reproductive functions in the resumption of postpartum fecundity. Am J Hum Biol [Internet] 21:559–566. Available from: http://doi.wiley.com/10.1002/ajhb.20907
Veile A, Martin M, McAllister L, Gurven M. 2014. Modernization is associated with intensive breastfeeding patterns in the Bolivian Amazon. Soc Sci Med [Internet] 100:148–158. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0277953613005820
Voigt EA, Ovsyannikova IG, Kennedy RB, Grill DE, Goergen KM, Schaid DJ, Poland GA. 2019. Sex Differences in Older Adults' Immune Responses to Seasonal Influenza Vaccination. Front Immunol [Internet] 10. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2019.00180/full
Washecka R, Behling A. 2002. Urologic complications of placenta percreta invading the urinary bladder: a case report and review of the literature. Hawaii Med J [Internet] 61:66–9. Available from: http://evols.library.manoa.hawaii.edu/bitstream/10524/53705/2002-04p66-69.pdf
Werner-Seidler A, Afzali MH, Chapman C, Sunderland M, Slade T. 2017. The relationship between social support networks and depression in the 2007 National Survey of Mental Health and Well-being. Soc Psychiatry Psychiatr Epidemiol 52:1463–1473.
Wetterö J, von Löhneysen S, Cobar F, Kristenson M, Garvin P, Sjöwall C. 2021. Pronounced Diurnal Pattern of Salivary C-Reactive Protein (CRP) With Modest Associations to Circulating CRP Levels. Front Immunol 11:1–11.
Whitacre CC. 2001. Sex differences in autoimmune disease. Nat Immunol 2:777–780.
Wira CR, Rodriguez-Garcia M, Patel M V. 2015. The role of sex hormones in immune protection of the female reproductive tract. Nat Rev Immunol [Internet] 15:217–230. Available from: http://www.nature.com/articles/nri3819
Won D Il, Kim S, Lee EH. 2018. Neutrophil oxidative burst as a diagnostic indicator of IgG-mediated anaphylaxis. Blood Res 53:299–306.

Wood SN, Pya N, Säfken B. 2016. Smoothing Parameter and Model Selection for General Smooth Models. J Am Stat Assoc [Internet] 111:1548–1563. Available from: https://www.tandfonline.com/doi/full/10.1080/01621459.2016.1180986

Wyatt S, Silitonga PII, Febriani E, Long Q. 2021. Socioeconomic, geographic and health system factors associated with rising C-section rate in Indonesia: a cross-sectional study using the Indonesian demographic and health surveys from 1998 to 2017. BMJ Open [Internet] 11:e045592. Available from: https://bmjopen.bmj.com/lookup/doi/10.1136/bmjopen-2020-045592

Yu-Lee LY. 2002. Prolactin modulation of immune and inflammatory responses. Recent Prog Horm Res [Internet] 57:435–455. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12017556

Zuk M, McKean KA. 1996. Sex differences in parasite infections: Patterns and processes. Int J Parasitol 26:1009–1024.

Zuk M, Simmons LW, Rotenberry JT, Stoehr AM. 2004. Sex differences in immunity in two species of field crickets. Can J Zool 82:627–634.