LIFE HISTORY TRADEOFFS BETWEEN TESTOSTERONE AND IMMUNE FUNCTION AMONG SHUAR FORAGER-HORTICULTURALISTS OF AMAZONIAN ECUADOR

by

THERESA E. GILDNER

A DISSERTATION

Presented to the Department of Anthropology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

June 2018

DISSERTATION APPROVAL PAGE

Student: Theresa E. Gildner

Title: Life History Tradeoffs Between Testosterone and Immune Function Among Shuar Forager-Horticulturalists of Amazonian Ecuador

This dissertation has been accepted and approved in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Anthropology by:

J. Josh Snodgrass	Chairperson
Lawrence S. Sugiyama	Core Member
Frances J. White	Core Member
Jennifer C. Ablow	Institutional Representative

and

Sara D. Hodges	Interim Vice Provost and Dean of the Graduate School

Original approval signatures are on file with the University of Oregon Graduate School.

Degree awarded June 2018.

DISSERTATION ABSTRACT

Theresa E. Gildner

Doctor of Philosophy

Department of Anthropology

June 2018

Title: Life History Tradeoffs Between Testosterone and Immune Function Among Shuar Forager-Horticulturalists of Amazonian Ecuador

The sex hormone testosterone supports male reproduction. However, testosterone is hypothesized to suppress immune activity, resulting in a tradeoff between energetic investment in reproductive effort and immune function. The Immunocompetence Handicap Hypothesis (ICHH) therefore argues that testosterone-linked masculine traits honestly signal health status to prospective mates, as only uninfected males should be able to maintain high testosterone levels. Still, this proposed tradeoff remains poorly tested among human men, especially among natural fertility populations experiencing high infectious disease burdens. This dissertation therefore tested the ICHH among indigenous Shuar men of Amazonian Ecuador. Specifically, this project examined testosterone variation patterns and assessed how male investment in reproductive effort is associated with reproductive success and immune function.

The first study tested testosterone level variation among Shuar men in relation to body composition, age, and style of life factors. This study demonstrated that age and BMI interactions shape testosterone levels in complex ways, such that the relationship between body composition and testosterone profile varies throughout the life course. The second study investigated whether individual reproductive success was significantly influenced by masculine trait development and parasite load. These results failed to support the hypotheses that masculine traits increased reproductive success or honestly signaled lack of parasitic disease. Instead, a significant positive association was observed between a composite score of masculine traits and *Ascaris lumbricoides* infection load; suggesting that male investment in reproductive effort may increase parasitic infection risk.

The final study assessed whether testosterone levels were negatively associated with four measures of immune function (parasite load, C-Reactive Protein [CRP], Immunoglobulin-G [IgG], and Immunoglobulin-E [IgE]). Testosterone levels were inversely associated with CRP levels and a positive relationship between testosterone levels and *Trichuris trichiura* infection load was documented, suggesting increased investment in reproductive effort may suppress some aspects of immune function and increase parasite burden. Overall, these studies fail to support the ICHH, but do indicate a context-dependent tradeoff between energetic investment in male reproductive effort and some aspects of immune function; thereby demonstrating complex interactions between physical characteristics, physiological processes, and immune activity in human men.

This dissertation includes unpublished, co-authored material.

CURRICULUM VITAE

NAME OF AUTHOR: Theresa E. Gildner

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene, OR
University of Notre Dame, Notre Dame, IN

DEGREES AWARDED:

Doctor of Philosophy, Biological Anthropology, 2018, University of Oregon
Master of Science, Biological Anthropology, 2013, University of Oregon
Bachelor of Science, Biology and Anthropology, 2011, University of Notre Dame

AREAS OF SPECIAL INTEREST:

Human Biology
Public Health and Epidemiology
Behavioral and Reproductive Ecology
Evolutionary Theory
Infectious/Parasitic Disease
Global Aging

PROFESSIONAL EXPERIENCE:

Research Assistant, Shuar Health and Life History Project (SHLHP), University of Oregon, 2011 – Present

Research Assistant, Study on global AGEing and adult health (SAGE), World Health Organization, 2011 – Present

Research Assistant, Building the Methodological Toolkit in Biological Anthropology: Dried Blood Spot Methods Development for Addressing Key Evolutionary and Biocultural Questions, University of Oregon, 2016 – 2017

Research Assistant, The Role of Arts Programs in Fostering an Organizational Culture of Patient-Centered Care and an Environment of Healing in Hospitals and Hospices, University of Oregon, 2013 – 2014

Undergraduate Research Supervision and Thesis Advising, Department of Anthropology, University of Oregon, 2014 – 2018

Instructor, Department of Anthropology, University of Oregon, 2015 – 2018

Training Assistant, Huerto de la Familia, Eugene, Oregon, 2013 – 2017

Graduate Employee, Department of Anthropology, University of Oregon, 2011 – 2018

Organizing Member, Association of Anthropology Graduate Students, University of Oregon, 2012 – 2014

Graduate Teaching Assistant, Department of Anthropology, University of Notre Dame, 2011

Undergraduate Teaching Assistant, Department of Biology, University of Notre Dame, 2010

GRANTS, AWARDS, AND HONORS:

Dissertation Research Fellowship, University of Oregon, 2017

Doctoral Dissertation Improvement Grant, National Science Foundation, 2017

Dissertation Fieldwork Grant, Wenner-Gren Foundation, 2016

Sasakawa Young Leaders Fellowship, 2015

Dissertation Fieldwork Grant, The Lewis and Clark Fund for Exploration and Field Research, 2015

Graduate Student Summer Research Award, Center for Latino/a and Latin American Studies, University of Oregon, 2015

William S. Pollitzer Student Travel Award, American Association of Physical Anthropologists, 2014

Department of Anthropology Travel Award, University of Oregon, 2014

Juda Memorial Endowment Fund, University of Oregon, 2014

Health Education Award for Travel, University of Oregon, 2014

Global Oregon Summer Research Award, University of Oregon, 2013

Department of Anthropology Travel Award, University of Oregon, 2013

McFee Award, University of Oregon, 2013

Health Education Award for Travel, University of Oregon, 2013

Department of Anthropology Travel Award, University of Oregon, 2012

Health Education Award for Travel, University of Oregon, 2012

Graduate Research Fellowship Program Honorable Mention, National Science Foundation, 2012

Graduate Teaching Fellowship, University of Oregon, 2011 – 2018

Cum Laude, University of Notre Dame, 2011

Undergraduate Research Opportunity Program Research & Materials Grant, University of Notre Dame, 2010

NSF-REU Fellow in Biocultural Anthropology, University of Notre Dame, 2010

Dean’s List, University of Notre Dame, 2007 – 2011

University Scholarship, University of Notre Dame, 2007 – 2011

PUBLICATIONS:

Gildner, T. E., Liebert, M. A., Schrock, J. M., Urlacher, S. S., Harrington, C. J., Cepon-Robins, T. J., ... & Snodgrass, J. J. (2018). Salivary testosterone and adiposity among Shuar men: Body composition variation in an indigenous, subsistence population. American Journal of Human Biology, 30(2), 15.

Gildner, T. E., Urlacher, S. S., Liebert, M. A., Cepon-Robins, T. J., Shrock, J. M., Madimenos, F. C., … & Sugiyama, L. S. (2018). Lifestyle Changes and Immune Function: Associations between Immunoglobulin E Levels and Market Integration among Indigenous Shuar from Amazonian Ecuador. American Journal of Physical Anthropology, 165(Supplement 66), 98.

Cepon-Robins, T. J., Gildner, T. E., Shrock, J. M., Eick, G., Bedbury, A., Liebert, M. A., … & Snodgrass, J. J. (2018). Immune response to soil-transmitted helminths is associated with reduced intestinal inflammation: Preliminary

evidence from Amazonian Ecuador. American Journal of Physical Anthropology, 165(Supplement 66), 44.

Liebert, M. A., Cepon-Robins, T. J., Gildner, T. E., Urlacher, S. S., Madimenos, F. C., Harrington, C. J., … & Snodgrass, J. J. (2018). Diurnal cortisol rhythms and helminth infection intensity among the indigenous Shuar of Amazonian Ecuador: Examining immune-endocrine interactions in a subsistence-based population. American Journal of Human Biology, 30(2), 26.

Madimenos, F. C., Liebert, M. A., Urlacher, S. S., Harrington, C. J., Cepon-Robins, T. J., Gildner, T. E., … & Sugiyama, L. S. (2018). Shuar Childhood and Adolescent Bone Density Documented Across Varying Degrees of Market Integration. American Journal of Physical Anthropology, 165(Supplement 66), 164.

Stagaman, K., Cepon-Robins, T. J., Liebert, M. A., Gildner, T. E., Urlacher, S. S., Madimenos, F. C., ... & Bohannan, B. J. (2018). Market Integration Predicts Human Gut Microbiome Attributes across a Gradient of Economic Development. MSystems, 3(1), e00122-17.

Urlacher, S. S., Ellison, P. T., Sugiyama, L. S., Pontzer, H., Eick, G., Liebert, M. A., ... & Snodgrass, J. J. (2018). Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists. Proceedings of the National Academy of Sciences, 201717522.

Arokiasamy, P., Kowal, P., Capistrant, B. D., Gildner, T. E., Thiele, E., Biritwum, R. B., ... & Guo, Y. (2017). Chronic noncommunicable diseases in 6 low- and middle-income countries: findings from wave 1 of the World Health Organization's study on global Ageing and adult health (SAGE). American journal of epidemiology, 185(6), 414-428.

Gildner, T. E., Kowal, P., & Snodgrass, J. J. (2017). Testing associations between cardiovascular health and cognitive performance in the Study on global AGEing and adult health. American Journal of Human Biology, 29(2), 17.

Gildner, T. E., Liebert, M. A., Cepon-Robins, T. J., Bribiescas, R. G., Urlacher, S. S., Shrock, J. M., ... & Snodgrass, J. J. (2017). Associations between testosterone levels and parasite load: Testing life history tradeoffs among indigenous Shuar men from Amazonian Ecuador. American Journal of Physical Anthropology, 162(Supplement 64), 194.

Cepon-Robins, T. J., Gildner, T. E., Liebert, M. A., Urlacher, S. S., Madimenos, F. C., Harrington, C. J., ... & Sugiyama, L. S. (2017). The Shuar Health and Life History Project: Tapeworm infection and demographic/lifestyle

measures among an indigenous population from Amazonian Ecuador. American Journal of Human Biology, 29(2), 7.

Liebert, M. A., Urlacher, S. S., Madimenos, F. C., Gildner, T. E., Cepon-Robins, T. J., Harrington, C. J., ... & Snodgrass, J. J. (2017). Diurnal cortisol rhythms among the indigenous Shuar of Amazonian Ecuador: individual differences by age, sex, and body composition across the lifespan. American Journal of Human Biology, 29(2), 28.

Madimenos, F. C., Liebert, M. A., Urlacher, S. S., Cepon-Robins, T. J., Gildner, T. E., Harrington, C. J., ... & Sugiyama, L. S. (2017). Documenting the Changing Reproductive Landscape among Shuar Females from Amazonian Ecuador. American Journal of Physical Anthropology, 162(Supplement 64), 272.

Hallett, E., Eick, G., Barrett, T., Liebert, M., Cepon-Robins, T., Gildner, T. E., ... & Snodgrass, J. J. (2017). The Shuar Health and Life History Project: Chronic helminth infection is associated with kidney function in an indigenous Ecuadorian forager-horticulturalist population. American Journal of Human Biology, 29(2), 19.

Gildner, T. E., Cepon-Robins, T. J., Liebert, M. A., Urlacher, S. S., Madimenos, F. C., Snodgrass, J. J., & Sugiyama, L. S. (2016). Regional variation in Ascaris lumbricoides and Trichuris trichiura infections by age cohort and sex: effects of market integration among the indigenous Shuar of Amazonian Ecuador. Journal of physiological anthropology, 35(1), 28.

Gildner, T. E. (2016). Book Review: Global Aging: Comparative Perspectives on Aging and the Life Course (by Suzanne R. Kunkel, J. Scott Brown, and Frank J. Whittington). American Journal of Human Biology, 28(3), 445.

Urlacher, S. S., Liebert, M. A., Josh Snodgrass, J., Blackwell, A. D., Cepon-Robins, T. J., Gildner, T. E., ... & Sugiyama, L. S. (2016). Heterogeneous effects of market integration on sub-adult body size and nutritional status among the Shuar of Amazonian Ecuador. Annals of Human Biology, 43(4), 316-329.

Urlacher, S. S., Snodgrass, J. J., Liebert, M. A., Cepon-Robins, T. J., Gildner, T. E., & Sugiyama, L. S. (2016). The application of knemometry to measure childhood short-term growth among the indigenous Shuar of Ecuador. American journal of physical anthropology, 160(2), 353-357.

Urlacher, S. S., Blackwell, A. D., Liebert, M. A., Madimenos, F. C., Cepon-Robins, T. J., Gildner, T. E., ... & Sugiyama, L. S. (2016). Physical growth of the shuar: Height, weight, and BMI references for an indigenous amazonian population. American Journal of Human Biology, 28(1), 16-30.

Gildner, T. E., Liebert, M. A., Kowal, P., & Snodgrass, J. J. (2016). Study on global AGEing and adult health (SAGE): Cross-cultural associations between cognitive function and diminished physical performance among older adults. American Journal of Human Biology, 28(2), 278.

Gildner, T. E., Liebert, M. A., Bribiescas, R. G., Sugiyama, L. S., Urlacher, S. S., Cepon-Robins, T. J., ... & Snodgrass, J. J. (2016). Diurnal testosterone variation among indigenous Shuar men from Amazonian Ecuador. American Journal of Physical Anthropology, 159(Supplement 62), 154.

Cepon-Robins, T. J., Gildner, T. E., Liebert, M. A., Urlacher, S. S., Madimenos, F. C., Sugiyama, L. S., & Snodgrass, J. J. (2016). Soil-transmitted helminth infection and immune function: Ascaris lumbricoides, but not Trichuris trichiura, is associated with lower adult-levels of C-reactive protein among the Shuar of Ecuadorian Amazonia. American Journal of Human Biology, 28(2), 271.

Sugiyama, L. S., Liebert, M. A., Madimenos, F. C., Blackwell, A. D., Urlacher, S. S., Cepon-Robins, T. J., ... & Snodgrass, J. J. (2016). From foraging to Facebook: Market integration and health among the Shuar of Southeastern Ecuador. American Journal of Physical Anthropology, 159(Supplement 62), 307.

Liebert, M. A., Madimenos, F. C., Urlacher, S. S., Gildner, T. E., Cepon-Robins, T. J., Snodgrass, J. J., & Sugiyama, L. S. (2016). The embodiment of market integration: Modeling cultural consonance among Shuar children of Amazonian Ecuador. American Journal of Physical Anthropology, 159(Supplement 62), 208.

Madimenos, F. C., Liebert, M. A., Urlacher, S. S., Cepon-Robins, T. J., Gildner, T. E., Harrington, C. J., ... & Sugiyama, L. S. (2016). Bone mineral density is associated with waist circumference and blood pressure but not lipid profile among the Amazonian Shuar of Ecuador. American Journal of Physical Anthropology, 159(Supplement 62), 215.

Gildner, T. E., Barrett, T. M., Liebert, M. A., Kowal, P., & Snodgrass, J. J. (2015). Does BMI generated by self-reported height and weight measure up in older adults from middle-income countries? Results from the study on global AGEing and adult health (SAGE). BMC Obesity, 2(1), 44.

Gildner, T. E., Cepon-Robins, T. J., Liebert, M. A., Urlacher, S. S., Snodgrass, J. J., Madimenos, F. C., & Sugiyama, L. S. (2015). The Shuar Health and Life History Project: Market integration, age, and intestinal helminth intensity among an indigenous lowland Ecuadorian population. American Journal of Human Biology, 27(2), 269.

Gildner, T. E., Liebert, M. A., Kowal, P., & Snodgrass, J. J. (2015). Is employment status cross-culturally associated with cognitive function among older adults: Results from the Study on global AGEing and adult health (SAGE). American Journal of Physical Anthropology, 156(Supplement 60), 145.

Brigham, R., Gildner, T. E., Liebert, M. A., Kowal, P., & Snodgrass, J. J. (2015). Study on global AGEing and adult health (SAGE): The effects of social cohesion and personal relationships on depression diagnosis among older adults in India. American Journal of Human Biology, 27(2), 263.

Snodgrass, J. J., Liebert, M. A., Gildner, T. E., & Kowal, P. (2015). Study on global AGEing and adult health (SAGE): The role of income on depression diagnosis among older adults in six middle income countries. American Journal of Human Biology, 27(2), 287.

Liebert, M. A., Gildner, T. E., Kowal, P., Chatterji, S., & Snodgrass, J. J. (2015). Associations between socioeconomic status and obesity in low- and middle-income countries: Results from the Study on global AGEing and adult health (SAGE). American Journal of Physical Anthropology, 156(Supplement 60), 204.

Cepon-Robins, T. J., Gildner, T. E., Liebert, M. A., Urlacher, S. S., Madimenos, F. C., Snodgrass, J. J., & Sugiyama, L. S. (2015). The Shuar Health and Life History Project: Testing the Hygiene Hypothesis—Soil-transmitted helminth infection and immune dysregulation among the Shuar of Amazonian Ecuador. American Journal of Physical Anthropology, 156(Supplement 60), 103.

Urlacher, S. S., Snodgrass, J. J., Liebert, M. A., Cepon-Robins, T. J., Gildner, T. E., & Sugiyama, L. S. (2015). The Shuar Health and Life History Project: Childhood somatic resources, linear growth, and immune function among the Shuar. American Journal of Physical Anthropology, 156(Supplement 60), 310.

Gildner, T. E., Liebert, M. A., Kowal, P., Chatterji, S., & Josh Snodgrass, J. (2014). Sleep duration, sleep quality, and obesity risk among older adults from six middle-income countries: Findings from the study on global ageing and adult health (SAGE). American Journal of Human Biology, 26(6), 803-812.

Gildner, T. E., Liebert, M. A., Kowal, P., Chatterji, S., & Snodgrass, J. J. (2014). Associations between sleep duration, sleep quality, and cognitive test performance among older adults from six middle income countries: results from the Study on Global Ageing and Adult Health (SAGE). Journal of clinical sleep medicine: JCSM: official publication of the American Academy of Sleep Medicine, 10(6), 613.

Cepon-Robins, T. J., Liebert, M. A., Gildner, T. E., Urlacher, S. S., Colehour, A. M., Snodgrass, J. J., ... & Sugiyama, L. S. (2014). Soil-transmitted helminth prevalence and infection intensity among geographically and economically distinct Shuar communities in the Ecuadorian Amazon. The Journal of parasitology, 100(5), 598-607.

Colehour, A., Meadow, J. F., Cepon-Robins, T. J., Gildner, T. E., Liebert, M. A., Urlacher, S. S., ... & Sugiyama, L. S. (2014). Local domestication of microbes via cassava beer fermentation. PeerJ PrePrints.

Gildner, T. E., Liebert, M. A., Kowal, P., Chatterji, S., Snodgrass, J. J. (2014). Study on global AGEing and adult health (SAGE): Associations between income adequacy and well-being. Gerontologist, 54, 178-178.

Gildner, T. E., Liebert, M. A., Kowal, P., Chatterji, S., Snodgrass, J. J. (2014). The Study on global AGEing and adult health (SAGE): The effect of sleep quality and duration on obesity levels among older adults from six middle income countries. American Journal of Human Biology, 26(2), 265.

Urlacher, S. S., Liebert, M. A., Cepon, T. J., Snodgrass, J. J., Gildner, T. E., Colehour, A. M., ... & Sugiyama, L. S. (2014). Childhood immune function and growth: Insight from repeat measures among the Amazonian Shuar. American Journal of Human Biology, 26(2), 283.

Liebert, M. A., Snodgrass, J. J., Urlacher, S. S., Cepon-Robins, T. J., Gildner, T. E., Madimenos, F. C., ... & Sugiyama, L. S. (2014). The Shuar Health and Life History Project: The role of market integration and life history trade-offs on diurnal cortisol among Indigenous Shuar children of Amazonian Ecuador. American Journal of Human Biology, 26(2), 270.

Cepon-Robins, T. J., Liebert, M. A., Gildner, T. E., Madimenos, F. C., Urlacher, S. S., Blackwell, A. D., ... & Sugiyama, L. S. (2014). The Shuar Health and Life History Project: Chronic and infectious disease burden among the Shuar – The complicated nature of epidemiological transitions. American Journal of Human Biology, 26(2), 261.

Olson, W. J., Liebert, M. A., Gildner, T. E., Kowal, P., Snodgrass, J. J. (2014). The Study on global AGEing and adult health (SAGE): Depression and body composition among aging populations. American Journal of Human Biology, 26(2), 276.

Madimenos, F. C., Liebert, M. A., Cepon-Robins, T. J., Gildner, T. E., Urlacher, S. S., Blackwell, A. D., ... & Sugiyama, L. S. (2014). The Shuar Health and Life History Project: Hypertriglyceridemia among Cross-Cutucu Shuar - Exploring etiologies and prevention strategies. American Journal of Human Biology, 26(2), 271.

Urlacher, S. S., Liebert, M. A., Cepon, T. J., Snodgrass, J. J., Gildner, T. E., Colehour, A. M., ... & Sugiyama, L. S. (2014). Measurement of short-term linear growth using knemometry among the Shuar. American Journal of Physical Anthropology, 153(Supplement 58), 258.

Sugiyama, L. S., Madimenos, F. C., Liebert, M. A., Urlacher, S. S., Cepon-Robins, T. J., Blackwell, A. D., ... & Snodgrass, J. J. (2014). Getting a grip on phenotypic quality: Shuar Health and Life History Project analysis of grip strength as an indicator of phenotypic quality and health. American Journal of Physical Anthropology, 153(Supplement 58), 249.

Gildner, T. E., Liebert, M. A., Kowal, P., Chatterji, S., Snodgrass, J. J. (2013). Effect of sleep quality and duration on cognitive function among older adults from five middle income countries: Results from the Study on global AGEing and adult health (SAGE). American Journal of Human Biology, 25(2), 258-259.

Hawkins, L. M., Snodgrass, J. J., Gildner, T. E., Liebert, M. A., Kowal, P., & Chatterji, S. (2013). The Study on global AGEing and adult health (SAGE): Socioeconomic status, urban-rural differences, and sleep in older adults from five middle income countries. American Journal of Human Biology, 25(2), 259.

Cepon-Robins, T. J., Gildner, T. E., Liebert, M. A., Colehour, A. M., Urlacher, S. S., Snodgrass, J. J., ... & Sugiyama, L. S. (2013). The Shuar Health and Life History Project: Market integration, avoidance behavior, and intestinal helminths among an indigenous lowland Ecuadorian population. American Journal of Human Biology, 25(2), 253-254.

Liebert, M. A., Snodgrass, J. J., Cepon-Robins, T. J., Gildner, T. E., Mathur, A., Williams, S. R., ... & Chatterji, S. (2013). The relationship between clinical markers of frailty and measured physical activity using accelerometers: Results of a SAGE sub-study among older adults in India. American Journal of Physical Anthropology, 150(Supplement 56), 180.

Liebert, M. A., Snodgrass, J. J., Urlacher, S. S., Cepon-Robins, T. J., Colehour, A. M., Gildner, T. E., & Sugiyama, L. S. (2013). The Shuar Health and Life History Project: The psychosocial stress response of children from varying degrees of market integration in an indigenous lowland Ecuadorian population. American Journal of Human Biology, 25(2), 264-265.

Gildner, T. E., Wolf, A., Sheridan, S. G., & Ullinger, J. M. (2011). There’s nothing humerus about cremation: a study of distal end humeral burn patterns. American Journal of Physical Anthropology, 144(Supplement 52), 142.

ACKNOWLEDGMENTS

This dissertation would not have been possible without the generosity, kindness, and patience of the Shuar who participated in my research. Thank you for your willingness to provide yet another saliva sample and for teaching me about your beautiful culture. I would also like to express my gratitude to the Jempekat family, Berta and Guimo Fernandez, Marcia Salinas Puente, Lauro Lopez, and Favio Chinkim; thank you all for being my “Ecuadorian family”.

I am also extremely grateful to my advisors, Josh Snodgrass and Larry Sugiyama, for their invaluable mentorship during graduate school. Josh, thank you helping me grow as a scientist; without your continuous guidance, support, and encouragement none of this would have been possible. Larry, thank you for always stepping up and being there for me, both in the field and in my professional life. Thanks also to Frances White for her statistical assistance and Rick Bribiescas for sharing his insights and time.

Also, a huge shout out to my Shuar Health and Life History Project team mates: Melissa Liebert, Tara Cepon-Robins, Sam Urlacher, Joshua Schrock, Felicia Madimenos, Chris Harrington, and Dorsa Amir. I couldn’t have done this research without your friendship and willingness to help me collect data. I am also indebted to Geeta Eick for her assistance in the laboratory during sample analysis.

This research was supported in part by the Wenner-Gren Foundation for Anthropological Research (Grant #9231), the National Science Foundation (Doctoral Dissertation Improvement Grant #1650674), the Ryoichi Sasakawa Young Leaders Fellowship Fund, the Lewis & Clark Fund for Exploration and Field Research, and the Department of Anthropology and the Center for Latino/a and Latin American Studies at

the University of Oregon.

Additionally, I never would have finished this dissertation without the love and support of my family and friends. Thank you to my Eugene crew for keeping me sane and grounded, with a special thanks to Monya Anderson for being such an amazing roommate for five years. Thanks also to Melissa Kanter for the years of friendship, football comradery, and book recommendations; you’ve always been there for me, no matter the distance, and known exactly the right thing to say. I also owe a huge debt of gratitude to my incredible family. Thank you to my amazing parents, Joe and Beth Gildner, for your unwavering support of my aspirations at all stages of my education (even when you don’t understand exactly what it is I am doing). I am also very grateful for the love of my siblings (Megan, Katie, Joe, and Molly), I truly can’t imagine life without each of you. Finally, I want to express my love and appreciation to my husband, Matthew Nagy. Matt, thank you for your selflessness and constant encouragement during this long process, I never would have reached the finish line without you.

Dedicated to my family: to my parents, who instilled my love of biology and learning;
to my siblings, whose intelligence and talents are continual sources of inspiration;
and to Matt, who helps me see the bigger picture.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

DISSERTATION OVERVIEW AND THEORETICAL FRAMEWORK

Dissertation Organization

This dissertation is organized into seven chapters that examine life history tradeoffs between human immune function and reproductive effort, in addition to exploring the influence of these tradeoffs on human sexual selection. The present chapter provides background information on the global impact of infectious disease, evolutionary life history theory (LHT), and the hypothesized associations between sexual selection and immune function. Chapter II presents an overview of male reproductive endocrinology and the influence of sex hormones on the immune system. It then discusses how interactions between male hormone levels and immune function are hypothesized to affect reproductive function, and how lifestyle change associated with economic development might influence these associations. Chapter III describes the study population that is the focus of this dissertation, the indigenous Shuar of Amazonian Ecuador.

Chapter IV presents unpublished, co-authored research examining variation in Shuar male testosterone levels across the lifespan, and how this variation is influenced by age, body composition, and level of Market Integration (MI: the degree that one produces for and consumes from the market economy). Chapter V includes unpublished, co-authored research examining associations between anthropometric signals of developmental testosterone, age-specific reproductive success, and immune function. Chapter VI presents unpublished, co-authored research testing the hypothesis that testosterone is negatively associated with immune function, as measured by select immune markers and by infectious disease burden of an important class of parasites – soil transmitted helminths. Chapter VII draws important connections between key findings and presents promising directions for future research.

Introduction

Although humans share a long coevolutionary history with infectious and parasitic disease agents, the adoption of intensive agriculture facilitated the emergence of widespread endemic disease and the potential for pandemics (Armelagos & Cohen, 1984; Cohen & Crane-Kramer, 2007; Perlman, 2013). The ability to effectively grow select highly-productive domesticates led to socio-ecological conditions that facilitated the emergence and spread of infectious disease. These conditions included the emergence of large, more densely settled sedentary populations, increased reliance on domestic animals and disease transmissions from these domesticates, poor sanitation and the accumulation of human and animal waste, exposure to vectors when breaking sod, use of feces as fertilizer, and the contamination of water sources (Armelagos et al., 1991; Armelagos & Cohen, 1984; Barrett et al., 1998; Cockburn, 1971; Cohen & Crane-Kramer, 2007; McDade & Adair, 2001; Nicastri et al., 2001; Patz et al., 2000; Perlman, 2013; Sattenspiel, 2000).

The emergence of periodic epidemics (e.g., influenza, smallpox, measles, rubella, and polio) appear to be linked with these lifestyle changes (Armelagos et al., 1991; Armelagos & Cohen, 1984; Barrett et al., 1998; Cockburn, 1971), substantially increasing human suffering and mortality rates. Perhaps most famously, an outbreak of bubonic plague in the 14th century is estimated to have killed nearly one third of Europe's population (Gage & Kosoy, 2005). Likewise, the 1918 Spanish influenza killed ~50-100 million people (more than twice as many as the First World War) with some nine such influenza pandemics recorded over the last 300 years (Morens & Fauci, 2007). Against such diseases, Jenner developed the first widely used vaccine – for smallpox – in 1796; additional vaccines soon followed, inoculating individuals against a range of devastating diseases (e.g., rabies, cholera, diphtheria, plague, typhoid, tuberculosis, measles, mumps and rubella) (Plotkin, 2011). Similarly, although antibiotic substances have a much longer history of use across human cultures, widespread medical reliance on these compounds increased in 20th century with the identification of penicillin in 1928 (Aminov, 2010). This discovery was quickly followed by the development of many other common antibiotics (Aminov, 2010).

In the wake of these medical advances, many in the medical community became increasingly convinced during the mid-20th century that advances in the development of vaccines and antibiotics would soon successfully overcome infectious diseases, at least in wealthier countries (Burnet, 1951, 1962; Fauci, 2001). Bolstered by what appeared to be the successful control of smallpox, polio, and other diseases, infectious disease was thought to be increasingly a thing of the past (Burnet, 1951, 1962; Fauci, 2001). In 1972, Nobel laureate Macfarlane Burnet and his co-author, David White, predicted that "the future of infectious diseases will be very dull" (Burnet & White, 1972).

However, subsequent events have demonstrated the hubris of those who expected medical advances to essentially control infectious disease. Those confident in the end of infectious disease failed to appreciate the complexity of human-pathogen interactions, particularly the coevolutionary arms race evident in these relationships. Most pathogens have short generation times and large population sizes relative to their hosts (Altizer et al., 2003; Ewald, 1983, 1995; Schmid-Hempel, 2011; Wiley and Allen, 2009). Ecological changes often result in strong selection pressures that accelerate pathogen evolution, resulting in novel strategies to evade host immune responses (Altizer et al., 2003; Ewald, 1983, 1995; Schmid-Hempel, 2011; Wiley and Allen, 2009). Thus, the rapid evolution of pathogens in response to changes within their human hosts did not emerge with the advent of antibiotics and vaccines, but is an evolved feature of pathogen life history.

Hosts have evolved counterstrategies, including an adaptive immune system, to control and eliminate infections (Altizer et al., 2003; Ewald, 1983, 1995). For example, the human major histocompatibility complex (MHC), which is comprised of 224 genes and over 3.6 million base pairs, demonstrates selection favoring diversity to efficiently combat rapidly coevolving pathogens (Altizer et al., 2003; Horton et al., 2004). The MHC encodes cell surface proteins critical for the acquired immune system to recognize self and foreign pathogens, with its most diverse loci having upwards of 1000 known alleles, hypothesized to facilitate rapid responses to quickly evolving pathogens (Altizer et al., 2003; Horton et al., 2004).

In addition to biological adaptations, humans also respond to disease through altering their environment to reduce infection risk (e.g., sewage disposal, basic hygiene, clean water, and insect-proof houses), yet many of these preventative strategies are only available to individuals in high-income countries (Ewald, 2002). Still, these host responses to infection alter the selection pressure acting on pathogens, leading to ongoing pathogen evolution to overcome human biological and technological disease interventions (Ewald, 1983, 1995; Schmid-Hempel, 2011; Wiley and Allen, 2009). This rapid evolution results in a moving target which changes much faster than technological advances; thereby making the complete elimination of disease difficult (Ewald, 1983, 1995; Schmid-Hempel, 2011; Wiley and Allen, 2009). Moreover, it is now evident that non-compliance and overuse of antibiotic treatment has (ironically) contributed to the current re-emergence of infectious disease and rising rates of drug-resistant pathogens (e.g., tuberculosis, *Clostridium difficile*, Methicillin-Resistant Staphylococcus Aureus [MRSA], *Streptococcus pneumoniae*) (Armelagos et al., 1991; Barrett et al., 1998; Boni & Feldman, 2005).

In humans, demographic features and socio-economic disparities also play a key role in maintaining global infectious disease levels. The global population has exploded in the past century, growing from 2 billion in 1927 to ~7 billion in 2013, with the population expected to grow to 9.8 billion by the year 2050 (United Nations Department of Economic and Social Affairs, 1999, 2017). This massive population increase has important implications for social inequality and health (e.g., Osei-Atweneboana et al., 2012; WHO, 2016). Disease burdens among the poor – particularly in low-income countries – often remain “hidden” and attract national attention only when transmission to other, less poor segments of the population bring these conditions to larger public awareness (Nicastri et al., 2001; Wiley & Allen, 2009). Thus, infectious disease morbidity and mortality are disproportionally high in economically developing low- and middle-income countries (LMICs), particularly in tropical regions where disease-causing microbes and vectors flourish.

Many infectious diseases common in LMICs result in high morbidity but relatively low mortality, and so garner comparatively little research and intervention investment from the global medical community. This lack of attention and the prevalence of these diseases in tropical areas has led the World Health Organization (WHO) to label this set of illnesses “Neglected Tropical Diseases” (NTDs) (WHO, 2016). This group of diseases includes 13 types of infection: Buruli ulcer, Chagas disease, cholera, dengue fever, dracunculiasis, lymphatic filariasis, human African trypanosomiasis, leishmaniasis, leprosy, onchocerciasis, schistosomiasis, soil-transmitted helminths, and trachoma (Aagaard-Hansen & Chaignat, 2010; WHO, 2016). These illnesses cost LMICs billions of dollars annually and primarily impact people living in poverty (often those lacking adequate sanitation and living in close contact with infectious disease vectors and domestic animals), consequently affecting local and global rates of economic development (WHO, 2016). Over 70% of nations affected by NTDs are low- and lower middle-income countries, and 100% of low-income countries are affected by five or more different NTDs (WHO, 2006).

Evidence indicates that 141,800 deaths were attributable to NTDs in 2013 (Herricks et al., 2017); yet, mortality numbers alone do not capture the magnitude of NTD costs in long-term suffering, morbidity, lost production, and educational attainment. For instance, about 57 million life years are lost to premature disability and death because of NTD infections across the 149 countries in which they are endemic, affecting over 1.4 billion people worldwide (Kealey, 2010; WHO, 2015). More recent estimates suggest that NTDs resulted in 79 million disability-adjusted life years (DALYs; a metric of disease burden where one DALY can be thought of as one lost year of ‘healthy’ life) in

2015 (GBD, 2016). Morbidity associated with NTD infection also has several poverty-promoting effects, including decreased work productivity and reduced future wage-earning capacity (Hotez et al., 2008).

High rates of parasitic infection among the world's most marginalized populations ultimately result in impaired physical and cognitive function, limited economic productivity, and death, making it difficult for effectively trapping these groups to escape in a cycle of disease and poverty (CDC, 2011). It is therefore unsurprising that these conditions rank near the top of all global health threats (Hotez, 2017). Still, despite widespread prevalence, neglected tropical diseases receive little attention in part because endemic disease suffered by poor people in lower income countries often remain invisible to decision-makers and those who allocate the majority of global medical resources (Aagaard-Hansen & Chaignat, 2010). Funding for research and public health interventions has instead been concentrated on the 'Big Three' – tuberculosis (TB), malaria, and HIV/AIDS – which are responsible for greater mortality levels (Kealey, 2010).

However, it has become increasingly clear that the Big Three predominantly occur in populations also suffering from high rates of NTD infection, with soil-transmitted helminths representing the most common parasites found in HIV/AIDS-, tuberculosis-, and malaria-infected populations (Hotez et al., 2006). For instance, a close geographic overlap between hookworm and malaria has been demonstrated across Africa (Hotez et al., 2006). The alleviation of many parasitic illnesses could potentially improve the prognosis of TB, malaria, or HIV/AIDS cases by reducing co-infection rates and alleviating NTD symptoms (e.g., anemia) which exacerbate the progression of Big Three infections (Kealey, 2010; Hotez et al., 2006). It has therefore been suggested by Hotez and colleagues that a successful global fight against HIV/AIDS, tuberculosis, and malaria requires concurrent treatment of NTDs, thus shifting attention to a larger battle against "a new 21st century 'Gang of Four'" (Hotez et al., 2006). Yet, this public health focus on the Gang of Four has yet to be matched by an equal emphasis in research efforts.

Research Goals and Theoretical Framework of this Dissertation

This dissertation research seeks to contribute data useful in the management of parasitic disease by examining how physiological factors and lifestyle patterns shape male health in an indigenous, Ecuadorian population experiencing high parasite loads (Blackwell et al., 2010; Cepon-Robins et al., 2014). Specifically, it uses evolutionary LHT to understand how lifestyle factors shape interactions between individual physiology and immune function (i.e., parasitic disease risk). It also tests hypothesized life history tradeoffs between reproductive effort and immune function that underlie several models concerning male disease risk.

Life History Theory and Immune Function

Life history theory is the branch of evolutionary theory that provides the theoretical framework for understanding how selection shapes the timing and context sensitive allocation of energy to critical life functions (e.g., growth, reproduction, senescence rate) (Bogin et al., 2018; Hill, 1993; Hill & Kaplan, 1999; Stearns, 1977). Typically, these critical life functions are broadly divided into somatic and reproductive effort. Somatic investments include growth, body maintenance, and immune function.

Reproductive investments are comprised of mating effort (e.g., finding, acquiring, and guarding a mate), the act of reproduction itself, and parental care (Hill, 1993; Kaplan et al., 2000). Somatic investment can be considered investment in future reproduction through extended longevity; conversely, reproductive investments represent current procreative efforts (Hill, 1993; Kaplan et al., 2000).

Within the life history of a given species, the fitness payoffs of different energy allocations will vary based on evolutionarily relevant conditions, so selection is expected to produce adaptations which allocate energy in a context-dependent manner (Kaplan & Gangstead, 2005; McDade, 2005). Depending on ecological conditions, an organism may forgo somatic investment in favor of immediate reproduction (e.g., if there are high rates of predation and they may not survive long enough for somatic investments to pay off reproductively) (Hill, 1993; Kaplan et al., 2000; Stearns, 2000). Thus, the timing of life events in strongly influenced by social and environmental factors, and these must be considered in studies examining different investment strategies.

A key premise of LHT is that energy is limited, so energy used for one function cannot be used for another (Bogin et al., 2018; Nylin & Gotthard, 1998; Kaplan & Gangestad, 2005; Stearns, 1977, 1989, 2000). Energetic tradeoffs between various traits are therefore inevitable, and selection is expected to produce mechanisms that preferentially allocate resources to different biological functions (Hill, 1993; Kaplan et al., 2000; Snodgrass, 2012; Stearns, 1989). An additional limiting resource is time, the allocation of which is also expected to entail tradeoffs (Kaplan et al., 2000). A life history tradeoff is defined as a situation in which an energetic investment that enhances one function detrimentally affects another (Hill & Kaplan, 1999; Kaplan et al., 2000; Stearns, 1977, 1989, 2000). These tradeoffs are expected in all important life history physiological processes, or natural selection would drive all fitness associated costs to optimization (Kaplan et al., 2000).

Still, these tradeoffs vary between individuals and within an individual at different life stages. For instance, sick individuals will allocate energy differently than healthy conspecifics (Kaplan & Gangestad, 2005), and these resource allocation patterns will be shaped in part by the duration and severity of infection (Behnke et al., 1992; Medzhitov et al., 2012; Sheldon & Verhulst, 1996). Further, tradeoffs are dependent on energy availability. Tradeoffs are more evident at stressful times when energy is particularly constrained (e.g., in the winter or during pregnancy or lactation) (Demas et al., 1997). The likelihood of a life history tradeoff is also dependent on the energetic cost of a given function.

For example, immune function represents a very costly somatic investment, and investment in immunity typically results in energetic tradeoffs with other expensive biological functions (Biolo et al., 1997; Demas & Nelson, 2012; Lochmiller & Deerenberg, 2000; Muehlenbein et al., 2010; Sheldon & Verhulst, 1996; Wolowczuk et al., 2008; Zuk & Stoehr, 2002). Systematic infection can increase metabolic costs by 50% in chickens (Lochmiller & Deerenberg, 2000). Furthermore, experimental infection increased basal metabolic rate (BMR) in birds 5.27% for every degree C increase. Over the course of 15 days this amounted to approximately 70 kJ, or the cost of producing one egg (Eraud et al., 2005). Similarly, sparrows injected with non-pathological immune stimulant exhibited a resting metabolic rate (RMR) increase of 29%, and this also appeared to impair egg production (Martin et al., 2003). In mice, experimental infection significantly increased energy and oxygen consumption (measured via indirect calorimetry) (Demas et al., 1997). These results collectively suggest the mounting an immune response is energetically expensive and may compromise investment in other physiological functions.

These patterns are also apparent in humans. Fever increases BMR by 10-15% for every degree increase in humans (Roe & Kinney, 1965). A similar study documented that mild respiratory infection increased RMR by an average of 8% in healthy men, but increases of up to 14% were also observed (Muehlenbein et al., 2010). In addition, isolated mitochondria exposed to the protein immune mediator interleukin (IL)-1 or tumor-necrosis factor (TNFα) increased their respiratory rate by 30% and IL-6 infusions in health adults can increase RMR by 25% (Lochmiller & Deerenberg, 2000). Chronic infection also appears to increase the rate of gluconeogenesis by up to 150-200% to fuel an effective immune response. This increased production rate depletes individual energy stores, and may decrease body weight by 15-30% over the course of an infection (Lochmiller & Derenberg, 2000). It is therefore apparent that immune responses are extremely costly and may utilize energy that other physiological functions rely on, resulting in tradeoffs that detrimentally impact other important functions, including reproduction.

For instance, across many species (i.e., fence lizards, trout, mice, several species of birds) males exhibit higher parasite loads than females, likely due to males having greater relative preferential investment in reproductive effort over immune function and longevity than females (Campbell et al., 2001; Klein, 2000; Poulin, 1996; Zuk & McKean, 1996). In addition to shaping key tradeoffs, the energetic costs of early disease exposure and poor nutrition also clearly influence the timing of life history events. Animals – including humans – born to malnourished mothers or who experience high pathogen burdens early in life exhibit decreased growth rates and altered immune development trajectories compared to individuals who didn't face these challenges (e.g., Blackwell et al., 2010; De Onis et al., 1993; Gluckman et al., 2007; McDade, 2005; Urlacher et al., 2016, 2018). Thus, immune function is a strong determinant of investment in other physiological functions and appears to influence the timing of important life history events.

Another factor shaping the influence immune function exerts on life history is the relative cost of different immune activities. For example, humans exhibit two basic types of immune responses: (i) continually active "innate" immunity (e.g., immune responses that target pathogens to which an individual is continuously exposed), or (ii) "adaptive" immune responses that are only activated when a pathogen is detected (Hall & Guyton, 2011; Schmid-Helprin & Ebert, 2003). These distinctive immune responses incur different energetic costs. Innate immune responses have lower developmental costs (e.g., are less costly to continuously maintain), but are generally thought to be more expensive to deploy and may also result in collateral host tissue damage (Hall & Guyton, 2011; Lochmiller & Deerenberg, 2000; Schmid-Helprin & Ebert, 2003). Conversely, adaptive immunity represents a more effective, targeted response to pathogens that is less costly to deploy, but more energetically expensive to maintain (Hall & Guyton, 2011; Schmid-Helprin & Ebert, 2003). Given this variation in immune costs, the type of immune response a human host mounts in response to illness varies according to infection severity, duration, and host energetic status (i.e., resources available to the host to mount an effective immune response) (Behnke et al., 1992; Medzhitov et al., 2012; Sheldon & Verhulst, 1996).

In addition to context-dependent immune responses, most species (including humans) appear to respond to endemic disease risk through altered life history trajectories. For example, in areas experiencing a high pathogen load, individuals within a species may reproduce earlier in life before disease exposure substantially impairs reproductive function, as opposed to individuals who invest more energetically into growth and delay reproduction (Minchella, 1985). There is some evidence to suggest that humans exhibit these LHT strategies. For example, research suggests that a positive correlation is apparent on a global scale between environmental stressors – including parasite virulence – and fertility (Guégan et al., 2001; Migliano et al., 2007). This illustrates how integrating LHT and energetic tradeoffs into immunology studies can clarify the timing of important life events such as reproduction.

In addition, different species are expected to utilize different immune strategies depending on their life history trajectories. “Fast-living” species with high reproductive rates and decreased longevity appear to rely more upon nonspecific inflammatory immune responses. Conversely, “slow-living” species with lower reproductive rates and extended life spans are expected to exhibit increased reliance on long-lasting costly (but more effective) acquired immune responses, because they will likely live long enough for the investment in immune function to pay off reproductively (Lee, 2006). This variation illustrates the utility of using LHT to understand species-specific immune strategies.

Life history theory can also be implemented to better understand the timing and strength of immune responses to infection. To be adaptive, the benefits of mounting a given level of immune response to clear an infection must outweigh the costs (i.e., tradeoffs with other physiological processes and possible incidental damage to host tissue) (Sheldon and Vehulst, 1996). Therefore, the maximum immune response is not necessarily the optimal response: if the maximum immune response diverts too much energy from other crucial functions the individual may instead mount a smaller immune response or simply tolerate the infection (Viney et al., 2005; Zuk and Stoer, 2002). This suggests that immune strategies are not generic, but are strongly influenced by unique environmental and physiological constraints; these responses should therefore be considered using a LHT approach.

Immune and Physiological Regulation

Given the high energetic costs associated with immune responses, immune function is tightly regulated within species. These responses are strongly influenced by endocrine messengers called hormones, which function to deliver environmental and physiological information to different cells throughout the body (Kaplan & Gangestad, 2005; McDade, 2005). These hormone messengers typically elicit a cellular response (e.g., protein synthesis) in response to their chemical message (Ellison, 2009). Hormone levels vary within and between individuals, life stages, and different species; this variation impacts immune status through the shaping of life history tradeoffs and the triggering of unique responses in response to individual contexts.

There are several types of hormones, including steroids (Ellison, 2009). Steroid hormones (e.g., testosterone, estrogens, progesterone) have several important functions, including sexual differentiation, gamete production, regulation of the female menstrual cycle and pregnancy, and shaping body composition (Ellison, 1993; Wallen & Hasset, 2009; Wells, 2010). Sex hormones profoundly influence physical development, from shaping genitalia development to influencing the distribution of adipose tissue (Wallen & Hasset, 2009; Wells, 2010). The effects of these hormones are also largely responsible for sex differences that emerge during puberty, leading to divergent patterns in both physical development and general behavioral patterns (Wallen & Hasset, 2009). Many of these factors have important implications for an individual's ability to acquire a mate, hence shaping reproductive success.

Types of Sexual Selection

Reproductive success (or the number of surviving offspring an individual produces) is heavily influenced by sexual selection. Sexual selection was first defined by Darwin as the competition for access to mates, particularly high-quality mates with desirable traits that can be inherited by offspring (Darwin, 1871). There are two general types of sexual selection: intra- and inter-sexual selection. Intra-sexual selection is selection for traits because they increased their bearer's ability to gain sexual access to the opposite sex via competition with same-sex competitors (Darwin, 1871; Freeman & Heron, 2007; Futuyma, 2009). For example, the results of intra-sexual selection include dominant male elephant seals physically driving away other males from a section of beach, thus ensuring exclusive access to a group of females (Cox & Le Boeuf, 1977).

In contrast, inter-sexual selection refers to selection for traits that increase fitness because they are preferred by members of the opposite sex. As a result, the trait spreads throughout the population and may become exaggerated over time, even if it has no survival value (Darwin, 1871; Fisher, 1915, 1930; Freeman & Heron, 2007; Futuyma, 2009). The classic example of inter-sexual selection is the female peahen's preference for the highly exaggerated tail of male peacocks. One possible mechanism accounting for this type of selection was proposed by Fisher as the "sexy son hypothesis", which states that an essentially arbitrary preference by one sex could become linked with expression of a trait in the other sex (Fisher, 1915, 1930). According to this hypothesis, mate choice occurs to ensure that offspring are born with the desired trait and therefore have the best chance for reproductive success; thus, this preference for exaggerated traits may lead to a process of "runaway" sexual selection (Fisher, 1915, 1930).

An alternate explanation for the mechanisms driving intra-sexual selection is that members of the opposite sex prefer these traits because they serve as signals of heritable high-fitness attributes, such as greater parasite resistance (Hamilton & Zuk, 1982). In contrast to the sexy son hypothesis, according to this model trait preference is not arbitrary. Rather, mate choice for these traits is thought to increase offspring genetic quality (Hamilton & Zuk, 1982). On this view, signals of health and immune function are hypothesized to play an important role in mate selection. It is therefore important to distinguish between signals and cues used in mate choice. As defined by biologists, signals are features or behaviors that have evolved because they indicate some underlying quality; further, the information conveyed by signals alters the behavior of perceivers (Bradbury & Vehrencamp, 1998; Smith, 2003). Conversely, cues have not specifically evolved to carry an explicit meaning or impart certain information on behalf of the organism possessing that trait (Bradbury & Vehrencamp, 1998; Smith & Harper, 2003).

Building upon these definitions, costly signaling theory contends that traits associated with desirable genetic qualities or behaviors (e.g., ability to provision offspring with material resources) can evolve into exaggerated displays, which serve as "honest" signals of underlying genotypic or phenotypic traits (Grafen, 1990; Zahavi & Zahavi, 1997). According to this model, these costly signals must be authentic representations of individual health and genetic quality, or they would cease to relay useful information to perceivers (Grafen, 1990; Zahavi & Zahavi, 1997). Thus, to avoid being tricked by a false signal from a lower quality individual, it is hypothesized that members of the opposite sex select signals that cannot be faked by cheaters. In other words, the high costs incurred by the development and maintenance of a costly signal necessarily prevents lower quality individuals from imitating it, while the fitness costs of the display to the bearer are offset by increased mating opportunities (Grafen, 1990; Zahavi & Zahavi, 1997). This high cost ensures the signal's honesty, and these costly signals can therefore be reliably used during mate selection to recognize desirable mates.

Evidence for both intra- and inter-sexual selection are also apparent in humans, with men and women exhibiting strong preferences for certain physical cues in prospective mates (e.g., waist-hip-ratio, facial symmetry, clear skin) (Grammer & Thornhill, 1994; Marlowe & Wetsman, 2001; Singh, 1993; Sugiyama, 2005, 2015; Wetsman & Marlowe, 1999; Yu & Shepard, 1998). The psychology generating these preferences are hypothesized to be universal to men and women, with increasing empirical evidence that this is the case. However, the degree to which a given preference is expressed is also hypothesized to be context sensitive, reflecting conditions affecting tradeoffs among preferences based on local socio-ecological variables (Sugiyama, 2004, 2005, 2015; Tooby & Cosmides, 1992). For instance, diet, physical work patterns, pathogen exposure, and psychosocial stress have all been shown to affect hormonal indices of female fertility and fecundity, suggesting that reproduction is shaped by context-dependent energetic constraints (Ellison, 1993, 2001, 2003; Jasienska, 2009; Vallegia & Ellison, 2009). While male reproduction does not appear to be as sensitive to energetic conditions, there is evidence to suggest that men with less nutritious diets, elevated disease risk, and higher activity levels, experience physiological changes (such as reduced testosterone levels) that decrease investment in reproductive effort to acquire mates (Bribiescas, 1996), likely due in part to increased immune activity.

Immune Tradeoffs and Mate Choice

The immune and reproductive systems are closely linked, with hormones driving this relationship. For instance, sex hormones impact secondary sexual characteristic development (traits unique to each sex that are not directly involved in reproduction), simultaneously influencing immune function and mate selection (Klein & Nelson, 1999). For example, the hormone testosterone is thought to mediate tradeoffs between reproductive effort and immunity in adult males. Work with non-human animals has shown testosterone enhances the development of male secondary sexual characteristics, but with immunosuppressive effects (Adamo et al., 2001; Klein, 2000, 2004; Poulin, 1996; Zuk & McKearn, 1996).

Although not yet well-documented in humans, these patterns are likely to be evolutionarily relevant as men exhibit a much higher ratio of lean muscle mass to fat than do women, reflecting sex differences in body composition that underlie reproductive strategies. On average, men have about 15-20% greater body mass than women (Puts, 2016); for example, Wells (2012) documented a mean lean mass of 50.8 kg in men compared to 38.7 kg in women. Men have about 61% greater muscle mass than do women, translating to about 90% greater upper body strength and 60% greater lower body strength than women (Puts, 2016; Lassek & Gaulin, 2009). Conversely, women have about 40% greater adipose tissue than men, with much of it concentrated in the gluteal femoral region (Lassek & Gaulin 2009). These differences appear to be driven by sex-specific selection pressures, including those favoring higher levels of fat in women (to buffer and support the high costs of female reproduction) and increased musculature in men (thought to be indicative of male-male competition to enhance male competitiveness in acquiring mates) (Lassek & Gaulin, 2009; Puts, 2010, 2016; Sell et al., 2012, Wells, 2007).

Moreover, it is likely this strong selection favoring musculature in men is mediated through the anabolic effects of testosterone, potentially at the cost of immune function (Bribiescas, 2001). In addition to muscle mass, testosterone has been linked with a variety of other phenotypic traits, often used to measure degree of individual masculinity (Gallup & Frederick, 2010; Sell et al., 2009). These include height, grip strength (a proxy of overall physical strength), chest compression (a measure of upper body strength), upper arm circumference (associated with amount of musculature), and 2D:4D digit ratio (an indicator of prenatal testosterone exposure) (Gallup & Frederick, 2010; Sell et al., 2009). While a large literature exists testing hypotheses about female preferences for male masculine traits and their relation to male mating strategies (e.g., Sugiyama 2005, 2015 for review), little research has tested the hypothesized tradeoffs between testosterone, immune function, and key aspects of human life history.

Despite limited research documenting tradeoffs between testosterone and immunity in humans, a large literature is based on the premise that testosterone is immunosuppressive. For example, females are hypothesized to prefer masculine secondary sexual characteristics linked with testosterone levels because they provide honest signals of heritable immunocompetence (Folstad & Karter, 1992). Based on this logic, studies have tested whether women display a significant preference for masculine male features, but with mixed results (DeBruine et al., 2006; Gangestad & Thornhill, 2003; Scott et al., 2014; Thornhill & Gangestad, 2006). Further, some studies in high-income nations have found no relationships between testosterone-linked traits and health, or found only a weak association (Kalick et al., 1998; Pound et al., 2014; Rhodes et al., 2003). A major limitation of previous work is that few studies have tested these hypotheses in a natural fertility, subsistence based, energy constrained population with high infectious disease burden, conditions most relevant to the evolution of hypothesized testosterone-mediated costly signals (Sugiyama, 2015; Thornhill & Gangestad, 2006).

Previous work indicates that natural fertility, subsistence populations (e.g., Shuar and Tsimane forager-horticulturalists) are characterized by increased immune system activation costs in response to elevated infectious disease exposure, including parasitic infection (Blackwell et al., 2010; Hotez et al., 2008; Hotez & Kamath, 2009). Further, given the long coevolutionary history humans share with parasites, and the costs of immune defense, parasitic disease may have particularly important implications for evolved life history tradeoffs (Hurtado et al., 2008; Jackson et al., 2009). Given this long period of coexistence, parasitic disease is also hypothesized to have played an important role in human evolutionary processes, including sexual selection favoring traits indicative of good health. Still, the relationship between testosterone and immune function in humans is unclear.

This dissertation therefore seeks to clarify how testosterone profiles and various masculine traits (thought to have evolved as costly signals of good health to prospective mates) are associated with infectious disease risk (as determined through the assessment of evolutionary relevant immune markers: parasite load and the concentrations of three key antibodies). This research was conducted among the Shuar of Amazonian Ecuador with the goal of elucidating how physiological patterns and lifestyle factors shape male health outcomes; thereby reducing disease risk – especially NTD infections – among the Shuar, and other populations experiencing a high infectious disease burden.

Research Goals and Hypotheses

Aim: This study will test relationships among between testosterone profile, immune function, and parasite load among indigenous Amazonian Shuar men living in a natural fertility, high pathogen environment. It thereby tests hypothesized tradeoffs among testosterone, immune function, and reproductive effort that underlie human life history. The data produced will better capture how individual biology and socio-ecological factors influence the relationship between male mating effort and immune function. Drawing from previous research, the primary goals and hypotheses of the proposed research are as follows:

Goal 1. To compare testosterone level variation among Shuar men. The hypothesis that will be tested is: H_1: Salivary testosterone levels will be positively correlated with household level of economic development and adiposity, but negatively correlated with age.

Goal 2. To determine if individual reproductive success is significantly influenced by signals of developmental testosterone and parasite load. The following hypotheses will be tested: H_2: Age-specific reproduction (number of children by age cohort) will be positively related to indicators of developmental testosterone (height, grip strength, chest compression, upper arm circumference, 2D:4D digit ratio); and, H_3: Masculine features are reflective of increased resistance to the effects of parasitic infection (e.g., during key developmental periods). Therefore, parasite load will be negatively associated with indicators of developmental testosterone.

Goal 3. To test if testosterone is negatively associated with immune function. The hypothesis to be tested is: H_4: Circulating testosterone (measured from saliva) is immunosuppressive and therefore will be negatively associated with immune response as measured by: (i) an acute inflammatory marker C-reactive protein (CRP); (ii) a marker of acquired humoral immunity (immunoglobulin G [IgG]), and; (iii) an indicator of macro-parasitic infection (immunoglobulin E [IgE]). Testosterone will decrease during immune system activation and therefore be negatively associated with (iv) parasite infection load.

This dissertation combines methodology from several disciplines to better examine evolutionary links among ecological variation, sexual selection, behavioral endocrinology, and human biology. Although methods differ somewhat across these fields, they share a fundamental basis in evolutionary theory. This research synthesizes complementary hypotheses and methods from these disciplines to provide evolutionary relevant data on how potential tradeoffs affect individual health (e.g., parasitic disease) and phenotypic traits that underlie hypotheses about mate choice, intra-sexual competition, and the evolution of potentially costly signals in humans. This conceptual integration of interrelated branches of evolutionary research is analytically powerful, and works toward the theoretical unification in the emerging field of human evolutionary biology.

Transition to Chapter II

Chapter I has outlined the theoretical framework and research goals of the dissertation research. This chapter has also explored the global burden imposed by high infectious disease rates, especially neglected tropical diseases in lower-income nations. In addition, the costs of immune function have been briefly considered and potential tradeoffs between investment in mounting an immune response and other functions (e.g., reproduction) have been highlighted. The next chapter will delve further into these relationships through a more in-depth exploration of the human immune system and a discussion of how sex hormones influence various immune mechanisms. The role these associations play in human sexual selection will also be further explored. Finally, the concept of market integration will be introduced and the influence of this process on hormone levels and health outcomes will be discussed.

CHAPTER II

HORMONE LEVELS, IMMUNE FUNCTION, AND MARKET INTEGRATION

Introduction to Human Endocrinology and Sexual Selection

The Role of Sex Hormones in Human Biology

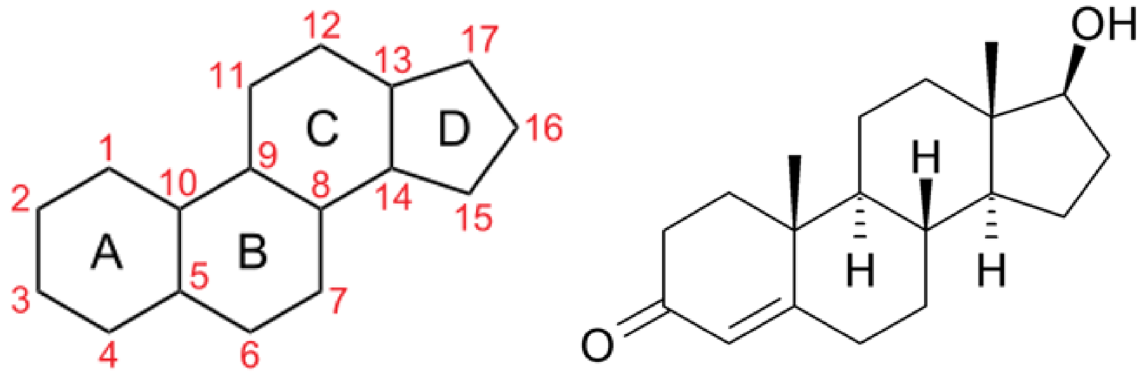
Hormones function as chemical messengers in the body, relaying information about environmental conditions to various body tissues. Hormones themselves do not directly cause physiological changes, rather each cell must "decide" how to respond to the information provided by the hormone messenger. This often results in a cascade of biological processes, culminating in the production of specific proteins or enzymes in response to different hormone levels (Ellison, 2009). There are several types of hormones, including steroid hormones.

Steroid hormones are small lipophilic molecules able to cross cell membranes and bind to intracellular receptors; these hormone-receptor complexes can then bind directly to DNA sequences (e.g., promoter regions) and initiate the transcription of specific protein products (Hall & Guyton, 2011; Wallen & Hasset, 2009). Steroid hormones are often carried in the blood by carrier proteins (e.g., sex hormone binding globulin [SHBG] or albumin) which render them biologically inactive and unable to cross cell membranes. These bound hormones serve as reservoirs which survive in the blood for longer periods of time than unbound hormones (Hall & Guyton, 2011; Wallen & Hasset, 2009). Typically, less than 10% of sex hormones are unbound in the blood, the rest are bound to carrier proteins (Bribiescas, 2010; Wallen & Hasset, 2009).

An important class of steroid hormone are the sex hormones. These hormones are comprised of progestins (mostly progesterone), androgens (such as testosterone), and estrogens (estradiol, estrone, and estriol) (Wallen & Hasset, 2009). Sex hormones share 18 carbon atoms in common, and are differentiated by the number of total carbon atoms (18-21) and the side groups found at their biologically active site (Wallen & Hasset, 2009). All sex hormones are produced *de novo* from cholesterol in the gonads or adrenal cortex and are reduced from 21 carbon progestin into their final form (Figure 2.1a); thus, these hormones can only lose, never gain, carbon atoms (Wallen & Hasset, 2009). The impact of sex hormones on various tissues is also influenced by the amount of aromatase present. Aromatase is an enzyme that reduces the number of carbon atoms in a sex hormone (e.g., converts testosterone to estrogen); thus, tissues exposed to the same hormone concentrations may exhibit very different responses based on the level of aromatase present (Wallen & Hasset, 2009).

The concentration of sex hormones in circulation is typically regulated by a negative feedback system. Specifically, sex hormone production is stimulated by luteinizing hormone (LH) and follicle stimulating hormone (FSH), which are released from the anterior pituitary. The production of these gonadotropin hormones is triggered by gonadotropin releasing hormone (GnRH), released from the hypothalamus. Increased sex hormone levels tend to suppress GnRH release, which consequently inhibits the release of LH and FSH, and decreases sex hormone production. Still, positive feedback does sometimes occur in the release of sex hormones, such as the surge of female reproductive hormones that trigger ovulation (Hall & Guyton, 2011; Wallen & Hasset, 2009).

Sex hormones have several important effects. They direct sexual differentiation, development, and reproductive function in both sexes (Hall & Guyton, 2011). In addition, they shape fat distribution patterns in men and women. This is dependent on the distribution of sex hormone receptors in various body tissues, but generally testosterone and estrogen influence abdominal and gluteofemoral fat distribution, respectively (Wells, 2010). Both men and women produce all sex hormone types, but the relative amount of different sex hormones varies between the sexes (Wallen & Hasset, 2009). Still, these hormones do have some similar effects in men and women. For example, testosterone strongly influences libido in both sexes (Bribiescas, 2001). Additionally, androgens initiate and estrogens end puberty in both men and women (Ellison, 2009). Yet, different levels of sex hormones do result in significant sex differences.



(a) (b)

Figure 2.1. (a) Basic cholesterol steroid hormone skeleton; (b) molecular structure of testosterone. Figure modified from OU Open Textbooks.

Male Reproduction: The Effects of Testosterone over the Lifespan

Male sexual development is characterized by the activity of androgens, including the hormone testosterone. Testosterone (Figure 2.1b) is well known for playing a key role in energy allocation and the development of male characteristics, including energetically expensive traits like increased musculature (Bribiescas, 2001, 2010), but the effects of this hormone begin in utero (Figure 2.2). Mullerian regression hormone causes the degeneration of rudimentary female reproductive organs, and Y chromosome genes cause the production of testes determining factor, which promotes the development of Leydig cells (producers of androgens) and Sertoli cells (important in sperm production) (Bribiescas, 2009; Hall & Guyton, 2011). The production of androgens supports the development of male reproductive organs, and androgen concentrations surge during the first six months of life to approximately half the levels observed in adults (Chemes, 2001; de Zegher et al., 1992; Dunkel & Huhtaniemi, 1990; Waldhauser et al., 1981).

Androgen levels then typically decline and remain low throughout childhood, likely due to the extreme sensitivity of the hypothalamus to sex hormone levels (Bribiescas, 2009; Hall & Guyton, 2011). At puberty the hypothalamus becomes desensitized to sex hormone concentration, perhaps due to the increased production of androgens by the adrenal cortex during adrenarche, and androgen levels subsequently increase in males. This increase promotes the development of sex-specific male characteristics, such as the growth of the penis, testes, and scrotum, a deeper voice (with enlargement of the larynx), and increased musculature (Hall & Guyton, 2011). Androgen levels remain elevated throughout adulthood and continue to shape traits linked with male biology and reproduction (Bribiescas, 2009).

It is important to note that the majority of androgen level research has been largely restricted to high-income populations, and it is unclear how much variation exists between groups living in distinctive physical and social environments. In particular, what constitutes "typical" testosterone levels during puberty and adulthood remain unclear (Bribiescas, 2001; Gould & Petty, 2000; Shores et al., 2012; Snyder et al., 1999); still, testosterone elicits similar physical effects cross-culturally (Bribiescas, 2001; Chemes, 2001; de Zegher et al., 1992; Dunkel and Huhtaniemi, 1990; Waldhauser et al., 1981). For example, across all populations testosterone exhibits a diurnal pattern, fluctuating predictably across a 24-hour period (Bribiescas and Hill, 2010). Testosterone levels peak in the morning (likely to mobilize energy for use through the day) and decline throughout the day (Bribiescas and Hill, 2010).

In addition, the effects of testosterone on physical processes also appear to be similar across ecological settings (Bribiescas, 2001, 2010). Androgens have anabolic effects that are energetically costly; by some estimates, anabolic processes account for ~ 20% of male basal metabolic rate (BMR) (Bribiescas, 2001, 2010). Males also exhibit increased BMR and red blood cell counts relative to females, likely due to the anabolic effects of androgens (Hall & Guyton, 2011), creating sex differences in energetic requirements (e.g., related to reproductive function).

Reproductive function is strongly influenced by several factors, including energetic status (e.g., nutrition and activity levels). In general, female fertility appears to be much more sensitive to current energetic status than males. This sensitivity has likely evolved to prevent investment in an energetically expensive pregnancy when conditions are not ideal and the odds of a successful pregnancy are low (Ellison et al., 1993; Ellison, 2003, 2008, 2009; Jasienska, 2001). Conversely, male fertility appears to much less sensitive to energetic constraints. Sperm production in mammalian males (including humans) accounts for a very low proportion of overall individual energetic costs (Bagatell & Bremner, 1990; Elia, 1992).

Men exhibit robust rates of sperm production even under taxing energetic circumstances, indicating that spermatogenesis, unlike ovulation, is not especially sensitive to acute energetic conditions (Bribiescas, 2001; Muehlenbein & Bribiescas, 2005). Fasting may suppress testosterone levels, and extreme physical activity levels (e.g., marathon runners) may decrease sperm counts, but these reduced levels still fall within clinically acceptable limits (Bribiescas, 2009). In fact, some forms of anabolic exercise appear to increase testosterone levels (Bribiescas, 2009). However, male fertility does appear to be influenced slightly by nutrition. Vegetarian diets low in protein may decrease testosterone levels and low zinc intake may decrease sperm count, but these changes rarely have a significant impact on male fertility (Christiansen, 1991; Bribiescas, 2001).

There are, however, certain aspects of the mating effort (i.e., time and energy spent finding, attracting, and defending a mate) on which males generally appear to expend more resources compared to females (Bribiescas, 2001, 2010). The development of masculine traits and behavior associated with mating effort appears to be strongly controlled by individual physiology (Bribiescas, 2010; Muehlenbein & Bribiescas, 2005), and is thus likely regulated by mechanisms sensitive to environmental factors.

Testosterone, for example, plays a key role in the development of male physical traits and behaviors associated with reproductive effort, including energetically expensive traits like increased musculature (Bribiescas, 2001, 2010; Figure 2.2). It is therefore evident that males and females exhibit very different reproductive strategies and that sex hormone levels strongly influence these patterns.

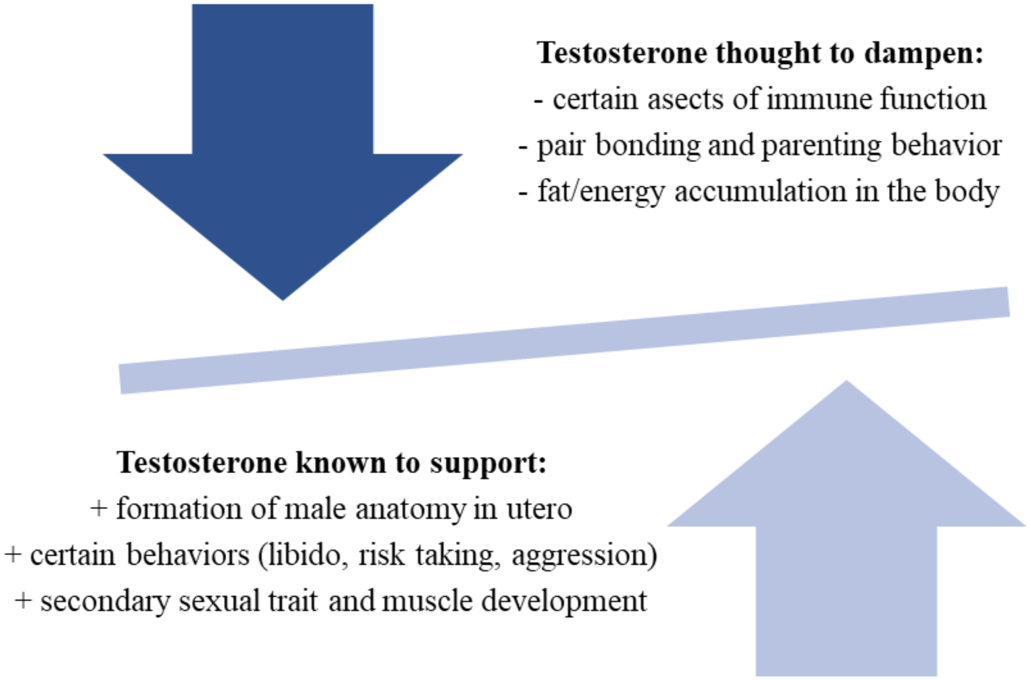


Figure 2.2. Proposed effects of the sex hormone testosterone.

Sexual Selection

The process of sexual selection, defined as the preference of one sex for certain traits in members of the opposite sex, is now recognized by many as a critical process driving the evolution of phenotypic complexity and diversity (Freeman & Heron, 2007; Futuyma, 2009; Miller & Todd, 1995). For example, finding, attracting, and protecting a high-quality mate is a very difficult and important aspect of reproductive success. Choosing the best possible reproductive partner is crucial for passing on adaptive traits and producing healthy offspring that will survive and also reproduce (Darwin 1871, Symons, 1979). Several forms of sexual selection (including various intra- and inter-sexual mating strategies) have therefore evolved, ultimately enhancing an individual's ability to accurately assess and prefer cues of mate value (defined as the number of healthy future offspring an individual is likely to produce) (Darwin 1871, Symons, 1979).

There are two general forms of sexual selection: intra- and inter-sexual selection. Intra-sexual selection refers to competition within one sex for access to members of the opposite sex (e.g., fighting among males for access to a group of females) (Darwin, 1871; Sugiyama, 2015). This type of competition can be very costly, potentially resulting in serious injury and even death; selection is therefore thought to produce adaptations that enable an individual to accurately assess their own mate value relative to potential rivals (Palowski & Dunbar, 1999; Puts, 2010). This saves time and energy, in addition to reducing physical risk by preventing competition with stronger rivals.

There is some evidence that elevated testosterone levels support intra-sexual competition in humans. Testosterone has been associated with aggression (related to competition) in men. For instance, previous research clearly demonstrates that testosterone levels increase in males prior to competition and remain elevated in winners (Booth et al., 1989; Gladue et al., 1989), both in physical (Elias, 1981) and non-physical contests (Mazur et al., 1992, 1997). However, this research has largely been limited to high-income populations, and studies in lower-income groups have documented different patterns, such as larger spikes in testosterone levels following physically-demanding horticultural activities (i.e., tree cutting) in comparison to a competitive event like soccer (Trumble et al., 2013). Still, changes in testosterone supporting this type of subsistence activity also likely have fitness benefits, including an increased ability to support a family.

In both cases, competition and horticultural activities, an increase in testosterone is hypothesized to support energy mobilization in muscle cells, likely involving glucose mobilization and amplified muscle metabolism in anticipation of physical exertion (Tsai & Sapolsky, 1996). Interestingly, this effect is even evident among sports fans, such that testosterone levels increase among fans of a winning team and decreases among those of the losing team (Bernhardt et al., 1998). This suggests direct competition is not required for testosterone levels to change dramatically. Cumulatively these short-term changes are thought to enhance male reproductive success through supporting male efforts to acquire mates (Wingfield et al., 1990).

In contrast to direct competition with members of one's own sex, inter-sexual selection refers to the evolution of traits used to attract members of the opposite sex (e.g., brightly colored features that function only to entice mates) (Darwin, 1871; Sugiyama, 2015). While these attractive traits may increase an individual's mating opportunities and success, they may also negatively impact survivability (e.g., due to the energetic requirements of maintaining these characteristics) (Kokko & Brooks, 2003). Many of the traits used in inter-sexual selection are therefore considered to be "costly". Costly signals are defined as traits linked with good genes (sometimes in the form of elaborate displays), which act as signals of an individual's underlying phenotypic and genotypic qualities (Grafen, 1990; Zahavi & Zahavi, 1997).

An important aspect of costly signaling theory is the premise that these signals must be "honest", accurately reflecting individual health and genetic quality. However, one would expect that the preference for costly signals would lead to the evolution of false signals by lower quality individuals (Grafen, 1990; Zahavi & Zahavi, 1997). To avoid being tricked by a false signal, it is hypothesized that members of the opposite sex are able to identify honest signals that cannot be faked. This means that the high cost associated with the development and maintenance of a signal which reflects underlying phenotypic or genotypic quality prevents lower quality individuals from imitating it, thus ensuring the signal's "honesty" (Grafen, 1990; Zahavi & Zahavi, 1997). Therefore, these signals can be reliably used during mate selection to identify desirable mates, and the fitness costs of the display to the bearer are offset by increased mating opportunities.

The development of costly signals in males has been linked with testosterone levels, and it appears that females may use certain testosterone-linked traits as honest signals of underlying male genetic quality (Folstad & Karter, 1992). In other words, only high-quality males should be able to produce these testosterone-linked masculine characteristics. However, the high energetic demands of these traits may also impact male health (Folstad & Karter, 1992; Klein, 2004; Zuk, 2009), as outlined by the Immunocompetence Handicap Hypothesis.

The Immunocompetence Handicap Hypothesis (ICHH)

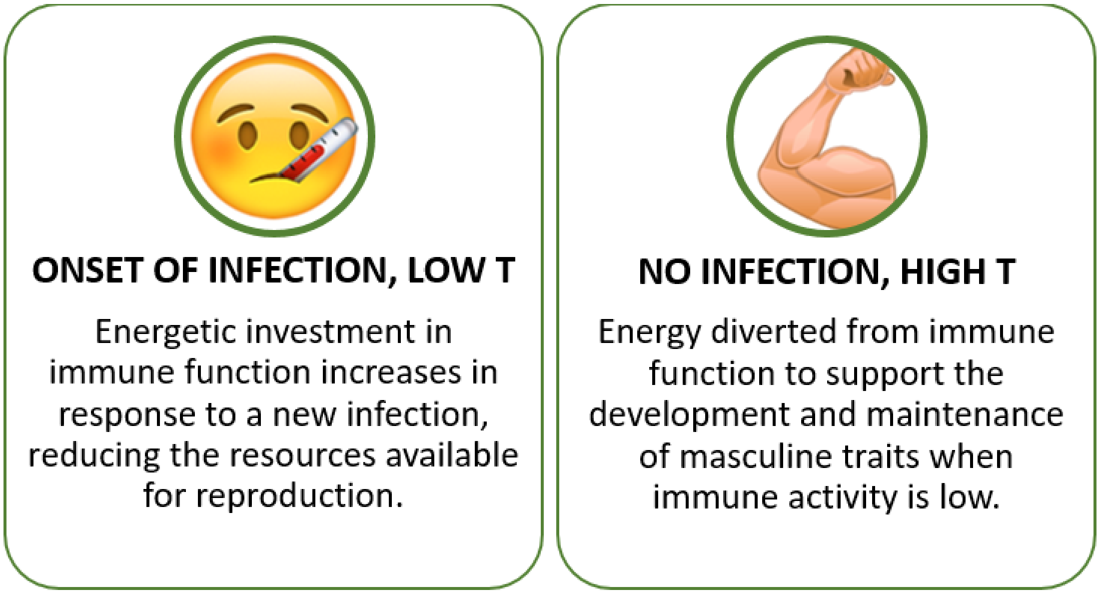
Sex hormones, such as testosterone, appear to influence immune function through binding directly to hormone receptors on many types of immune cells (Fish, 2008; Grossman, 1985; Klein, 2000). Differences in sex hormone profiles therefore have important consequences for immune function, resulting in immunological differences between males and females (in humans and other species). In general, females tend to exhibit a more robust immune response, including more effective presentation of foreign material by immune cells and increased humoral responses (Ahmed et al., 1985; Klein, 2000).

In comparison, males are more often exhibit increased disease risk, and research using animal models suggests that males of many species (i.e., fence lizards, trout, mice, several species of birds) exhibit higher levels of parasitic infection compared to females of the same species (Campbell et al., 2001; Klein, 2000; Poulin, 1996; Zuk & McKean, 1996). This higher level of infection in males has been attributed by many to the immunosuppressive effects of testosterone, which has been hypothesized to increase investment in male mating effort at the expense of other important physiological functions (Adamo et al., 2001; Bouman et al., 2005; Bribiescas, 2009; Morales-Montor et al., 2004). This elevated investment in reproductive effort may decrease overall life span (due to reduced energy allocation to somatic maintenance), but if the male lives long enough to sire more offspring than competitors the investment in high testosterone levels will enhance reproductive fitness and be favored by natural selection (Adamo et al., 2001).

This hypothesized relationship between testosterone and male health is also thought to influence sexual selection patterns. It has been proposed that the close association between reproductive status, immune function, and testosterone levels allow females to use masculine traits – linked with testosterone levels – as an honest signal of underlying male genetic quality. Specifically, females are expected to prefer high quality mates with ‘superior genes’, and to be sensitive to cues of phenotypic condition indicative of this quality, including size, strength, and facial masculinity (Buss & Schmitt, 1993; Folstad & Karter, 1992; Gangestad et al., 2010; Thornhill & Gangestad, 2003). These traits are thought to be influenced by testosterone, and to reflect a male’s ability to withstand health insults (including parasitic infection) (Frederick & Haselton, 2007; Gallup & Frederick, 2010; Gangestad & Buss, 1993; Hamilton & Zuk, 1982; Sugiyama, 2005).

This hypothesis, termed the Immunocompetence Handicap Hypothesis (ICHH), contends that elevated testosterone levels (across species) support the development and maintenance of male secondary sexual characteristics; further, this increased reproductive investment is only possible if relatively little energy is required for immune function (i.e., due to the good health of the male) (Folstad and Karter, 1992). Thus, males are selected for based on the production of costly and conspicuous testosterone-linked secondary sexual characteristics, because these traits reflect an individual’s ability to convert ecological resources into somatic reserves without interruption from immune challenges (Grossman, 1985; Klein, 2000, 2004). Increased investment in these sexual cues should therefore be indicative of superior immune function (e.g., increased disease resistance or more effective infection responses) (Folstad and Karter, 1992). Cheaters should be prevented because their overall reproductive success will be detrimentally affected by chronically elevated testosterone levels and disease burdens exacerbated by the immunosuppressive effects of the hormone (Roberts et al., 2004).

However, this pattern is subject to change within an individual as pathogen load varies over time. For example, as parasite load increases during the course of infection in a male, a shift to less testosterone production and an increased investment in immune function should be observed (Figure 2.3). Once the infection is cleared, individual testosterone levels should rise, marking increased investment in mating effort (Folstad and Karter, 1992). High circulating testosterone levels are thus hypothesized to indicate low current parasite load according to this model (Folstad and Karter, 1992). Although it is possible for males with exaggerated secondary sexual characteristics to temporarily experience lower testosterone levels during periods of intense infection, according to this model, males with more developed masculine characteristics should generally exhibit increased resistance to parasitic illness compared to other males. Females should therefore be able to use testosterone-linked masculine traits to ascertain heritable health and immunocompetence (Stoehr & Kokko, 2006).



ONSET OF INFECTION, LOW T

Energetic investment in immune function increases in response to a new infection, reducing the resources available for reproduction.

NO INFECTION, HIGH T

Energy diverted from immune function to support the development and maintenance of masculine traits when immune activity is low.

Figure 2.3. Effects of testosterone (T) levels on male secondary sexual characteristics and immune function, as predicted by the Immunocompetence Handicap Hypothesis (ICHH).

There is some empirical evidence to support the ICHH. Studies suggest that men experience decreased testosterone levels during periods of illness or somatic injury, with a rebound in testosterone levels after the individual recovers (Dym & Orenstein, 1990; Muehlenbein et al., 2010; Spratt et al., 1993; Spratt, 2001). For example, empirical evidence indicates that experimentally induced infection decreased testosterone levels by 10% in men, with some participants experiencing an average decrease of up to 30% (Muehlenbein et al., 2010). Another human study found that women responded more effectively to seasonal influenza vaccines, perhaps due to a testosterone induced upregulation in genes involved with lipid biosynthesis in men; gene expression that is negatively associated with antibody responses in vaccinated men (Furman et al., 2014). These testosterone-linked changes in gene expression have been linked with a decreased ability to neutralize viruses in men (Furman et al., 2014), potentially driving sex differences in immune responses.

Females of many species also appear to be highly sensitive to subtle variations in male secondary sexual characteristics and testosterone levels as predicted by the ICHH (Klein, 2000; Sheldon & Vehulst, 1996). From a proximate perspective, females may use changes in visual, auditory, olfactory, or behavioral characteristics to discern between healthy and parasitized males (Buchanan et al., 1999; Kavaliers et al., 1997; Kiesecker et al., 1999; Penn & Potts, 1998; Zuk et al., 1990). For example, one study demonstrated that female mice found experimentally infected male mice to be less attractive, but their attractiveness was restored when these males were injected with testosterone, despite continued infection (Roney, 2009).

Additional support for the ICHH has come from work examining the relationship between testosterone and level of asymmetry, a phenotypic indicator of quality. Fluctuating asymmetry (defined as small random deviations from perfect symmetry in bilaterally-paired features) has been linked with developmental disruptions, especially infection and high levels of stress during key developmental periods (Valen, 1962). High levels of fluctuating asymmetry are therefore viewed as indicators of developmental instability (Palmer & Strobeck, 1986; Møller & Thornhill, 1998). According to the ICHH, only high-quality males should be able to withstand the immunosuppressive effects of elevated testosterone levels and develop with minimal disruptions and immune insults (e.g., low levels of fluctuating asymmetry).

In other words, only symmetrical males, signaling high phenotypic quality, should be able to afford to maintain high levels of testosterone (which may compromise immune function) (Kirchengast & Christiansen, 2017). There is some preliminary research among humans supporting this hypothesis. Work among !Kung San men indicates that high phenotypic quality – as measured by low degree of asymmetry – was significantly associated (albeit weakly) with higher testosterone levels (Kirchengast & Christiansen, 2017). Still, little work has been done examining these tradeoffs in humans and alternative hypotheses for female preferences have been proposed.

For example, it has been suggested that high testosterone levels do not always honestly represent infection status. Testosterone levels may instead be honestly correlated with dominance rank, which may or may not correlate with health status. It is therefore possible that dominant, high testosterone males are not more resistant to initial infection and thus exhibit low pathogen loads (as posited by the ICHH), but rather are better able to cope with the negative effects of parasitic infection. There is some evidence to support this model. For instance, among mammals, polygynous species or seasonal breeders are expected to experience higher levels of intrasexual competition, and consequently exhibit elevated testosterone levels (e.g., to maintain physical traits and behaviors linked with dominance). This pattern is hypothesized to result in higher levels of immunosuppression and lead to the increased disease burdens observed in males of many species, including crickets, lizards, birds, mice, baboons, and chimpanzees (Adamo et al., 2001; Ahmed et al., 1985; Fish, 2008; Hamilton & Zuk, 1982; Klein, 2000; Roberts et al., 2004).

Moreover, research among non-human primates indicates that dominant males consistently display higher testosterone levels than subordinate males, yet also harbor significantly higher parasite loads (Hausfater & Watson, 1976; Muehlenbein, 2006; Muehlenbein & Watts, 2010). This pattern suggests that these high-ranking males invest in reproduction at the expense of immune function and consequently exhibit both elevated testosterone levels and infectious disease burdens, yet are still able to acquire mates through retaining a dominant position within the group (Muehlenbein & Watts, 2010). However, other studies have documented no significant relationship between parasite burden and rank among non-human primates (Müller-Graf et al., 2009); it is therefore unclear that social rank and infection risk are uniformly related across species.

A third hypothesis posits that it is individual energetic budgets that most strongly influence disease risk. This hypothesis contends that some males have inherently larger energetic budgets due to some underlying, partly genetic aspect of their physiology, allowing them to invest sufficiently in both immune function and the development of ornamentation (Roney, 2009). However, these individuals may at times be forced to invest more heavily in reproductive effort to outcompete other large budget males. This would result in an immune profile more closely resembling a small budget male (who could not afford to invest as much in ornamentation). Thus, this model suggests nothing about pathogen load and testosterone level, but makes two alternative hypotheses. First, size of ornamentation should positively correlate with male energetic budget. Second, females should selectively mate with males who exhibit large ornaments to ensure these large energy budgets are passed onto their offspring (Roney, 2009). Further work is therefore needed to test these alternative hypotheses, particularly in humans.

Studies testing the ICHH in human populations would particularly benefit by testing the association between testosterone levels and an evolutionary-relevant measure of immune function, such as parasite load. Evidence suggests that parasitic infection may be a particularly important measure within the context of life history tradeoffs given the long coevolutionary history humans share with parasites, and the costs of infection and immune defense (Hurtado et al., 2008; Jackson et al., 2009). Given this long period of coexistence and the rapid evolution of pathogens, parasitic disease is hypothesized to have played an important role in human evolutionary processes, including sexual selection favoring traits indicative of parasite resistance. In addition, it has been suggested that parasite disease risk has shaped aspects of human behavior - including likelihood of group xenophobia - to avoid infection in areas with high rates of transmission (Brown & Fincher, 2016; Fincher et al., 2008; Fincher & Thornhill, 2008; Thornhill & Fincher, 2015). Still, the relationship between testosterone and immune function with regards to human parasitic infection remains poorly studied and studies are needed to clarify the role testosterone plays in parasitic disease risk and other aspects of immune function.

Human Immune Function Overview

The human immune system is a complex, synergistic system composed of two primary branches: innate and adaptive (or acquired) immunity (Table 2.1). In a healthy individual these two parts operate in concert to resist pathogens. It is necessary to have a basic understanding of these components to fully grasp the energetic costs of immunity and how different microbes and parasites trigger distinctive immune responses.

Table 2.1. Basic characteristics of innate and acquired branches of the immune system.

	Innate Immunity	**Acquired Immunity**
Response speed	Immediate (minutes to hours)	Longer lag (hours to days)
Specificity to antigen	Low: non-specific	High: specific to initiating antigens
Response potency	Lower	Higher
Memory (in subsequent infections)	No (same response as previously)	Yes (rapid, amplified response)
Component types	Epithelial barriers, macrophages, dendritic cells, mast cells, granulocytes	B & T lymphocytes, antibodies, activated T cells

Innate Immune Responses

Innate immunity is comprised of general immune responses that are not specialized to specific pathogens. For example, these responses include all defenses encoded in the germline of the host (e.g., epithelial barriers and mucosal linings that sweep away inhaled particles). Innate immunity also ensures the continual presence of certain proteins in blood that can attach to foreign compounds and destroy them (Alexander et al., 2010; Chaplin, 2006; Hall & Guyton, 2011; Wilder, 1998). This class of immune cells includes lysozymes, monocytes, macrophages, granuolocytes, dendritic cells, and natural killer cells. These cells often attack pathogens in circulation through phagocytosis of the microbe itself or by lysing an infected cell (Alexander et al., 2010; Chaplin, 2006; Hall & Guyton, 2011; Wilder, 1998).

Innate immune responses are also closely linked with inflammation, a typical response to injury or infection characterized by pain, swelling, heat, and redness (Akira et al., 2006; Newton & Dixit, 2012). Inflammation occurs when innate immune cells detect infection or tissue damage. Specifically, immune cells residing in tissues (macrophages, mast cells, dendritic cells, etc.) or circulating immune cells (leukocytes) identify a pathogen or cellular damage with pattern recognition receptors (PRRs) that are sensitive to pathogen-associated molecular patterns or damage-associated molecular patterns released by injured cells (Akira et al., 2006; Newton & Dixit, 2012). Activated PRRs then bind together and these large complexes initiate signaling cascades and vasodilation which facilitate the recruitment of leukocytes to the area (Akira et al., 2006; Newton & Dixit, 2012). In addition to inflammation, innate immunity also includes the cell-surface receptors that bind to molecular patterns expressed on surfaces of invading microbes, further activating the immune system, including stimulating more specific adaptive responses (Alexander et al., 2010; Chaplin, 2006; Hall & Guyton, 2011; Wilder, 1998).

Adaptive Immune Responses

In contrast to the general responses characteristic of innate immunity which are effective against many foreign microbes, adaptive immunity directly targets specific microbes through the production of antibodies or activated lymphocytes that seek out certain pathogens and destroy them (Hall & Guyton, 2011). More specifically, this branch of immunity is composed of B cells (responsible for humoral immunity) and T cells (associated with cell-mediated immunity). Both T and B cells originate in bone marrow, and B cells mature there while T cells migrate and mature in thymus (Alexander et al., 2010; Chaplin, 2006; Hall & Guyton, 2011; Wilder, 1998). However, before B or T cells can have any effect, the adaptive immune response must be activated following the invasion of the body by a pathogen.

Adaptive immunity activation occurs through the recognition of specific antigens (generally proteins or polysaccharides) that are contained in the chemical makeup of a microbe. B lymphocytes bind with antigens that match their receptor, the bound cells then activate adjacent B cells, thereby triggering the rapid production of pathogen-specific antibodies (e.g., various immunoglobulins). These antibodies act mainly in two ways. First, they direct attacks on the pathogen, via inactivating the foreign agent (e.g., covering toxic sites) or occasionally by lysing the pathogen directly. Second, antibodies activate the “complement system”, which has several pathways for destroying invaders (e.g., activating phagocytosis by macrophages and neutrophils or via the creation of a lytic complex which directly ruptures the cell membrane) (Alexander et al., 2010; Chaplin, 2006; Hall & Guyton, 2011; Wilder, 1998).

In addition to activating humoral (B cell) immunity, antigens can also be recognized, processed, and presented at the cell surface by so-called antigen-presenting cells (typically macrophages, B lymphocytes, or dendritic cells). Once the antigen is presented by these cell types they can initiate the second type of adaptive immunity: cell-mediated immune responses. A helper T cell with matching receptors will bind to the antigen-presenting cell and secrete a variety of protein mediators called cytokines (e.g., interleukins [IL]) that in turn act on other immune cells (Hall & Guyton, 2011). Some of these cytokines stimulate the growth and proliferation of cytotoxic T cells, which directly targets and kills the specific pathogen. Others stimulate the growth and proliferation of suppressor T cells, which are believed to prevent the cytotoxic T cells from causing excessive damage to the host. Other cytokines stimulate continued B-cell growth and differentiation to form plasma cells and antibodies (Hall & Guyton, 2011). Cytokines also activate the macrophage system, slowing their migration to affected areas to reduce their accumulation and reduce inflammation, and directing them to more effectively target and destroy invading pathogens. Finally, the cytokines provide positive feedback to the helper T cells themselves, stimulating their continued activation and ramping up the entire immune response (Hall & Guyton, 2011).

The cytokines produced by helper T cells can elicit a wide variety of responses, leading to the division of helper T cells into two categories: Th1 or Th2 (Figure 2.4). In general, the activation of one category of helper T cells suppresses the activity of the other. The Th1 response, commonly associated with viral and bacterial infection, is linked with the production of pro-inflammatory cytokines and is largely reliant on the action of neutrophils (which kill invading pathogens via phagocytosis). Conversely, Th2 responses are generally linked with parasitic infection, anti-inflammatory cytokine production (resulting in the down regulation of inflammation levels), and the generation of humoral immune responses (Alexander et al., 2010; Chaplin, 2006; Hall & Guyton, 2011; Wilder, 1998). Activating the correct pathway is critical to effectively deal with a current infection while mitigating damage to host tissue. Cell-mediated immunity also ensures that the host can quickly control future reinfection. This involves the production of T lymphocyte memory cells, specialized cell clones of activated T lymphocytes. These memory cells spread throughout the lymphoid tissue of the body, triggering a rapid immune response if that pathogen invades the host again (Hall & Guyton, 2011). This feature of adaptive immunity is the part stimulated by vaccination, ensuring the immune system is primed to effectively respond to future disease exposure (Hall & Guyton, 2011).

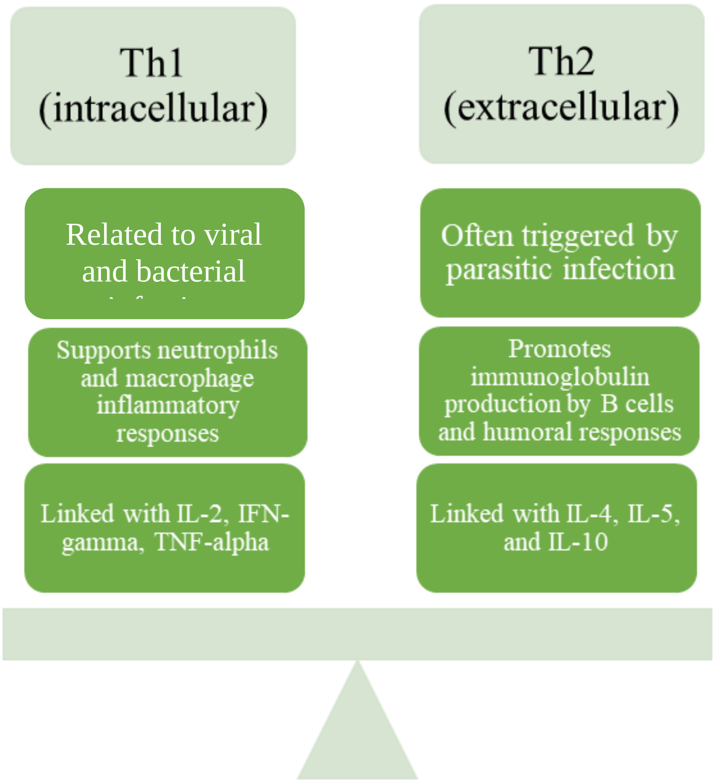


Figure 2.4. Basic characteristics of Th1 and Th2 immune responses.

In addition to differing in function and microbes targeted, innate and adaptive immune responses are shaped by current circumstances, such that low intensity infections may be tolerated if the costs of complete pathogen removal outweigh the benefits (Sheldon & Vehulst, 1996). Thus, optimal immune responses are context dependent, rather than generic, and a strong immune response is not necessarily the best option (Zhao et al., 2017). It is possible that elevated immunity requires such a large amount of energetic investment that the potential benefits of mounting such a response are not worth the tradeoffs with other physiological demands (Viney et al., 2005; Zuk & Stoehr, 2002). The cost of host resistance must therefore be less than that incurred by infection in terms of overall reproductive success to be evolutionary beneficial. Finally, pathogens typically have short generations and evolve rapidly; thus, host immune responses must be flexible to effectively deal with continually evolving microbes (Minchella, 1985). These various factors must be integrated to successfully mount an immune response, and it appears that host physiology (e.g., hormone signals) plays a large role in shaping the type and strength of these responses.

Interactions Between the Sex Hormones and the Immune System

Sex Hormone Influence on Immune Responses

The strength and type of a particular immune response across many taxa appears to be influenced by host hormone levels, including sex hormones (Fish, 2008; Grossman, 1985; Klein, 2000). As previously discussed, hormones serve as a widespread indicator of physical and environmental conditions, which are important in directing an immune response (e.g., signaling the amount energy available to mount an immune response). Thus, many types of immune cells (e.g., cells in the thymus, spleen, and bone marrow) have hormone receptors, including those specific to sex steroids (Grossman, 1985; Klein, 2000), and immune cell proliferation and number are thought to be sensitive to sex hormone levels. For example, T cell numbers in women increase during the follicular phase when estrogen levels are high, but decrease in the luteal phase when estrogen levels fall (Fish, 2008). Immune function therefore seems sensitive to alterations in hormone levels across the lifespan.

Hormone profiles, and subsequent immune responses, change predictably at different life stages. Sex hormones decrease with age in women, and often decrease in older men as well (although there is some context-dependent variation across populations) (Bribiescas, 2010; Ellison, 2009). These alterations may subsequently affect immune function. For example, estrogen levels decrease in women following menopause, and a decrease in T cell numbers has simultaneously been observed (Bouman, 2005). Another example of immune changes during life course is the unique case of pregnancy. A fetus contains foreign DNA, and may therefore be targeted as an invader by the maternal immune system. To prevent this from happening, inflammatory immune responses are suppressed during pregnancy, likely due to elevated progesterone levels (an anti-inflammatory hormone) (Ellison, 2009). More specifically, inflammatory Th1 responses are inhibited to protect the fetus. However, this increases the risk that the mother may be detrimentally affected by diseases targeted by Th1 activation (e.g., toxoplasmosis) (Roberts et al., 1996). It is therefore evident the association between sex hormones and immune status is a complex and highly dynamic process that changes throughout the course of an individual's lifespan.

Basic Sex Differences in Immunity

The consequences of hormone level variation are also evident in immunological differences between males and females, in both humans and non-human animals (Ahmed et al., 1985; Fish, 2008; Grossman, 1985; Klein, 2000). For example, females of many taxa exhibit more efficient Th2 responses, and often demonstrate increased production of IgG and IgM and anti-inflammatory cytokines (e.g, IL-4 and IL-6) compared to male conspecifics (Ahmed et al., 1985; Klein, 2000). It has been hypothesized that this trend allows females to respond more effectively to parasitic infections, while males are more likely to mount inflammatory responses, perhaps negatively affecting disease progression and contributing to symptoms (Klein, 2004). However, the stronger immune function typically observed in females is also a two-edged sword, and females in many species are more likely to experience autoimmune diseases due to overactive immune responses (Campbell et al., 2001; Klein, 2004; Ngo et al., 2014; Shoenfeld et al., 2012).

In comparison, evidence indicates that males across many animal taxa (lizards, birds, rodents, fish) exhibit higher disease rates, likely due to the immunosuppressive effects of elevated testosterone levels (Campbell et al., 2001; Klein, 2000; Poulin, 1996; Zuk & McKean, 1996). In general, high levels of testosterone are associated with higher parasite and disease burdens (Klein 2000, 2008). In addition, increased testosterone appears to reduce overall T cell count, decrease T and B cell and antibody production, and perhaps even increase the likelihood of immune cell apoptosis (Ahmed et al., 1985; Campbell et al., 2001; Fish, 2008). Several studies using animal models have documented that castration is linked with improved immune function, while injection with endogenous testosterone appears to reverse these effects (Ahmed et al., 1985).

Few studies, however, have tested these associations in humans, and those that have often have produced mixed results. For instance, recent work testing the association between testosterone levels and immune function among indigenous Tsimane of Bolivia documented complex relationships, suggesting testosterone differentially influences distinct immune activities (Trumble et al., 2016). This study demonstrated that higher testosterone levels downregulated more costly aspects of immune function (as hypothesized by the ICHH), but had no effect on less costly forms of immune activation, suggesting that the influence of testosterone may vary based on immune response type (Trumble et al., 2016). It may therefore be more accurate to label the effects of testosterone as immunomodulatory, rather than immunosuppressive.

Moreover, immune responses are extremely complex and exceptions do exist; estrogens are not always protective and testosterone is not always immunosuppressive. For example, high estradiol levels are associated with increased risk of certain parasitic infections (e.g., *Taenia* [a genus of tapeworm] and toxoplasmosis) (Klein, 2000). Parasites may also manipulate host sex hormone levels to favor their proliferation, further complicating the relationship between hormone profile and infection intensity (Dunlap & Schall, 1995). Some immune reactions also appear to vary between the sexes based on the specific type of parasite. For instance, IFN gamma levels are significantly higher in females than males during malarial infection, but this pattern is reversed during toxoplasmosis (Escobedo et al., 2010). This suggests that in some cases it is not host sex, but the type of parasite that determines the immune response elicited.

The effect of hormones on immune function is further complicated by the fact that hormones rarely act in isolation; instead changes in one hormone level are likely to impact other hormones, perhaps leading to further feedback and change (Morales-Montor et al., 2004). This makes these complex associations even more difficult to determine. This is especially true in humans where changes in diet, activity, environmental conditions (e.g., day length and temperature), and other lifestyle factors can alter hormone levels (Bribiescas, 1996; Ellison & Panter-Brick, 1996), subsequently impacting individual development and immune function. Therefore, changes in lifestyle can drastically alter physiological parameters and immune activation patterns.

Cultural Transition Terminology

Populations in transition from traditional subsistence production to more urban lifestyles generally experience rapid economic, social, and nutritional changes including increased consumption of market foods, agricultural intensification, and greater travel (Strachen, 1989). Studies examining these changes use several different terms to describe this phenomenon, including “urbanization”, “Westernization”, “modernization”, “industrialization”, “acculturation”, and “market integration”. These labels are typically used interchangeably as they all refer to the collective process during which political, economic, ecological, and sociocultural changes within a population alter individual behavior and well-being patterns. Typically, these terms collectively refer to transitions from a more traditional, subsistence strategy within a local ecology to increased reliance on technologies, goods, and services from non-local sources. These various views also all share an interest is determining how these processes influence human health (McDade, 2001; McDade & Nyberg, 2010).

There are, however, critical differences between these terms. Each concept is based on a slightly different definition and conceptualization of how sociocultural change manifests; thus, each term emphasizes a distinctive set of drivers and outcomes related to the process of sociocultural change. These differing interpretations of sociocultural change ultimately lead to confusion and an inability to realizably compare results across studies and populations (Lopez-Class et al., 2011; McDade & Nyberg, 2010; Thomson & Hoffman-Goetz, 2009). It is therefore necessary for studies addressing sociocultural transitions to clearly define the terminology used and discuss how this conceptualize how drives data collection and interpretation.

In this dissertation, the term “Market Integration” (MI) is used. This theoretical framework centers on the establishment of market-based trade system, and is defined as the degree to which people consume from (e.g., ownership of market goods and reliance on market foods) and produce for (e.g., sale of goods or participation in wage labor) a market economy (Godoy & Cardenas, 2000; Godoy, 2001; Godoy et al., 2005; Lu, 2007; McDade & Nyberg, 2010). This conceptualization of sociocultural change is particularly useful as participation in a market economy has been shown to affect individual well-being, interactions with the environment, and group dynamics within indigenous populations (Godoy et al., 2005). Populations may choose to enter the market economy for several reasons, including: encroachment by other groups, resource depletion, the draw of diverse food items, and the allure of foreign goods (Godoy et al., 2005). The reason for MI shapes how the population subsequently interacts with the environment and can result in both positive and negative consequences for individual well-being.

Market Integration and Human Health

Market Integration and Infectious Disease Risk

Market Integration-induced cultural change affects the way a group understands and interacts with their environment (Huss-Ashmore, 1992). Thus, lifestyle changes associated with MI have the potential to drastically impact population health and well-being. Evidence indicates that MI affects a range of health conditions across ecologically and culturally diverse populations, including groups residing in Samoa (e.g., Baker et al., 1986; Hanna & Fitzgerald, 1993; James et al., 1987; McDade, 2000, 2001, 2002), the Philippines (e.g., McDade et al., 2008; Kuzawa et al., 2012), Amazonian South America (e.g., Blackwell et al., 2009; Fitton, 2000; Liebert et al., 2013; McDade et al., 2005; Urlacher et al., 2016), Siberia (e.g., Leonard et al., 2002; Snodgrass et al., 2007), and Papua New Guinea (e.g., Dennett & Connell, 1985).

The health changes documented in these studies include: the development of chronic disease (Baker et al., 1986; Dressler, 1999; Dressler et al., 1987; Gohdes, 1995; Bindon, 1995; Shephard & Rode, 1996; Stein et al., 2005), childhood growth patterns (Blackwell et al., 2009; Fitton, 2000; McDade, 2000, 2001, 2002; Piperata et al., 2011; Stein et al., 2005; Urlacher et al., 2016), and mental well-being (Berry & Kim, 1998; James et al., 1987; McDade et al., 2001, 2002; Pearson et al., 1993). According to some studies, the transition to a market economy has been shown to result in stunted childhood growth (Blackwell et al., 2009; Fitton, 2000), elevated chronic disease risk (e.g., type II diabetes, obesity, cardiovascular disease, and hypertension) (Baker et al., 1986; Liebert et al., 2013; Shephard & Rode, 1996; Snodgrass et al., 2007; Valeggia et al., 2010), and increased psychosocial stress levels (Dressler, 1988; Graves & Graves, 1975; Hanna & Fitzgerald, 1993; McDade, 2001, 2002). However, others have found that MI improves health through the facilitating access to nutritious food sources, health care, community sanitation, and more educational and employment opportunities (Godoy et al., 2005; Mascie-Taylor et al. 2004; McDade & Adair, 2001; Steckel, 1995; Strachen, 1989). It is therefore unclear whether MI leads to net improvements or deficits in indigenous population health over time (Godoy & Cardenas, 2000; Godoy et al., 2005; McDade & Nyberg, 2010).

Some researchers have proposed that indigenous populations have developed subsistence strategies well-suited to their specific environment and MI-induced changes disrupt this balance, thus negatively impacting population health and well-being (Bodley, 2014; Wirsing, 1985). Conversely, others have suggested that many indigenous groups are not well-adapted to their environment and therefore have high mortality rates and disease burdens (Dennett and Connell, 1985) On this view, MI improves overall health through the reduction of child mortality rates and malnutrition, while also providing access to medical care (Dennett and Connell, 1985). A final group of researchers contend that MI produces mixed effects on indigenous health, with some changes improving well-being while others produce worse health outcomes (Berry et al., 1986; Godoy et al., 2005; McElroy, 1990; Steffen et al., 2006).

To take one example, infectious disease risk appears to fit within this final category, with MI producing both beneficial and negative health effects (Morens et al., 2004). Specifically, some changes liked with MI (e.g., improved sanitation systems, clean water, houses with paved floor and screens, the commercialization of hygiene products, and improved medical care access) typically decrease risk of infection from a range of pathogens (e.g., cholera, Chagas disease, Dracunculiasis, etc.) (Godoy et al., 2005; Mascie-Taylor et al. 2004; McDade and Adair, 2001; Sattenspiel, 2000; Strachen, 1989). However, other changes (e.g., high population densities, increased travel, increased reliance on domesticated animals, the creation of standing water vector breeding sites from deforestation) can also favor pathogen transmission (e.g., malaria, viral infections, lymphatic filariasis, etc.) (Armelagos et al., 1991; Godoy et al., 2005; Sattenspiel, 2000). It is therefore apparent that behavioral patterns and interactions with the environmental are shaped by lifestyle change, and in turn strongly influence both chronic and infectious disease risk in groups undergoing MI.

Market Integration and Testosterone Levels

Environmental conditions and lifestyle patterns linked with MI also appear to influence male endocrinology. One obvious example is the difference in testosterone levels typically observed between industrial and non-industrial populations. Men from high-income, industrial populations tend to exhibit higher testosterone levels, likely due to differences in diet and activity patterns (Bentley, 1993; Bribiescas, 1996, 2010; Ellison, 2009; Harman, 2001). Specifically, a highly nutritious diet during development and adulthood in association with a more sedentary lifestyle are thought to fuel increased investment in male secondary sexual characteristics (e.g., musculature) and result in higher testosterone levels (Bribiescas, 2010). Men in non-industrial populations, on the other hand, are more likely to experience less nutritious diets, elevated disease exposure, and higher activity levels, all factors believed to reduce testosterone levels, thus decreasing investment in secondary sexual characteristics and resulting in lower testosterone levels (Bentley, 1993; Bribiescas, 1996, 2010; Ellison & Panter-Brick, 1996). These lower adult testosterone levels has been documented in several indigenous populations, including the Ache, Lese, !Kung, and Nepali (Bentley, 1993; Bribiescas, 1996, 2010; Ellison, 2009; Harman, 2001).

Males from higher income nations also tend to exhibit substantial declines in testosterone levels during aging. After the age of 40, testosterone appears to decline by an average of 1.2% per year and sex hormone binding globulin declines by an average of 1.4% annually (Bribiescas, 2005, 2010). However, this testosterone decline is not always apparent in nonindustrial populations (Bribiescas, 2010). Several factors appear to influence the degree of testosterone decline, if one occurs, including: the peak testosterone level attained during adulthood and the overall health of the male as he ages (e.g., healthier older men may exhibit less of a decline) (Ellison et al., 2002; Uchida et al., 2006). Finally, at all ages, sperm morphology and count appears to vary between individuals; this is likely due to differences in diet, activity, and ejaculation frequency (Bribiesas, 2001). Thus, culturally-determined lifestyle factors and environmental conditions strongly affect male sex hormone levels (and thus reproductive characteristics) throughout the lifetime of an individual.

Other social and behavioral factors also influence hormone levels. This nuanced relationship between testosterone production and individual behavior is illustrated by the association between fatherhood (paternal behavior) and testosterone levels. In general, fathers exhibit significantly lower testosterone levels compared to married or single nonfathers (Gettler, 2016; Gettler et al., 2011, 2013, 2014a; Kuzawa et al., 2009; Muller et al., 2009). This appears to be a dose-dependent effect determined by the time a father spends interacting with his children (Gettler et al., 2011, 2013, 2014a; Muller et al., 2009), and this testosterone decline has been attributed to a physiological shift favoring care-giving activities over mate-seeking behaviors (Gettler et al., 2011, 2013, 2014a). One longitudinal study among Filipino men clearly demonstrates these concurrent changes in hormone levels and male behavior (Gettler et al., 2011). This study found that men with higher testosterone levels were more likely to become father at follow-up and these men had significantly reduced testosterone levels compared to non-fathers (Gettler et al., 2011). The largest testosterone decline (especially in evening levels) are typically observed among men with newborns and who invest more hours per day in childcare (Gettler et al., 2011; Muller et al., 2009).

This fatherhood testosterone decline is strongest among populations in high-income nations, perhaps due to differences in family structure (e.g., living with nuclear families) and paternal investment (Gray & Campbell, 2009; Muller et al., 2009). Interestingly, married men who spent more time with their wives also exhibited decreased testosterone levels compared to non-married men or less attentive spouses, suggesting that spousal investment may also influence these patterns (Gray et al., 2002). Overall, these findings suggest that changes in testosterone levels may facilitate the transition between mating effort and paternal investment. This again highlights the complex and culturally-influenced relationship between behavior and hormone levels.

Transition to Chapter III

Chapter II has explored research assessing basic human endocrinology, immune function, and the associations between the two. This chapter has also discussed how the multifaceted relationships between hormone profiles and immune responses are affected by differences in culture and environmental interactions. Finally, the impact of altered lifestyle factors on these associations due to market integration was briefly introduced. The following chapter will build upon this literature through introducing how these variables and relationships are tested among the study population, the Shuar. The Shuar are an indigenous group currently experiencing a range of sociocultural and economic changes linked with market integration. The Shuar are therefore experiencing many of the environmental, lifestyle, and health changes explored in this chapter.

CHAPTER III

STUDY POPULATION: THE INDIGENOUS SHUAR OF AMAZONIAN ECUADOR

Indigenous Shuar of Amazonian Ecuador

The Shuar are an indigenous group belonging to the Jívaroan language family and numbering ~ 40,000 - 110,000 individuals (CODEMPE, 2012). Shuar, which means "people" in the Shuar language (Rubenstein, 2001), reside in the neotropical lowlands and Andean foothills of southeastern Ecuador and northeastern Peru (CODEMPE, 2012; Harner, 1972; Rubenstein, 2001; Urlacher et al., 2016). Shuar territory encompasses about 30,000 km^2 – over 10% of Ecuador's total area – with most Ecuadorian Shuar living in the provinces of Pastaza, Morona-Santiago, and Zamora-Chinchipe (Bennett et al., 2002; Laraque et al., 2007; Rubenstein, 2001).

A large proportion of Ecuadorian Shuar reside in the Morona-Santiago Province, which reaches from the eastern foothills of the Andes to the Peruvian border (Figure 3.1). This area is divided by the Cordillera de Cutucú mountain range (elevation 2,225 m), which extends north to south. Between the Andes and the Cutucú, lies the Upano River Valley (UV), the region in which most of the province's Shuar population is located (Bennett et al., 2002; Laraque et al., 2007; Rubenstein, 2001; Urlacher, 2016). The UV region is characterized by tropical low-montane forest, with an elevation of roughly 600 - 700 m, average daytime temperature of 24°C, and approximately 2200 mm of rainfall annually (Laraque et al., 2007; Urlacher, 2016).

To the east of the Cutucú range is the less densely populated and lower-lying "cross-Cutucú" region (CC). The CC is characterized by lowland neotropical forest, with an elevation of roughly 200 - 300 m, an average daytime temperature of 25°C, and approximately 3000 mm of rainfall annually (Laraque et al., 2007; Urlacher, 2016). Seasonality is relatively mild in both the UV and CC regions, with a slight peak in rainfall between April - July (Laraque et al., 2007). Until 2016, when a second road to the Canton of Taisha was opened to the north, the only road access to CC communities was via a road that terminated near the Peruvian border along the Morona river, followed by canoe and/or foot travel to villages. Alternately, some communities also have dirt airstrips providing access to light aircraft.

Despite residing in different ecological environments, UV and CC Shuar share most sociocultural features, including language. The Shuar language belongs to the Jívaroan language family, and Shuar are similar culturally to other Jívaroan-speaking peoples of Ecuador and Peru, including the Achuar (Descola, 1996), Shiwiar (Seymour-Smith, 1988; Sugiyama, 1996, 2004), Aguaruna (Berlin & Markel, 1977; Roche et al., 2008), and Huambisa (Berlin & Berlin, 1983). However, Shuar do have distinct cultural practices shaped by local environmental factors and their history of contact with non-Shuar (*apaci* in Shuar) colonists.

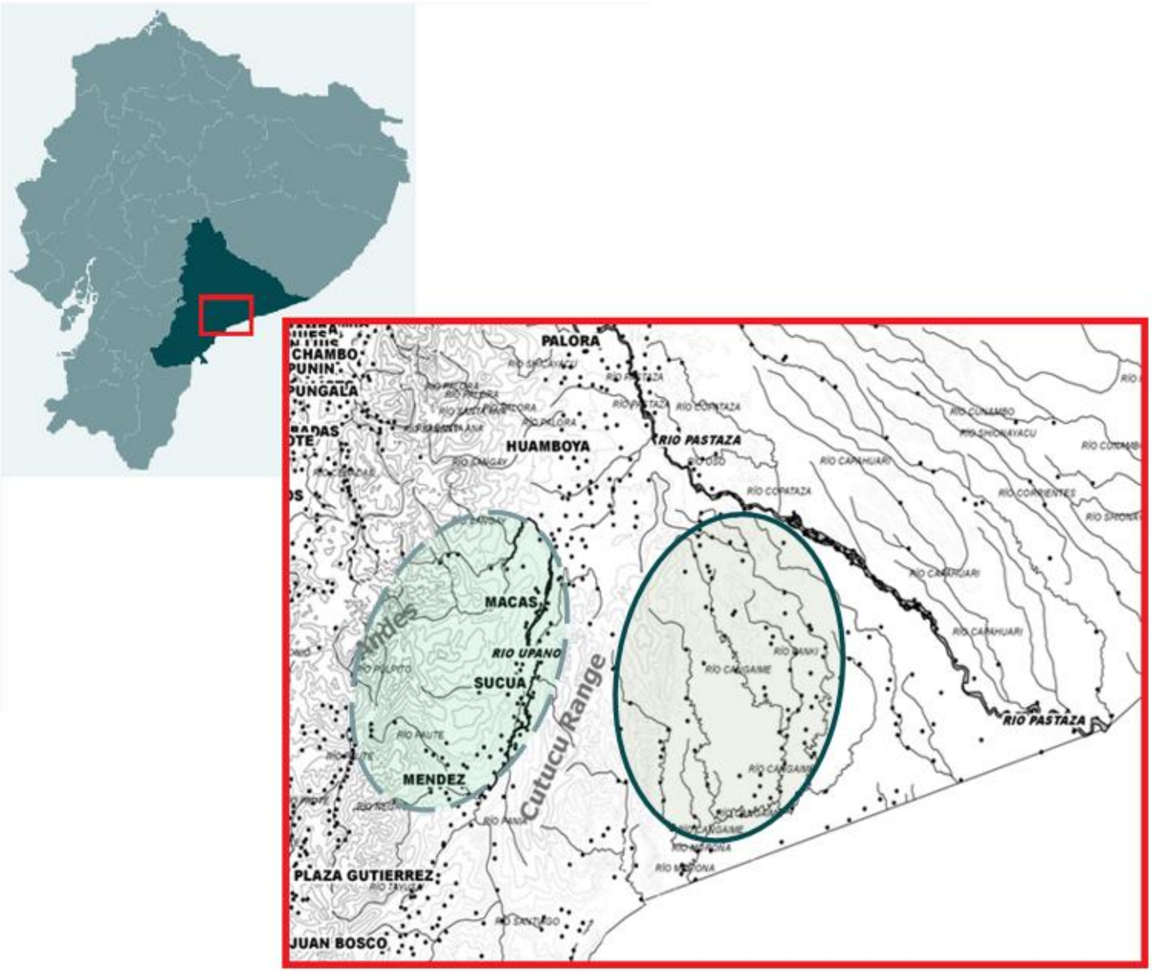


Figure 3.1 Map of Ecuador (*upper left*) with red rectangle indicating region of research (map retrieved from http://en.worldheadquarters.com/travel/ecuador and modified by this dissertation author). Boxed area in detail (*lower right*) showing details of the study area, including the Upano River Valley (dashed oval on left) and Cross-Cutucú (solid oval on right) regions (map modified for use by the Shuar Health and Life History Project).

Shuar Political, Subsistence, Health, and Demographic Patterns

Shuar Political Organization

According to 2010 census data, indigenous people account for 7% of the Ecuadorian population (~1.02 million individuals), an increase of 22.6% from the estimated indigenous population in 2001; a trend in population growth that has continued in recent years (Minority Rights Group International, 2014). Some estimates suggest that the Ecuadorian indigenous population is substantially higher than official census approximations (Minority Rights Group International, 2014). Current demographic patterns and population distributions have been strongly shaped by government dealings with indigenous groups. Historically, Ecuadorian indigenous lands were coveted by outsiders and indigenous populations were consequently pressed into low-paid work in mines, ranches, or plantations (Davis & Partridge, 1994). Missionaries served as a buffer in some populations, protecting vulnerable communities from exploitation, forced dislocation, and disease. However, this protection often came at the price of dependency on religious institutions and loss of traditional religions, practices, and values (Davis & Partridge, 1994). Although Shuar have remained fiercely independent, some of these patterns are also evident among the Shuar.

Traditionally the Shuar had no territorial or political center, instead the basic unit of Shuar social life was the politically and economically autonomous household, or small uxorilocal household cluster (Harner, 1972; Karsten, 1935; Stirling, 1938). According to some accounts, there was no single Shuar society; rather, the Shuar were divided into many independent social groups linked by fluid kinship networks, trading partnerships, and alliances (Rubenstein, 2012). Each social group was organized as a cluster of households, which would be abandoned and relocated regularly (Harner, 1972; Rubenstein, 2012). The Shuar household typically consisted of a husband, two or three wives, unmarried sons, daughters, and sons-in-law (Rubenstein, 2012). Men and women played distinct but complementary social roles which organized the local production of food, religion, and warfare (Rubenstein, 2012).

The first recorded Spanish contacts with Shuar occurred in 1549, when Hernando de Benavente descended the Rio Upano but was driven back by the Shuar. However, by 1552 the Spanish established towns along the Rio Upano to exploit gold deposits, primarily through the subjugation of local Shuar. In 1599 the Shuar organized a coordinated resistance, expelling the Spanish in a violent uprising (De Velasco, 1841; Harner, 1972; Jiménez de la Espada, 1965). During the 18th and 19th centuries missionaries made sporadic attempts to convert the Shuar, with little success, and it wasn't until the 1890s that permanent trade was established with Ecuadorians of partial Spanish descent. At this time, Ecuadorians of European ancestry began to migrate to the area from the highlands, taking on the name "*colono*" (or settler) (Rubenstein, 2001). The relationship between the Shuar and *colonos* began amicably and resulted in mutually beneficial trading partnerships, but quickly deteriorated due to land ownership disputes (Rubenstein, 2001).

Natural resource exploration, missionary activity, and border conflict with Peru subsequently increased interactions between Shuar and outsiders during the 1930s - 60s. The government also increased its presence in the area, exerting pressure to convert the Shuar to Christianity and have them adopt identities as citizens of the Ecuadorian nation (Perreault, 2003; Rubenstein, 2001). The Salesian Catholic order therefore began to establish missions, hospitals, and schools in the area (Lorenzo Garcia, 1985; Rubenstein, 2001). In 1935, the Ecuadorian president (José María Velasco Ibarra) created a Shuar land reserve giving the Salesians control over the area and its inhabitants. This arrangement profited the Salesians, who effectively controlled the land upon which its missions were built, resulting in profit and political clout (since the government needed the missionaries to establish a continuous presence to support its claim to the Amazon). In turn, the Salesians were expected in turn to convert the Shuar into Catholic Ecuadorian citizens (Lorenzo Garcia, 1985; Rubenstein, 2001).

The Salesians further sought to change traditional practices through the founding of missionary schools and the education of Shuar children (Rubenstein, 2001). Given the amount of official control the Salesians wielded over Shuar territory, efforts to convert/baptize the Shuar went relatively smoothly. Missionaries had legal control over Shuar land and facilitated trade opportunities, thereby organizing Shuar access to the market economy and ultimately providing an economic advantage to Catholic over non-Catholic Shuar. There was little resistance to the baptism ritual itself during this period of conversion, although the adoption of Catholicism resulted primarily in a syncretistic overlay of Catholicism upon the existing system of traditional spiritual and philosophical beliefs, much of which remains intact today; even now very few Shuar who self-identify as Catholic regularly attend church (Rubenstein, 2001). In addition, there were many practices explicitly condemned by the Church that the Shuar refused to abandon, including polygyny, shamanism, the consumption of hallucinogenic compounds for vision quests (Rubenstein, 2001). Although not as common today, these practices still exist among the Shuar.

By the mid-20th century, Shuar children who attended mission schools were being taught Catholicism and the Spanish language, and being prepared to participate more fully in Ecuadorian society (Rubenstein, 2001). The area also experienced economic restructuring during this time, altering land use patterns and increasing pressure on the Salesian order to protect Shuar interests from *colonos*. In the 1950s, the Ecuadorian economy was hard hit by waning international interest in Panama hats, a major export (Rubenstein, 2001; Salazar, 1981). Following this, the Centro de Reconversión Economica de las provincias de Azuay, Cañar, y Morona Santiago (CREA) was created to stimulate the local economy. The efforts of CREA included the active encouragement of struggling highland Ecuadorians to migrate and colonize the eastern lowlands of the country (in Shuar territory), where they were could produce agricultural products and raise cattle (Rubenstein, 2001; Salazar, 1981).

Later, in 1964, Ecuador's military leaders initiated an agrarian reform program to address a shortage of available agricultural land, which focused on resettling additional poor families to "vacant" territory in the eastern Ecuadorian lowlands, ultimately moving these families onto Shuar land (Wasserstrom, 2010). However, *colono* location efforts in Shuar territory were hampered by limited transportation access to the Morona Santiago region. This changed beginning in 1965, when authorities in Cuenca constructed a road system that eventually reached land deep inside Shuar territory (Wasserstrom, 2010). The increase in colonization following road construction amplified land disputes between Shuar and *colonos*. In response, Shuar were encouraged to move to permanent settlements ("*centros*"), typically centered around a chapel and sports field (Rubenstein, 2001).

Salazar (1981) reports that each *centro* was typically composed of 25 - 30 Shuar family households, governed by a local elected council, but current Shuar communities are often much smaller (although a few are much larger). The household or family represents the basic Shuar social and political unit, and group organization is hierarchical and democratic (Rubenstein, 2001). Each community chooses its own representatives (*síndicos*) every two years, elected by the *socios* (members) of the community; any respectable men or women in the community can be appointed as *síndico* (Rubenstein, 2001). Important community decisions are discussed at open community meetings at which all *socios* are expected to attend, and when appropriate a vote on important issues is taken, often after much debate.

In addition to the formation of *centros*, the political and economic changes of the mid-20th century also galvanized the Shuar to organize and centralize to better deal with *colonos* and the national government. The *Federación Interprovincial de Centros Shuar* (FICSH) was therefore established in 1964, to advance Shuar interests and more effectively manage interactions with the government and other agencies (Rubenstein, 2001). Representation within the FICSH is hierarchically organized, with representatives elected from *centros,* now often referred to as *communidades* (communities), that then comprise larger sub-regional associations (Davis & Partridge, 1994).

Since its formation, the FICSH has grown quickly, reaching over 260 *centros* by 1988, and now boasting a membership of over 600 communities (Rubenstein, 2001; Augustin Wachapa personal communication to Sugiyama, 2016). In 1967, the Federation received authority from the government to register births and soon after took charge of the education of Shuar children (Rubenstein, 2001). In addition, the FICSH successfully petitioned the Ecuadorian government for collective land rights in 1973, meaning land can now only be transferred amongst Shuar (Rubenstein, 2001), thereby protecting Shuar communities from colonists moving onto their land.

As the Federation assumed control over the Shuar reserve, the Catholic Church lost most of its influence in the area, although this religious institution remains an important resource for the Shuar government (Rubenstein, 2001). The FICSH also established an innovative bilingual radio school program in the 1970s, to bring formal Western education to Shuar who lived in remote areas of the territory without access to schools. Evidence indicates this radio program helped combat low schooling rates (due to difficulty in traveling to schools from remote areas) (Davis & Partridge, 1994; Rubenstein, 2001). The radio school was finally closed in 2001, and was replaced with classroom bilingual education programs (Grenoble & Whaley, 2006).

Soon after the formation of the FICSH, infrastructure development in the area increased, with the construction of a two-lane highway connecting settlements within the Morona-Santiago region to cities in the highlands in the 1970s. These building projects facilitated increased trade between the Shuar and cities in the highlands, leading to accelerated Market Integration (MI; the production for and consumption from a market-based economy). In other words, economic development and a reliance on market produced goods and services increased in the city of Sucúa and other Shuar urban centers, while dependence on traditional subsistence methods generally decreased (Rubenstein, 2001).

Traditional Subsistence Patterns

Prior to the 1930s, the Shuar resided in dispersed households and lived as semi-nomadic hunter-horticulturalists (Harner 1972; Dufour, 1992; Rubenstein 2001; Stirling, 1938). Shuar subsistence traditionally relied on a mix of slash and burn horticulture, hunting, gathering and fishing, a system well adapted to the lower tropical montane and floodplain forest environment that the Shuar inhabit (Harner, 1972; Pohle & Reinhardt, 2004). Plant gathering was essential for the Shuar diet and played an important role in both their material and spiritual culture. Collected plants were used primarily for food, construction material, and medicine. Food security among the Shuar depended largely on diverse plant cultivation in the forest and home gardens (called *aja* in Shuar or *chacra* in Spanish). *Chacras* continue to play an important role in the subsistence strategies of many Shuar, and an estimated 185 wild and cultivated plant species and breeds are currently used, primarily for food, construction, and medicine (Pohle & Reinhardt, 2004). Commonly consumed plant material includes: manioc/yuca, plantains, bananas, papaya, pineapple, maize, taro root/papa china, and sweet potatoes (Dufour, 1992; Harner, 1972).

In addition to plant foods, Shuar traditionally supplemented their diet by hunting, fishing, and gathering forest products (Harner, 1972), and many Shuar continue to rely on these practices. Today (as in the past), several species of wild animals are hunted, typically using guns, blowguns, sticks, machetes, and dogs. Commonly hunted species include: the nine-banded armadillo (*Dasypus novemcinctus*), black agouti (*Dasyprocta fuliginosa*), kinkajou (*Potos flavus*), green acouchi (*Myoprocta pratti*), paca (*Cuniculus paca*), South American coati (*Nasua nasua*), Spix's owl monkey (*Aotus vociferans*), red howler monkey (*Alouatta seniculus*), white-fronted capuchin monkey (*Cebus albifrons*), common woolly monkey (*Simia lagotricha*), red brocket deer (*Mazama americana*), collared peccary (*Pecari tajacu*), monk saki (*Pithecia monachus*), lowland tapir (*Tapirus terrestris*), and ocelot (*Leopardus pardalis*) (Zapata-Rios et al., 2009).

More recently, many Shuar families have also begun to raise poultry to supplement their diets (Pohle & Reinhardt, 2004; Rudel et al., 2002). Cows are also sometimes killed for a celebration, but are primarily raised for sale (Pohle & Reinhardt, 2004; Rudel et al., 2002). Furthermore, wild items foraged from local forests were an important source of nutrients in the past, and these foraging practices continue today in many Shuar communities. Approximately 32 wild plant species are foraged, including peach palm fruit and *hungurahua* palm heart (Pohle & Reinhardt, 2004). Palm weevil larvae (*Mukindi* in Shuar) and flying ants (*Week* in Shuar) represent an additional source of foraged sustenance.

Fishing represents another key source of protein in the Shuar diet, both traditionally and in many CC communities today. A variety of fishing techniques are used, including hook and line, nets, and *barbasco* poison (derived from the plant *Lonchocarpus urucu*) or *musu* poison. *Barbasco* fishing entails digging up the *barbasco* root from either wild or cultivated plants and pounding them to release their liquid into a river or stream. Incapacitated fish are then either speared (by men and boys) or scooped up in baskets (women and girls). Commonly consumed fish species include: various species of catfish (*Callichthyidae sp.*, *Cetopsidae sp.*, *Loricariidae sp.*), South American darters (*Crenuchidae sp.*), and characids (*Characidae sp.*) (Gray et al., 2015).

Traditional Shuar subsistence also centers around the production of manioc beer (*niahamnch* in Shuar, or *chicha* from Quechua), a drink that is typically consumed daily by the Shuar. *Chicha* in Quechua is a generic term for the traditional fermented beverages produced by indigenous groups throughout the Andes and Amazon basin. This beverage can be produced from several starchy plant crops (e.g., manioc, maize, peach palm fruit), and has a long history among indigenous groups (Moseley et al., 2005). Today, the Shuar continue to brew *chicha* from sweet manioc (*mama* in Shuar, yuca in Spanish; *Manihot esculenta*), a staple tuber crop in tropical climates. *Chicha* from manioc generally has a low alcohol content (2%–5%), and is distinguished by a milky consistency and often sour flavor (Harner, 1972).

Chicha is often prepared over a two to three-day period by Shuar women (Harner, 1972). First, the roots of the manioc are peeled, washed, and boiled until soft. Water is then drained off and the root mashed, while the brewer chews pieces of the manioc which are periodically spit into the mash. The finished mash is placed in a designated vessel to ferment for one to three days, depending on preference for sweet (little to no fermentation) or sour (highly fermented) *chicha* (Colehour et al., 2014). *Chicha* remains a key dietary component for most Shuar, with fermentation thought to improve the bioavailability and synthesis of essential vitamins and minerals (zinc, calcium, iron and magnesium) that may otherwise be difficult to acquire (Ahaotu et al., 2011; Boonnop et al., 2009; Colehour et al., 2014; Dilworth et al., 2013). This added nutrition is particularly important given that chronic nutritional stress among indigenous groups can stunt growth (Blackwell et al., 2009; Piperata et al., 2011).

However, while *chicha* is a key component of Shuar daily life and ceremonial rituals, the drink may also act to spread illness. The inclusion of saliva during production introduces oral bacteria to the beverage and pathogen exchange is also likely to occur during *chicha* consumption, when the drinking cup (*pilchis*) is passed around a social gathering and dipped repeatedly into the fermentation vessel after each individual takes a drink and the rim of the cup is wiped by the server's bare hands (Colehour et al., 2014). The frequency and amount of *chicha* consumed may therefore influence infection risk, Still, the traditional importance of *chicha* remains ingrained in most Shuar communities, influencing Shuar health.

Disease Risk and Traditional Treatments

The climate of the Amazonian rainforest makes it a highly pathogenic environment (Guzman et al., 1995; Pan et al., 2010), fostering the spread of several infection types among the Shuar. Particularly common illnesses include: acute respiratory infections, and parasitic infection (e.g., soil-transmitted helminths) (Cepon-Robins et al., 2014; Gildner et al., 2016a); moreover, vector-borne diseases (e.g., malaria, dengue, and yellow fever) have been documented in some CC communities (Jokisch & McSweeney, 2006). Acute illness symptoms also appear to be common among the Shuar. For instance, McDade and colleagues (2012) observed elevated immune inflammatory biomarker concentrations in roughly 27% of all Shuar samples, while Urlacher and colleagues (2018) documented elevated immune activity in approximately 25% of Shuar children.

The Shuar traditionally relied on both cultivated and wild plants as sources of medicine; although the number of medicinal plants reported to be used by the Shuar varies by study, as many as 275 medicinal plant species have been reported (Gerique, 2010; Pohle & Reinhardt, 2004; Tene et al., 2006). These plants are used to treat a variety of ailments, including parasitic infection (Gerique, 2010; Mali & Mehta, 2008; Pohle & Reinhardt, 2004). The eight species most commonly cited as viable parasite treatments include three herbs, two trees, two fruits, and one climbing vine, all consumed orally (Table 3.1) (Gerique, 2010; Mali & Mehta, 2008; Pohle & Reinhardt, 2004). Of the participants included in this dissertation, the most commonly cited traditional treatment for parasites was ingesting the milky sap of the *Wampu* tree (*Ficus cf. gomelleira Kunth*) (Gildner, unpublished data). In addition, several participants referenced the use of mashed papaya seeds as an effective way to expel intestinal parasites (Gildner, unpublished data[1]). These treatments contain compounds that disrupt the metabolic pathways or nerve-muscular activities of targeted parasites (Mali & Mehta, 2008).

Table 3.1. Medicinal plants the Shuar use to treat parasitic disease, adapted by the author.

Plant Name	**Plant Type**	**Uses**	**Application Technique**
Araceae (several species) (*Ushu* in Shuar)	Herb	Parasites (vomitive)	Raw tuber is orally consumed
Anthurium alienatum	Fruit	Burrowing insects (in skin)	Fruit crushed and placed on skin
Carica papaya	Fruit	Anthelmintic	Mashed seeds are orally consumed
Chenopodium ambrosioides (*Paico* in Quechua)	Herb	Parasites and diarrhea	Tea (mixed with *Verbena litoralis* sprouts
Croton lechleri (*Urush numi* in Shuar)	Forest tree	Anthelmintic	Latex (one teaspoon daily before breakfast)
Fevillea cordifolia (Javillo or Antidote Cocoon)	Climbing vine	Parasites and diarrhea	Mashed seeds are orally consumed
Ficus cf. gomelleira Kunth (*Wampu* in Shuar)	Forest tree	Stomach parasites	Dilution (oral): sap mixed with *chicha*
Piper stileferum Yunck (*Nampich* in Shuar)	Herb	Stomach ache & parasites	Infusion (oral) made from sap of roots

Although the Shuar pharmacopeia is large and often effective, Shuar transitioning to a more market integrated lifestyle generally exhibit increased reliance on Western biomedical drugs to treat parasite infections (e.g., albendazole). For example, anthelmintic drugs are now regularly administered to Shuar children in some communities during local school treatment programs (Cepon-Robins et al., 2014). The vast majority of Shuar participants have also stated they prefer medical drugs to traditional treatments (Gildner, unpublished data). Moreover, in more market integrated

---

[1] Data collected as part of a questionnaire designed to determine how Shuar participants understand parasitic infection spread, prevention, and treatment options.

areas interview respondents said the knowledge of how to prepare traditional parasite infection treatments had been lost in their community, whereas respondents living in more traditional communities could more often describe which plants to use and how to prepare the treatment (Gildner, unpublished data).

Shamanism also played an important role in traditional Shuar medicine and most illness were historically attributed to witchcraft or sorcery (Harner, 1972; Rubenstein, 2012). It was commonly believed that enemy shamans could curse someone by sending magical darts (*tsentsak*) to cause illness or death in those they afflict (Bennet, 1992; Harner, 1972). The consumption of narcotic beverages, under the direction of a shaman (*uwishin*), was common among Shuar to heal illness and achieve spirit visions (Bennet, 1992; Rubenstein, 2012). Frequently used hallucinogens include: ayahuasca (*natem* in Shuar; *Banisteriopsis caapi*) and Angel's trumpet (*Brugmansia suaveolens*) (Bennet, 1992; Harner, 1972).

During a healing ceremony, the shaman and patient consume the *natem*, which has a hallucinogenic effect ~10 - 20 mins after consumption. The shaman will then chant or sing to heal the patient (divining the cause of the ailment), suck out the poison or malevolent beings carried by the *tsentsak*, and vomit it up (Bennet, 1992). The purging caused by ingestion of ayahuasca may have the added benefit of clearing parasites and other pathogens responsible for intestinal illness. These ceremonies often last 3 - 6 hours, or until the hallucinogenic effect diminishes (Bennet, 1992).

Demographic Trends

Ethnographic accounts suggest that the Shuar population shrank significantly during the early 20th century (Harner, 1972; Karsten, 1936). This population decrease was likely due to disease transmission through greater contact with non-Shuar colonos. Between 1932 – 1937 non-Shuar colonos moved into the Upano Valley looking for gold and bringing several novel illnesses, including whooping cough, measles, and tuberculosis, resulting in the death of up to half the indigenous population (Harner, 1972). This is similar to mortality rates at contact reported ethnographically in other Amazonian societies experiencing introduced disease (e.g., Sugiyama & Chacon, 2000; Walker et al., 2015). High mortality levels during this time may also be associated with increased access to firearms through trade, which may have increased the lethality of raiding. Among the Shuar, the 1950 census recorded a mere 4,137 native “Jíbaro” speakers, although some of these individuals were also likely non-Shuar (INEC, 1951). This abrupt population decline was followed by two decades of slow recovery and then a remarkable population spike beginning in the 1960s and continuing today.

Rapid population growth beginning in the 1950s has been attributed to decreased levels of intra-tribal feuding and the increased access to life-saving medical services and treatments, particularly among infants (Harner, 1972; Jokisch & McSweeney, 2011). Consequently, the Shuar population roughly doubled during 1950s and 1960s, and then doubled again to approximately 30,000 individuals by the late 1980s (Hendricks, 1988). In a recent sample, approximately 53% of the Shuar population is under the age of 15 years of age (Blackwell, 2009). In fact, indigenous populations in the Ecuadorian

Amazon appear to be among the few groups in the world not obviously transitioning to lower fertility (Davis et al., 2015).

Rapid population growth may also tangentially influence infectious disease patterns among the Shuar. For instance, microscopic analysis of stool samples from 370 Shuar individuals (of both sexes and all ages) indicates that while helminth (parasitic worm) infection is fairly high at all ages, infants/children and juveniles display significantly higher whipworm loads than adults (Gildner et al., 2016a). This pattern has been attributed to the naïve immune systems of children coupled with poor sanitary habits that facilitate the consumption of fecal-contaminated soil and child-to-child transmission (Blackwell et al., 2011; Bundy, 1988). The growing number of Shuar children may therefore serve as a reservoir for endemic parasitic infection in Shuar communities. However, Shuar disease patterns and health outcomes also appear to be influenced by changes associated with access to state sponsored medical care.

Overview of Ecuadorian Healthcare

Ecuadorian healthcare has transformed substantially in the previous century as the political climate and public health sector have changed, with important implications for the health of indigenous populations. In particular, changes in government organization have had profound effects on the treatment of indigenous groups and the state benefits available to these communities (including healthcare). The Ecuadorian government adhered to a corporatist regime model during the 20th century until the 1980s (Perreault, 2003). Under this model social benefits were provided to all citizens (e.g., welfare, education, healthcare, and subsidies for food and energy) in exchange for political participation through the formation of state-sanctioned organizations. The goal of these policies was the formation of a more homogenous population (Stutzman, 1981; Yashar, 1998), with indigenous peoples expected to adopt identities as Ecuadorian citizens in exchange for these government provided benefits (Radcliffe, 1996; Radcliffe & Westwood, 1996). Under this corporatist governmental model, indigenous populations could access state benefits only if they formed officially recognized class-based labor organizations; this policy led to the country-wide creation of indigenous production cooperatives and community associations (Perreault, 2003).

However, similar to much of South America, this model began to fall apart in the 1980s due to Ecuador's worsening economic crises and national debt (Perreault, 2003). State reform began in 1979 with a return to civilian government, but continued slowly and was met opposition from labor parties and indigenous groups (Perreault, 2003). The nationwide economic crisis continued, worsening in the 1990s due to low oil prices, substantial public-sector wage growth, and low tax revenue (IMF, 2000). In response to the deteriorating economic situation, the new civilian administration systematically dismantled populist social programs (Hey & Klak, 1999), resulting in budgetary cuts for education, welfare, and public healthcare. However, Ecuador's fiscal decline continued. By 2000, the value of Ecuadorian currency (the sucre) had fallen precipitously and inflation had risen to 96.1% (Index Mundi, 2015).

In an attempt to reverse this severe economic decline, the Ecuadorian government adopted the U.S. dollar as the national currency in early 2000 (Acosta, 2001; Adam Smith Institute, 2014; North, 1999). This decision to dollarize the economy generally had the desired effects; dollarization slowed hyperinflation, stopped the plummeting value of the sucre, and stabilized the financial market, thereby helping to end the economic crisis (Adam Smith Institute, 2014). However, not all Ecuadorians initially benefitted from the shift to U.S. currency (Acosta, 2001; North, 1999). The adoption of the dollar resulted in thousands of people falling below the poverty line, and many Ecuadorians lost their savings due to over 20 major banks going bankrupt (Lind, 2003).

Governmental organization and economic conditions have also played an important role in shaping Ecuadorian healthcare. Like many countries, Ecuador has experienced massive nationwide health improvements since the 1960s with global medical advances (e.g., vaccine and drug development). Between 1960 and 2000, life expectancy increased by approximately 37%, infant mortality decreased 75%, and mortality in children under 5 years old decreased 82% (Pan et al., 2010). Furthermore, vaccination rates have improved (e.g., measles immunization rose from 24% in 1980 to 84% in 2000 to 95% in 2005) (UNSD, 2008; WHO & UNICEF, 2004).

In 2000, following the adoption of the dollar and subsequent economic stabilization, the Ecuadorian Congress passed a noteworthy health reform bill that sought to create a functional National Health Care System (e.g., through dramatically increasing the national health sector budget over the next few years and subjecting private services to government licensing standards) (López-Cevallos & Chi, 2009; Organización Panamericana de la Salud, 2008). As a result of these reforms, the Ecuadorian health sector is currently comprised of a mix of public and private institutions (both non-profit and for-profit), headed by the Ministry of Public Health and the Ecuadorian Social Security Institute (López-Cevallos & Chi, 2009).

Although general well-being has improved over the last several decades, health disparities persist. Fundamental problems still exist in the organization, management, and funding of the health sector that prevent equitable access to health services (Echeverría, 2002; Pan-American Health Organization, 2001). Evidence indicates that low-income and indigenous individuals are significantly less likely to utilize healthcare services, especially preventative care, shaping long term health outcomes in these populations (López-Cevallos & Chi, 2009). These healthcare access disparities are consistent with a larger global trend.

Research indicates that health outcomes are generally worse for indigenous communities (compared to non-indigenous communities), and that these patterns are evident across sociocultural and geographic contexts (Valeggia & Snodgrass, 2015). Poor health indicators common among indigenous populations include: reduced life expectancy, high maternal and infant mortality, malnutrition, stunted growth, high infectious disease burdens, and increased risk for chronic disease and depression (Gracey & King, 2009; King, 2009; Ohenjo et al., 2006; San Sebastián & Hurtig, 2007; Snodgrass, 2013; Stephens et al., 2006; Valeggia & Snodgrass, 2015).

Health disparities among indigenous and non-indigenous communities are also apparent among Ecuadorian populations, due in large part to an unequal distribution of healthcare services (INEC, 2001). Most healthcare improvements have been concentrated in coastal and mountain towns, especially in the major cities of Guayaquil, Cuenca, and Quito. In contrast, individuals living in the Amazon river basin (such as the Shuar) have significantly higher risks for poor health outcomes (e.g., chronic malnutrition, endemic parasitic disease, amoebiasis) (Buitrón et al., 2004; Orr et al., 2001; Quizhpe et al., 2003;

San Sebastián & Santi, 1999). These health disparities are also apparent among the Shuar; for example, higher rates of stunted growth have been documented among Shuar children compared to non-Shuar *colono* children (Blackwell et al., 2009).

Socioeconomic inequalities appear to drive the poor health patterns evident in many indigenous populations (e.g., differences in local sanitation and housing construction), including a general lack of medical resources in these communities (INEC, 2001). Today, the physical and geographic barriers to healthcare services vary widely between different Ecuadorian provinces. Several infectious diseases remain widespread in the country and deserve special medical consideration, including many parasitic diseases. For instance, sporadic malaria outbreaks and variety of helminthic diseases are recognized as being prominent health concerns (Guzman et al., 1995); although it should be noted that the distribution of these diseases varies widely across the diverse Ecuadorian eco-zones. This range of infectious diseases in Ecuador is due to diverse geography, abundant vector habitat, and logistically-complex public health issues. Apart from schistosomiasis and African trypanosomiasis, almost all major groups of human parasitic disease can be observed in Ecuador (Guzman et al., 1995).

Access to medical care services also varies widely between regions, including among the Shuar. More market integrated UV communities are often situated relatively close to urban centers and the hospitals, pharmacies, and clinics located there. However, CC Shuar living in more remote areas must often travel long distances (typically by foot and via boat and/or bus) at great personal expense to visit a hospital. Yet, the government does provide some medical services to the Shuar. For example, Ecuador enforces compulsory medical service for physicians and other healthcare professionals (through a program called “*medicatura rural*”) to improve the health status of rural populations. This program requires that medical students offer care for one year in remote areas of the country before they can be certified (Cavender & Alcan, 1998). Moreover, the Shuar have their own government-funded Health Director and the government financially supports basic regional clinics, including two large hospitals in Morona Santiago (in the cities of Sucúa and Taisha), although many Shuar still have trouble accessing these care centers. It is therefore apparent that changes in access to healthcare and subsequent health outcomes among the Shuar reflects collaborative efforts with the Ecuadorian government, as well as regional MI levels.

Market Integration, Lifestyle Change, and Health

Ecuadorian Amazonian populations (including the Shuar) are currently experiencing drastic socioecological changes. For example, resource extraction has accelerated in Amazonian Ecuador, resulting in recent conflict between Shuar and Ecuadorian officials over government attempts to allow Chinese companies to mine copper on Shuar land. These actions by the government threaten Shuar well-being and traditional cultural practices, although these clashes have also strengthened resolve and unity among Shuar seeking to protect their territories and preserve indigenous identity. In addition to threatening Shuar autonomy, mining industries are associated with severe health risks, including the release of toxic waste into the environment (Houck et al., 2013). Further, environmental contaminants have been linked with several health complaints such as skin mycosis, sore throat, headache, red eyes, ear pain, diarrhea, and gastritis (San Sebastián & Hurtig, 2005). Changes linked with MI therefore have important cumulative health consequences, via changes in healthcare access and routes of disease exposure (San Sebastián & Hurtig, 2004).

Furthermore, mining activities and other forms of environmental alteration associated with MI (e.g., deforestation for logging or to create grazing land for cattle) result in the deterioration of ancestral ecosystems and increase pressure on local wildlife and habitats, threatening traditional lifeways and subsistence strategies (Gracey & King 2009). For example, hunting (a crucial Shuar subsistence activity) may no longer be sustainable in some areas given current socioeconomic conditions (Zapata-Rios et al., 2009). This resource depletion is often largely attributable to increased population densities, which have increased demand and exhausted wildlife populations (Bennett & Robinson, 2000; Robinson & Bennett, 2004).

The depletion of important local resources is a common theme among indigenous groups undergoing MI, and often results in significant changes in population activity levels and diet (e.g., increased consumption of processed market foods) (Lagranja, et al. 2015; Lourenco et al., 2008; Nagata et al., 2011; Snodgrass, 2013; Snodgrass et al., 2006). Alterations in subsistence activities subsequently result in a loss of traditional ecological knowledge and generally increase the risk of chronic disease (e.g., obesity, cardiovascular disease, and type II diabetes) (Lagranja et al., 2015; Lourenco et al., 2008, Nagata et al., 2011, Snodgrass, 2013, Snodgrass et al., 2006).

Although the degree of socioecological changes varies across Shuar communities, both the UV and CC regions are currently experiencing rapid socioeconomic and environmental change resulting from MI. These changes include the construction of roads, the expansion of power grids (providing more communities with electricity), and access to novel goods, services, technologies, and modes of life (Liebert et al., 2013; Lu, 2007). The effects of MI dramatically influence all aspects of indigenous life, including subsistence activities, long-term health outcomes, and the provision of medical services. However, these changes, while occurring rapidly in many regions, are not uniform across the population. Due to this uneven access to market centers, Shuar communities exhibit varied levels of access to technology and connectivity to other regions (e.g., through road construction). Thus, the effects of MI generally vary between Shuar communities.

It should also be noted that a great deal of MI heterogeneity is evident within communities. Within both UV and CC communities, MI variation is apparent at the household level (Gildner et al., 2016a; Urlacher et al., 2016). Households exhibit marked differences in construction, income sources, diet, and ownership of market goods (Liebert et al., 2013; Urlacher et al., 2016). For example, many Shuar continue to reside in more traditional houses (e.g., constructed of wood or cane walls with dirt floors and thatched roofs), while others within in the same community may live in government-issued concrete houses. This variation results in very different individual developmental and health outcomes. For example, work among the Shuar suggests that household-level MI differences shape parasitic disease risk (Gildner et al., 2016a), as well as childhood growth patterns (Urlacher et al., 2016). Still, some general regional patterns are apparent between Shuar communities, likely due to differences in access to market centers.

Market Integration in the Upano Valley

Market integration is occurring rapidly across UV communities. The UV region is now connected by several roads (many constructed during the mid-2000s, some of them paved) and is the location of two market centers (Sucúa and Macas), with populations of ~12,600 and ~19,000 individuals, respectively (INEC, 2010). Furthermore, the UV is connected by a fairly reliable bus system to major urban centers (e.g., Quito and Cuenca). Connection to commercial centers has also altered subsistence activities such that many Shuar communities now exhibit high levels of wage labor (e.g., timber extraction), cash cropping (e.g., coffee and cacao), and commercial activity with wage labor (Lu, 2007). These activities have altered UV diets such that protein derived from domestic livestock has largely replaced hunting and fishing, although traditional horticulture remains an important source of sustenance (Liebert et al., 2013). Moreover, while most UV Shuar continue to live in rural areas, many of these communities now possess electricity, piped water, and schools.

Shuar residing in UV communities also generally experience several health benefits associated with MI, including local medical clinics, markets, and access to hospitals and other services available in larger cities (Liebert et al., 2013). Recent evidence suggests that Shuar with market access report purchasing Western medicines and domestic items more frequently than other indigenous Ecuadorian groups (Lu, 2007). However, several UV areas still lack proper sanitation and clean drinking water, facilitating infectious disease spread (Gildner et al., 2016a; Houck et al., 2013). In addition, changes in chronic disease patterns have been documented among other indigenous groups with shifts away from low-calorie plant foods to the increased consumption of market foods (Houck et al., 2013). Specifically, increased access to processed foods, refined sugars, and saturated fats, coupled with decreased activity levels has resulted in negative health outcomes, especially among adults (Houck et al., 2013).

While widespread MI-linked chronic disease has not yet been documented at high levels among UV Shuar, there is some evidence supporting a trend toward increasing metabolic risk factors among these communities (Liebert et al., 2013). It therefore appears that rapid MI among UV Shuar has health consequences.

Market Integration among Cross-Cutucú Communities

Changes linked with MI are also evident in CC communities, although the overall degree of economic development is much lower than that observed in the UV region. In contrast to the UV, until 2016 when a new dirt road was constructed between the UV and Taisha, the CC had only one road linking it to outside areas. Further, most CC communities do not have access to the electrical grid, and access to Western medical care is much more limited. Participation of CC Shuar in commercial activity is hampered by this lack of access to market centers. Travel to UV market centers such as Sucúa or Macas, currently requires a motor canoe ride ranging from 1 - 16 hours, depending on water levels, followed by an approximately seven-hour bus ride to Sucúa. Both these modes of transport require money, which presents a financial challenge for most CC Shuar who are primarily reliant on traditional subsistence and very limited agropastoral production for the market. Given these barriers, many CC Shuar continue to engage primarily in traditional subsistence activities and rarely visit large urban centers.

The generally slower pace of MI in the CC region has also resulted in notably different health patterns than evident among UV Shuar. Evidence suggests that, despite economic gains, stunting still affects ~26% of children under the age of five in Ecuador, due in large part to unequal access to resources and healthcare services (Larrea &

Kawachi, 2005), and that these high levels of stunted growth are also evident among the Shuar (Blackwell et al., 2009; Urlacher et al., 2016). These findings suggest that the economic inequality accompanying MI may have a deleterious effect on child nutrition, resulting in long-term health complications. A dual burden of infectious and chronic disease is therefore evident across the Shuar population, resulting from simultaneous over- and under-nutrition and the lifestyle changes associated with the economic and social forces of market integration (Blackwell et al., 2009, 2010; Cepon-Robins et al., 2014; Gildner et al., 2016a; Liebert et al., 2013; Urlacher et al., 2016, 2018).

The processes of MI will almost certainly continue to change in coming years. Interconnectivity between CC communities and market centers is changing notably each year since the establishment of the SHLHP in 2005, with continued urban development, road construction, and introduction of electricity into previously isolated areas. Ownership and use of electronic devices is rapidly increasing as well, even in remote areas with only power drawn from solar panels available to charge these devices. Increasing levels of MI will likely have mixed effects on Shuar health in coming years.

Market Integration and Shuar Health

Evidence indicates that MI has mixed effects on disease risk among the Shuar. For example, MI-linked housing factors influence the spread of several disease types, including: crowding related illnesses (e.g., respiratory infection, cold, diarrheal illness), building material related illness (e.g., allergies, asthma, parasitic disease), and water and sanitation related disease (e.g., vector transmission and fecal-oral disease) (Kroeger, 1980). Traditional Shuar houses tend to be oval shaped with walls of *chonta* palm staves, a roof of woven palm thatch, and a stamped dirt floor. The dirt floors are often swept multiple times a day and absorb any mess, but may still act to spread parasitic disease through the ingestion of contaminated soil (especially by children). In contrast, more market integrated Shuar often live in houses of cement or built with wooden walls and floors and corrugated zinc roofs, thereby reducing parasitic disease transmission resulting from contact with contaminated soil within the home.

Moreover, defecation habits shaped by MI are also associated with parasitic infection risk (e.g., the spread of helminths, parasite worms) (Fitton, 2000). Latrine use is rare in CC communities, but common in the UV. In the CC, dispersion of defecation into surrounding forested areas may limit helminth transmission. Conversely latrines cannot be fully disinfected, making it difficult to restrict helminth egg viability; these sites may therefore facilitate parasite transmission within UV communities. Further, latrines are often located in areas that increase risk of surface soil or water source contamination, increasing the number of potential infections routes. Increased travel and trade facilitated participation in market economies and developing infrastructure (roads, buses, etc.) may also facilitate disease spread in highly populated areas via the movement of goods, livestock, and contaminated food items (Petney, 2001). For example, UV Shuar have more regular access to market goods and livestock, and it is possible helminth infection may occur through increased contact with domestic animals or travel to commercial areas in these populations (Gildner et al., 2016a).

Market integration may also influence health patterns through altering hormone profiles. Several immune cells have hormone receptors, suggesting that hormones play an important role in shaping immune responses (Grossman, 1985; Klein, 2000). For example, the male sex hormone testosterone appears to have several immunomodulatory effects (Ahmed et al., 1985; Fish, 2008; Campbell et al., 2001). An important determinant of immune function, hormones are highly sensitive to environmental cues (Ellison, 2009), and are therefore likely to change in response to lifestyle changes resulting from MI. Previous work indicates that men living in traditional subsistence populations often exhibit significantly lower testosterone levels than men living in more industrialized communities at all ages, a pattern thought to reflect an adaptive decrease in reproductive effort in response to lower caloric intake, higher physical activity levels, and increased disease exposure (Bribiescas, 2009; Ellison & Panter-Brick, 1996).

This pattern has also been observed among the Shuar, with traditionally living Shuar men exhibiting significantly lower testosterone levels than American men (Gildner et al., 2016b). Market integration-induced changes in diet and activity patterns will therefore likely affect hormone levels and subsequent immune function among the Shuar, shaping long term health outcomes. Thus, while MI has several health effects, alterations in infectious disease transmission routes and hormone profiles represent two potential pathways through which MI-linked lifestyle change can impact Shuar health.

The Shuar Health and Life History Project

This dissertation builds upon the Shuar Health and Life History Project (SHLHP; http://www.bonesandbehavior.org/shuar/), which has worked with the Shuar since 2005. The SHLHP, co-directed by Lawrence Sugiyama and Josh Snodgrass of the University of Oregon, conducts annual fieldwork as part of a collaborative research endeavor including faculty, graduate, and undergraduate students from several universities. In addition, through time this project works has worked closely with various local Ecuadorian organizations, including the FICSH, the Ecuadorian health ministry, and community leaders and members.

The SHLHP has several overarching research objectives. First, the project seeks to determine how cultural and economic changes impact health and well-being. Second, the project seeks to clarify the mechanisms that regulate human life history, including age and context-sensitive tradeoffs among life functions, such as between investment in different branches of immunity, immunity and growth, and bone formation/reformation and reproduction, to name a few. Finally, health data collected as part of ongoing SHLHP research is used to provide information and feedback to Shuar participants and community partners. Ultimately these findings are used to solicit government healthcare support and inform the development of effective prevention and treatment efforts.

Previous project research has provided evidence of life history tradeoffs and the effects of MI-linked lifestyle changes on Shuar health, including: (i) tradeoffs between childhood growth and immune function (Blackwell et al., 2009, 2010, 2011; Urlacher et al., 2016, 2018); (ii) various effects of reproductive factors on skeletal health (Madimenos et al., 2011; 2012; 2015); (iii) changes in the relationship between parasite load and autoimmune disease risk (Cepon-Robins et al., 2014); and, (iv) links between psychological stress and immune function (Liebert, 2016). This dissertation contributes to previous SHLHP research by examining links among sociocultural change, immune function, endocrinology, and reproductive effort among Shuar men.

Transition to Chapter IV

Chapter III introduced the Shuar of Amazonian Ecuador, an indigenous population whose health is shaped largely by a combination of environmental and sociocultural factors. The high rates of infection (e.g., parasitic disease) characteristic of this part of the world, coupled with rapidly changing subsistence, political, and demographic patterns have important implications for Shuar disease patterns and long-term health outcomes. Specifically, the variable rates of market integration currently experienced by Shuar communities influence infection risk and treatment via rampant lifestyle and environmental change.

As a natural fertility population experiencing high parasite loads, the Shuar experience conditions relevant to those under which hypothesized life history tradeoffs between immune responsiveness and other costly processes evolved. Given this setting, the Shuar represent an ideal population in which to test life history tradeoffs between immune function and reproductive effort among men. Chapter IV details the first step of this investigation through the examination of diurnal testosterone variation in Shuar men with regards to age, body composition, and market integration level. This information highlights how variation in lifestyle factors (e.g., diet and activity patterns) influence important physiological parameters, ultimately impacting male health and reproductive fitness.

CHAPTER IV

DIURNAL TESTOSTERONE PATTERNS AMONG INDIGENOUS SHUAR MEN OF AMAZONIAN ECUADOR: INDIVIDUAL DIFFERENCES BY BODY COMPOSITION, AGE, AND STYLE OF LIFE

This chapter contains unpublished, co-authored material and was prepared with assistance from J. Josh Snodgrass, Lawrence S. Sugiyama, Melissa A. Liebert, Samuel S. Urlacher, Joshua M. Schrock, Dorsa Amir, Christopher J. Harrington, Tara J. Cepon-Robins, and Felicia C. Madimenos. The author of this dissertation is responsible for the study design, collection and analysis of stool samples, data analyses, and all write up for this paper. Sugiyama, Liebert, Urlacher, Schrock, Amir, Harrington, Cepon-Robins, and Madimenos aided in data collection in the field. Sugiyama is the field site director and provided editorial assistance on this manuscript. Snodgrass is the academic adviser for this dissertation, provided editorial assistance, and is the director of the Global Health Biomarker Laboratory where all samples were analyzed.

Introduction

Hormones are chemical messengers that relay information about environmental and physiological conditions throughout the body. These messengers do not themselves cause direct biological changes, instead each tissue must "decide" how to react to the endocrine messenger and respond accordingly (Ellison, 2009). Hormones are classified into several types, including steroid hormones (Hall & Guyton, 2011). An important class of steroid hormones are sex hormones (i.e., progestins, androgens, and estrogens) (Wallen & Hasset, 2009). Both men and women produce all sex hormone types, but the relative amount of different sex hormones varies between the sexes (Wallen & Hasset, 2009); these differences result in significant sex differences. For example, the androgen testosterone plays a crucial role in sexual differentiation, development, and reproductive function in males (Hall & Guyton, 2011).

Testosterone levels typically follow a predictable pattern throughout the male lifespan, although some individual variation is possible (e.g., in the case of intersex individuals) (Hall & Guyton, 2011). The production of androgens, including testosterone, typically surges during the first sixth months of life, supporting the development of male reproductive organs (Bribiescas, 2009; Hall & Guyton, 2011). Androgen levels then decline and remain low throughout childhood (Bribiescas, 2009; Hall & Guyton, 2011). At puberty this trend reverses and androgen levels increase, promoting the development of male sex characteristics (genital enlargement, a deeper voice, and increased musculature) (Hall & Guyton, 2011).

Androgen levels generally remain elevated throughout adulthood and shape male reproductive effort (Bribiescas, 2009), but appear decline during aging, particularly in men from high-income countries (Bribiescas, 1996). For example, work among American men between the ages of 39-70 found that testosterone declines by an average of 1.2% annually (Bribiescas, 2001; Drafta et al., 1982; Gray et al., 1991). However, it is unclear whether this age-related decline in testosterone is apparent in all populations.

In addition to changing throughout the lifespan, testosterone levels exhibit a diurnal pattern, fluctuating predictably across a 24-hour period (Barberia et al., 1973; Bribiescas & Hill, 2010; Rose et al., 1972; Resko & Eik-Nes, 1966). Testosterone levels typically peak in the morning (likely to mobilize energy for use through the day) and then decrease as an individual approaches sleep (Barberia et al., 1973; Bribiescas & Hill, 2010; Rose et al., 1972; Resko & Eik-Nes, 1966). Overall, these changes in testosterone throughout both the day and the life course are thought to reflect changes in male reproductive effort. Evidence indicates that higher testosterone levels are associated with a range of behaviors and physical traits and behaviors that influence male reproductive success, although these associations are very complex (Bribiescas, 2001).

For example, testosterone levels within a set range are required for typical aggressive behavior; however, slight testosterone concentration changes within this "normal" range do not alter subsequent aggressive behaviors (Sapolsky, 1997). Still, massive increases in testosterone exposure do usually increase aggression (Sapolsky, 1997). This multifaceted association between testosterone and aggression has been linked with male competitiveness, likely to enhance male reproductive success through supporting male competitive efforts to acquire mates (Booth et al., 1989; Gladue et al., 1989; Wingfield et al. 1990). Moreover, increased testosterone production during puberty directly contributes to the development of masculine traits generally considered to be attractive to potential mates (Bribiescas, 2009). Specifically, boys typically experience a significant growth spurt at puberty, while simultaneously exhibited marked changes in body composition (Bribiescas, 2009).

In particular, testosterone plays a key role the development of energetically expensive traits like increased musculature (Bribiescas, 2001, 2010; Hall & Guyton, 2011). Testosterone stimulates muscle anabolism via increased protein synthesis and glucose uptake (Bribiescas, 2001, 2010). Furthermore, testosterone is known to impact fat deposition patterns, influencing both the amount and location of fat accumulation (Wells, 2009). Testosterone triggers fat catabolism, promoting the use of fat cells as energy substrates which can be used in the building of skeletal muscle (Bhasin, 2003). Higher testosterone levels also inhibit the recruitment of adipose precursor cells (Bhasin, 2003). Together these effects facilitate muscular development and reduce adiposity. These testosterone-induced effects subsequently increase basal metabolic rate (BMR)

(Hall & Guyton, 2011); in fact, evidence suggests anabolic processes linked with testosterone may account for 20% of male BMR (Bribiescas, 2001, 2010).

Further, given the high energetic costs associated with testosterone activity, testosterone is believed to help shape energetic tradeoffs between competing somatic functions (Bribiescas, 2001, 2010; Hau, 2007). Elevated testosterone levels in adult men are hypothesized to favor energetic investment in male reproductive effort (e.g., more developed musculature and propensity for competitive behaviors) over other important physiological processes, such as immune responses targeting infectious disease (Bribiescas, 2009; Folstad & Karter, 1992). Specifically, increased energetic investment in reproductive effort (as indicated by elevated testosterone levels) is hypothesized to shift resources away from immune function, thereby suppressing immune responses to pathogens (Folstad & Karter, 1992).

Additional evidence that testosterone influences energetic tradeoffs comes from studies examining changes in testosterone in response to important life events. Research clearly demonstrates that men in long-term monogamous partnerships exhibit significant reductions in testosterone levels (Mazur & Michalek, 1998; van Anders & Watson, 2006). Likewise, fatherhood and spending time caring for children is also related to substantial decreases in testosterone (Gettler 2011, 2013, 2016). These testosterone drops are thought to reflect a shift from reproductive effort (i.e., behaviors associated with acquiring a mate) toward increased cooperation and investment in mates and children (Gettler, 2011, 2016).

Yet, testosterone level variation and the hypothesized role this hormone plays in human energetics appears to be shaped by environmental and social context. Testosterone levels vary across populations. For example, men living in subsistence populations often exhibit significantly lower testosterone levels than men living in more urbanized contexts, a pattern thought to reflect an adaptive modulation of reproductive effort in response to lower caloric intake, higher physical activity levels, and increased disease exposure (Bentley, 1993; Bribiescas, 1996, 2010; Ellison & Panter-Brick, 1996). Lower testosterone levels have been documented in several nonindustrial populations (e.g., the Lese, Nepali, Ache, and !Kung) compared to US and European men (Bentley, 1993; Bribiescas, 1996, 2010; Ellison, 2009; Harman, 2001).

Furthermore, men in nonindustrial populations do not seem to exhibit the marked decrease in testosterone during aging often observed in men living in high-income countries (Bribiescas, 2010). These patterns suggest that hormone levels across the life course are not uniform across all individuals, but rather vary according to social and environmental interactions (Ellison, 2009). It has therefore been proposed that testosterone levels adaptively respond to individual physiology and environmental conditions (Bribiescas, 2001, 2010). While short term changes in testosterone levels appear to be driven by behavior, diet, and activity patterns (Bishop et al., 1988; Spratt et al., 1988), some studies suggest that 40-50% of individual testosterone variation is accounted for by genetic inheritance (Meikle et al., 1986, 1988).

Differences in testosterone levels are also thought to influence body composition variation cross-culturally (Wells, 2010), such that the association between testosterone levels and body composition varies across populations. Previous work in high-income nations has documented an inverse relationship between testosterone levels and adiposity, particularly in overweight men (Kapoor et al., 2006; Mårin, 1995; Morh et al., 2006).

Excess fat accumulation, associated with the obesity pandemic, is associated with lower testosterone levels due to the aromatization of androgens into estrogens within adipose tissues (Bhasin et al., 2001; Kley et al. 1980, 1981; Morh et al., 2006; Pritchard et al. 1998).

Clinical research has further tested the association between adiposity and testosterone levels in industrialized settings through documenting the effects of testosterone treatment in obese men with especially low androgen levels. Studies in high-income nations have demonstrated that testosterone supplementation is generally associated with reduced adiposity, as determine by a variety of measures, including: waist to hip ratio, waist circumference, and total and visceral fat mass (Kapoor et al., 2006; Mårin, 1995). Conversely, suppression of testosterone production has been linked with decreased muscle mass and increased adiposity in healthy young men (Mauras et al., 1998). These findings cumulatively suggest that high testosterone levels may provide some health benefits in overnourished populations through decreasing adiposity levels.

Still, little work has been done to test these patterns in subsistence populations; most work has tested these links in high-income, sedentary populations, with excess available caloric energy availability. What research has been conducted suggests that, in contrast to findings in more urbanized countries, testosterone is positively related to adiposity in the relatively lean men living in subsistence groups (Alvarado, 2013; Bribiescas, 2001; Campbell et al., 2003, 2007; Gettler et al., 2010), indicating that energy status may regulate circulating testosterone levels such that men in better energetic condition exhibit higher testosterone levels. This is likely due to at least in part to tradeoffs between reproductive effort and other physical functions in energetically constrained conditions (Bribiescas, 2001). In other words, among men living in energetically limited conditions, those with greater energy reserves (i.e., adiposity) are better able to afford the energetic costs associated with elevated testosterone levels.

However, other studies in non-Western populations have failed to document any significant association between adiposity and testosterone levels (Alvarado et al., 2014; Gettler et al., 2014a). Therefore, while a strong negative association between testosterone and adiposity has been documented in high-income countries, there is less evidence currently available establishing a clear relationship between testosterone and adiposity in men living in more resource-limited populations. Furthermore, it is unclear how changes in economic development influence these associations. Populations in transition from traditional subsistence production to one in which there is greater production for, and consumption from the market economy (market integration: MI) generally experience a suite of associated economic, social, and nutritional changes including increased consumption of market foods, agricultural intensification, and greater travel (Godoy & Cardenas, 2000; Godoy, 2001; Godoy et al., 2005; Lu, 2007; McDade & Nyberg, 2010; Strachen, 1989).

Market integration induced cultural change affects the way a group understands and interacts with their environment (Godoy & Cardenas, 2000; Godoy, 2001; Godoy et al., 2005; Huss-Ashmore, 1992; Mascie-Taylor et al. 2004; Sattenspiel, 2000; Strachen, 1989). Thus, behavioral changes associated with MI have the potential to drastically alter adiposity and hormone levels (including testosterone) via changes in diet, activity patterns, medication use, and inadvertent exposure to exogenous hormones (e.g., in gels, pills, or food) (Bribiescas, 2009; Ellison, 2009; Ellison, 2009). Clarifying how the association between testosterone and body composition varies in different settings has important implications for male health. Biomedical research in high-income countries suggests that low testosterone increases of risk of several chronic diseases, including: type 2 diabetes, visceral adiposity, dyslipidemia, and metabolic syndrome (Khaw & Barret-Connor, 1988; Oh et al., 2002; Stellato et al., 2000). Thus, there is a growing interest in determining how testosterone levels influence chronic disease risk via alterations in body composition, as well as clarifying how these associations vary by individual economic development level and by age. These findings have the potential to inform the development of medical interventions designed to promote “healthy” hormone levels, as dictated by specific environmental and nutritional contexts.

The present study tests the relationship among testosterone levels, body composition measures, age, and MI level among Shuar men. The Shuar are an indigenous population living in the lowland region of Amazonian Ecuador experiencing rapid, yet uneven MI. Association among testosterone, adiposity, age, and lifestyle patterns has been understudied among indigenous groups, and the Shuar represent a particularly useful study population given that many Shuar communities continue to utilize traditional subsistence practices (Liebert et al., 2013; Urlacher et al., 2016). Previous research indicates that Shuar children living in more traditional communities generally exhibited lower nutritional status and body stature (i.e., were shorter and less heavy) compared to individuals in more market integrated areas (Urlacher et al., 2016), suggesting that traditional subsistence activities in this population result in energy constrained conditions.

This study investigates testosterone profiles across the life course among male participants, testing whether the Shuar exhibit testosterone patterns similar to those documented in other subsistence populations that rely heavily on traditional subsistence activities. Specifically, the objective and hypotheses of the present study are:

- ***Objective One*** **- To investigate the relationship between age and diurnal testosterone levels among the Shuar.**
    - **Hypothesis 1: Waking levels and total daily production of testosterone will be lowest among older men.** We hypothesize that waking testosterone levels and total daily testosterone production will by highest in young adult men and lowest among older men (i.e., decline linearly with age), as has been documented in high-income nations (Bribiescas, 2001; Drafta et al., 1982; Gray et al., 1991).
- ***Objective Two*** **- To examine links between adiposity and testosterone levels among the Shuar.** As has been documented among other indigenous populations (Alvarado, 2013; Bribiescas, 2001; Campbell et al., 2003, 2007; Gettler et al., 2010), adiposity levels indicate energetic condition and will therefore be positively associated with diurnal waking testosterone level, total testosterone production, and average morning and evening testosterone measures.
    - **Hypothesis 2a: Adiposity will exhibit a positive relationship with waking testosterone level and total daily testosterone production.** Body Mass Index (BMI; a proxy measure of body composition) will be positively associated with diurnal testosterone peak diurnal testosterone level and total daily testosterone production. In addition, due to documented age-associated changes (Ferraro et al., 2003) in BMI levels, we predict that there will be a significant age-by-BMI

interaction, indicating that the effect of BMI on testosterone profile is moderated by age.

  - **Hypothesis 2b: Adiposity will exhibit a positive relationship with average morning and evening testosterone level.** Accounting for covariates linked with testosterone levels, three adiposity measures (BMI, waist circumference, and percent body fat) will be positively associated with testosterone measures collected across the day.

- ***Objective Three*: to determine how MI levels are associated with testosterone profiles among Shuar men.**
  - **Hypothesis 3: Average morning and evening testosterone levels will be positively associated with MI level.** Nonindustrial populations often exhibit lower testosterone levels compared to high-income nations (Bentley, 1993; Bribiescas, 1996, 2010; Ellison, 2009; Harman, 2001), we therefore expect that testosterone concentrations will exhibit a positive relationship with MI level (e.g., due to reduced disease burdens, lower physical activities, and access to market foods). Furthermore, given that MI has been shown to influence BMI levels (e.g., Abraído-Lanza et al., 2005; Chen et al., 2012) and impact age-related testosterone patterns (Bribiescas, 2010), we predict that there will be significant age-by-MI, BMI-by-MI, and age-by-BMI-by-MI interactions, suggesting that the relationship between MI and testosterone profile will be moderated by age and BMI.

Materials and Methods

Participants

This study was conducted as part of the Shuar Health and Life History Project (SHLHP). The Shuar are natural fertility indigenous population of ~100,000 people distributed in more than 400 communities in the neotropical eastern foothills of the Andes and the Amazonian lowlands of southeastern Ecuador in the provinces of Pastaza, Morona-Santiago, and Zamora-Chinchipe (Bennett et al., 2002; Rubenstein, 2001). Traditionally, Shuar lived in scattered households reliant on horticulture, fishing, hunting, and foraging (Harner, 1972; Karsten, 1935; Rubenstein, 2001). Today most Shuar continue to rely on traditional cultigens, supplemented to various degrees by hunting and fishing, and mixed agro-pastoral production (Liebert et al., 2013).

The Shuar are also experiencing rapid socioeconomic and environmental change resulting from MI. These changes include the construction of roads, the expansion of power grids (providing more communities with electricity), and access to novel goods, services, technologies, and modes of life (Liebert et al., 2013; Lu, 2007). The effects of MI have considerable influence on all aspects of indigenous life, including subsistence activities, disease patterns, and the health care access. Interactions with non-indigenous populations and accelerating MI have also strongly shaped Shuar lifeways over the past several decades, altering traditional patterns.

Study Design and Sampling

This study employed a cross-sectional approach, with data collected over five field seasons (2012-14, 2016-17). Random sampling was not feasible across this large population, dispersed over relatively inaccessible areas, with sometimes divisive internal politics. Thus, through consultation with knowledgeable Shuar friends and political leaders, and the Dirigente de Salud of the Federación Interprovincial de Centros Shuar, a sample of communities were chosen based on their size, location, and agreement to collaborate on this research. Once communities agreed at an open community meeting that the SHLHP could conduct research in their community, all men were invited to participate in this study.

The sample included 137 male volunteers from 11 communities, aged 12 - 67 years old. The age of 12 was selected to capture those entering puberty at a younger age, thereby capturing the full range of testosterone profiles across key developmental reproductive transitions. Informed consent was obtained from adult participants and parental verbal consent and child assent were obtained for individuals under 14 (the local age of consent). The University of Oregon Institutional Review Board approved this study, and the Federación Interprovincial de Centros Shuar and local leaders authorized research in participant communities.

Field and Laboratory Procedures

Salivary testosterone collection and analysis

The author (TEG) and members of the SHLHP collected saliva samples from study participants twice a day for three successive days. To capture diurnal fluctuation, at least 1 mL saliva was collected before 9 am and after 4 pm for three consecutive mornings and evenings for a maximum of six samples per participant, following established protocol (Bribiescas, 1996). Participants were asked to provide saliva samples via passive drool, wherein they pooled saliva in their mouth and then gently “drooled” directly into a 2.0 mL vial. Direct collection by SHLHP researchers facilitated participant compliance and adherence to the study design, an important concern in diurnal hormone research (Adam & Kumari, 2009; Kudielka et al., 2003). On average, 5.25 samples were collected per participant. In addition, the exact time of sample collection, time to bed on previous night, and wake time that morning were recorded for each participant on all collection days. Samples were stored in a portable -20°C field freezer until transfer to Quito, where they were shipped on dry ice via courier service to the Global Health Biomarker Lab at the University of Oregon for analysis.

All testosterone samples (n = 719) were analyzed using commercially available enzyme immunoassay (ELISA) kits from Salimetrics (#1- 2402; State College, PA); this kit has been well-validated to quantitatively assess salivary testosterone. Samples were assayed in duplicate and those that did not meet the coefficient of variation (CV) and/or absolute value difference criterion were re-analyzed (Kim et al., 2015). Interassay coefficients of variation were 8.36% and 20.75% for high and low control samples, respectively; resulting in an overall interassay coefficient of 14.55%. The intraassay coefficient of variation was 8.44%. Analysis indicated that 1.68% of testosterone concentrations were below the lower limit of detection; for statistical analyses, these low values were substituted with the average lower limit of detection calculated (133.39 pmol/L).

Body composition

Body composition was determined through a variety of standard anthropometric measurement techniques (e.g., Lohman et al., 1988). Height was measured using a stadiometer (Seca Corporation 214, Hanover, MD) and a Tanita scale (model BF680W) was used to obtain participant weight and percent body fat. These height and weight measures were then used to calculate BMI (kg/m^2). The Tanita scale was also used to obtain percent body fat using bioelectric impedance analysis. However, it should be noted that this analysis is based on proprietary equations that were not specifically developed for use with this particular study population. Still, other studies examining associations between reproductive factors and body composition in South American subsistence populations have included this measure on a battery of physical measures to better capture total adiposity levels (Gurven et al., 2016).

Waist circumference (WC) was also measured twice using a tape measure, the average of these two values was then calculated and used during analysis. Finally, a composite body composition variable was created by computing z-scores for each adiposity measure and these three z-scores (BMI, percent body fat, and WC) were summed for each participant, resulting in a final composite z-score. All adiposity variable calculations and analyses were also conducted excluding participants under the age of 18 years old; however, these the results of these analyses did not differ substantially from the analyses including the younger individuals. All participants were therefore included to increase the sample size and analytical power.

Participant characteristics

Ages were determined by birthdates on government issued identification cards, and cross-checked by informants and existing SHLHP genealogical data, as has been previously described elsewhere (Blackwell et al., 2011; Liebert et al., 2013). Evidence indicates that testosterone levels decrease when a man marries or becomes a father (Gettler et al., 2011); marital status and number of children were therefore included in the regression models during analyses.

Market integration

Participants completed a structured interview based on a Material Style of Life (SOL) Index developed by SHLHP for use with Shuar after extensive qualitative research and pre-testing (e.g., Liebert et al. 2013), modeled on examples used in other populations (Bindon et al., 1997; Leonard et al., 2002). The SOL index includes a list of items associated with degree of participation in the market-based or subsistence economy divided into three scales (Table 4.1): Traditional Style of Life (T-SOL), Market-Integrated Style of Life (M-SOL), and Housing Style of Life (H-SOL). T-SOL and M-SOL are calculated as a weighted sum of the total number of traditional (e.g., blowgun, hunting dog, fishing nets) or market-integrated (e.g., radio, car, television) index items a household owns.

H-SOL is calculated as a composite score based on different aspects of household type, size, access to water source, etc. This approach is useful for capturing lifestyle variation with a few manageable variables (Liebert et al., 2013; Urlacher et al, 2016). In addition, a composite score was calculated by computing the z-scores of M-SOL and H-

SOL and adding these scores together to better capture cumulative factors associated with higher levels of MI (i.e., ownership of market produced items and aspects of household construction linked with higher MI). It is worth noting that while M-SOL and H-SOL are positively correlated with each other and negatively correlated with T-SOL (Liebert et al., 2013), it is possible for a household to report high scores on each scale, low on each scale, or any combination of scores. Thus, it is necessary to conduct separate analyses based on the T-SOL, M-SOL, and H-SOL scores.

Traditional-SOL (n=6)	**Market Integrated-SOL (n=12)**	**Housing-SOL (n=7)**
• Canoe • Blowgun • Firearm • Hunting dog • Fishing net • Fishing hook/line	• Propane stove • Chainsaw • Outboard motor • Radio • Mobile phone • Television • Computer • Refrigerator • Bicycle • Motorcycle • Car • Truck	• Houses (total number) • Rooms (total number) • Floors (0 = dirt; 1 = palmwood; 2 = milled lumber; 3 = concrete; 4 = tile) • Walls (0 = palmwood; 1 = mixed; 2 = milled lumber; 3 = cinder block) • Latrine (0 = none; 1 = pit; 2 = indoor toilet without water; 3 = outdoor toilet with water; 4 = indoor toilet with water) • Water Source (0 = river/stream; 1 = well/outdoor pipe; 2 = indoor pipe) • Electricity (0 = none; 1 = lights only; 2 = outlets)

Table 4.1. Household variables used in the calculation of Shuar Style of Life (SOL) measures.

Statistical Analyses

Multi-level model analyses were conducted to examine associations between diurnal testosterone parameters and participant age and BMI. In addition, linear regression analyses were run to determine the contribution of various adiposity measures and SOL factors to variation in average morning or evening testosterone levels. All multi-level model analyses were conducted using Stata 14, while the regression analyses were conducted using SPSS 25. Results were considered significant at $p < 0.05$. Finally, prior to analysis, data were examined for outliers, defined as ±3 SD above or below the mean. No outliers were observed. Additional diagnostic analyses revealed acceptable degrees of model linearity and heteroscedasticity.

Testosterone variables

To assess individual differences in testosterone levels, key diurnal testosterone indices were calculated from the salivary testosterone data. The present study used the following measurements: 1) decline in testosterone levels across the day (i.e., the diurnal slope); 2) total area under the testosterone curve with respect to the ground (AUC_g), a measure of total daily testosterone production; 3) average morning testosterone levels; and, 4) average evening testosterone levels.

Diurnal Testosterone Slope: The diurnal slope was calculated as a best fit line using a regression of each testosterone value onto hours since waking. Thus, waking testosterone levels acted as the intercept in these models (Measures A and B, Figure 4.1). Across the 137 participants, 12.53% of individual saliva samples were missing. However, analyses revealed that these missing data did not vary by age or BMI and appeared to be missing at random, these values were consequently accounted for with missing data techniques (see the *Missing Data* section below). Prior to analyses all testosterone data were assessed for normality. Due to a positive skew in the distribution of testosterone values, all measures were $\log_{10}$-transformed and retested for normality (Adam, 2006). Retested transformed variables were normal, with a skew between ±1; these transformed testosterone measures were subsequently used in all analyses.

Area Under the Curve with Respect to Ground (AUC_g): AUC_g provided an estimate of total daily testosterone production, as measured by the total area under the testosterone values across the day with respect to the ground (Adam & Kumari, 2009). Following standard procedures, this measure was calculated using a trapezoidal function (Pruessner et al., 2003). In other words, AUC_g values reflect the area of the trapezoid (Measure C, Figure 4.1) formed by morning and evening testosterone levels (two vertical lines) and the interim time between sample collection times (one horizontal line) (Ross et al., 2014). AUC_g was not calculated for a specific day if either the morning or evening saliva samples were missing (23.84%). This pattern of missing data did not differ significantly by age or BMI, and appeared to be missing at random. These values were accounted for with missing data techniques (see the *Missing Data* section below). Due to a positive skew in the distribution of AUC_g values, these values were square root transformed, resulting in a normal distribution with a skew between ±1. These transformed testosterone measures were subsequently used in all analyses.

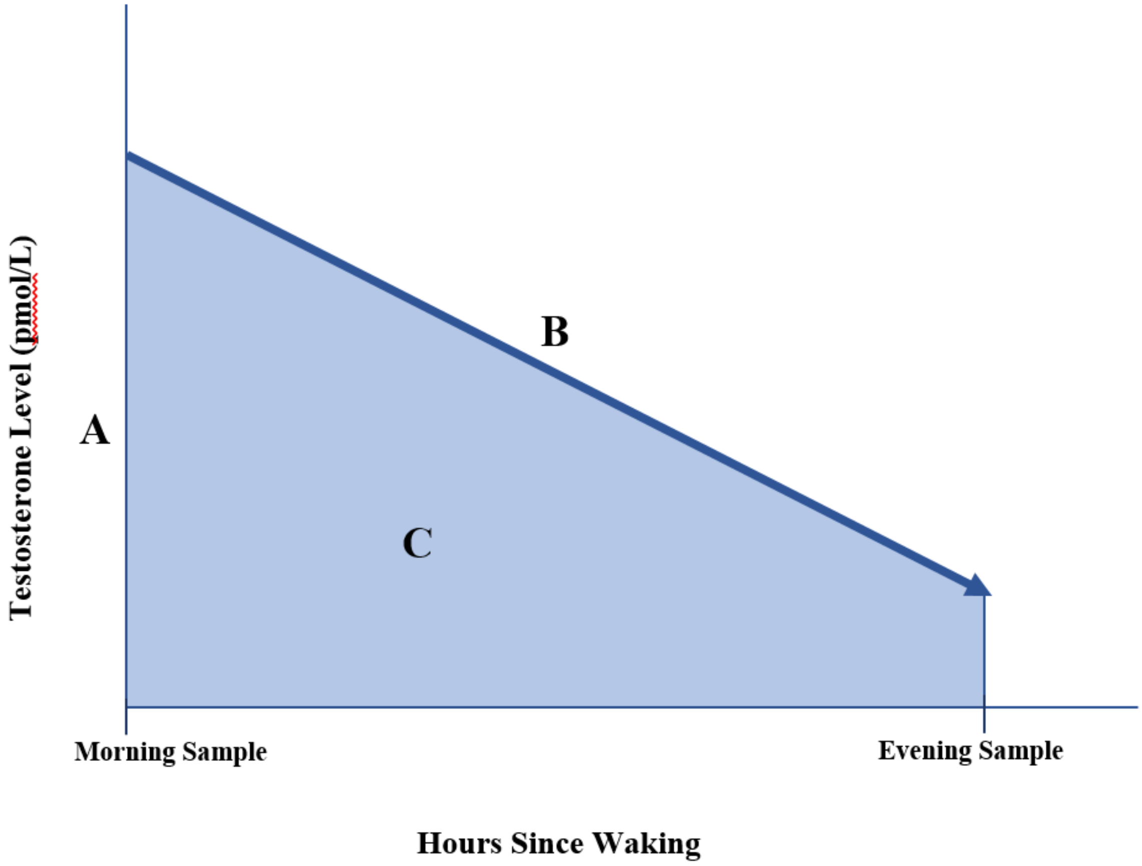


Figure 4.1. Key testosterone indices measuring various aspects of diurnal testosterone rhythms. Figure Legend: Measure A = testosterone levels at waking; Measure B = best fit line calculated from a regression of testosterone levels collected across the day onto hour since waking; Measure C = area of the trapezoid formed by morning and evening testosterone levels and the hours between their collection.

Average morning and evening testosterone levels: Average morning (AM) and evening (PM) values of participant testosterone were calculated from the three morning and three evening measures gathered across the three days of collection. These average levels were positively skewed and therefore square root transformed. Retested transformed variables were normal, with a skew between ±1; these transformed average measures were subsequently used during analyses.

Body composition variables

Participant BMI, percent body fat, and composite adiposity z-scores were all normally distributed and therefore were not transformed. However, WC exhibited substantial skew and was therefore $\log_{10}$-transformed, resulting in a normal distribution with a skew between ±1. Furthermore, preliminary analyses indicated multicollinearity between participant age and each of the adiposity measures. Unstandardized residuals were therefore calculated for BMI, percent body fat, WC, and composite adiposity z-scores removing the contribution of age to these measures. Finally, multicollinearity was also observed between BMI and percent body fat. An additional unstandardized residual was calculated for BMI removing the contribution of percent body fat (in addition to age) to this measure.

Style of Life (SOL) variables

Both the T-SOL and M-SOL indices were positively skewed and were therefore $\log_{10}$-transformed, resulting in normal distributions. H-SOL was normally distributed and therefore not transformed. Multicollinearity was not detected between SOL variables and any other measures of interest.

Covariates and interactions

Age and marital status were normally distributed and therefore not transformed. However, number of children was positively skewed and subsequently $\log_{10}$-transformed, resulting in a normal distribution with a skew between ±1. Preliminary analyses indicated that participant age exhibited multicollinearity with both marital status and number of children; unstandardized residual variables were therefore calculated for these two variables, removing the contribution of age.

Missing data

A large proportion of participants (35.77%) were missing at least one variable. For example, 35% of participants were missing WC measures, 25.5% were missing percent body fat information, 23.8% were missing an AUC_g measure, 23.4% were missing information on BMI, 12.5% were missing one of the six saliva samples used to calculate testosterone diurnal slope, and 12.4% were missing data on H-SOL. All other variables were missing ~6% of cases or less. Little's Missing Completely at Random (MCAR) Test (Little, 1988) was run to assess whether these data were missing at random. Accounting for all variables included in the final models, results indicate that the data are missing completely at random ($p > 0.05$).

Still, separate variance t-tests conducted to test how each variable influenced missingness in the other variables documented significant relationships, suggesting that certain variables may be predicting some of the missingness evident in the dataset. In particular, age significantly predicted missingness in BMI, WC, percent body fat, and composite adiposity z-score ($p < 0.05$). Similarly, T-SOL significantly predicted missingness in BMI and evening average testosterone level ($p < 0.05$), WC predicted missingness in evening testosterone ($p = 0.018$), marital status predicted missingness in M-SOL and T-SOL ($p < 0.05$), and number of children predicted missingness in BMI ($p = 0.001$).

Multiple imputation analysis was therefore used to create a complete dataset by replacing each missing value with a set of probable values (i.e., within a set range of possible numbers), resulting in valid statistical inferences that properly reflect the existing uncertainty caused by missing values (Rubin 1978, 1986). This technique is commonly used to handle missing data in similar data sets (Van der Heijden et al., 2006). The complete pooled dataset resulting from multiple imputation was used during analysis, and these results were compared to those from the original (incomplete) data set. In each case, the results from the analyses using the imputed data did not differ substantively from the those produced from the original data. The results from the analyses using the complete datasets produced from multiple imputation are therefore presented here.

Statistical models

Descriptive Statistics: Basic descriptive statistics were estimated for age, number of children, marital status, each of the adiposity variables, the SOL measures, and average morning, evening, diurnal slope, and AUC_g testosterone levels.

Objectives One & Two, Multilevel Growth Curve Models: To assess associations between age, BMI, and diurnal testosterone levels among the Shuar, a series of multilevel growth curve models (based on previous steroid hormone research) were used to describe the diurnal testosterone patterns of the Shuar (Adam, 2006; Nyberg, 2012). For the present study, a series of three-level multilevel models were used to estimate both testosterone indices (at waking/diurnal slope and AUC_g). In each model, the various levels reflected distinct sources of variation, including within-individuals (Level-1), between-individuals (Level-2), and between-households (Level-3). Preliminary analyses identified significant correlations between testosterone values across collection days for each index: at waking/diurnal slope (r = 0.60-0.76; $p < 0.01$) and AUC_g (r = 0.72-0.79, $p < 0.01$). Given that testosterone values were significantly correlated across days and testing between-day variance was not one of the study objectives, day-to-day variance was not examined in these analyses.

In the waking/diurnal slope model, Level-1 considered repeated measures within an individual; specifically, the $\log_{10}$-transformed testosterone values were included as the outcome variable and time (calculated as minutes since waking to time of collection) was the predictor variable. Thus, testosterone levels at waking represented the model intercept and the change in testosterone levels throughout the day is captured in the linear growth coefficient (i.e., slope), which was estimated by fitting a best fit line through the morning and evening testosterone values (Adam & Gunnar, 2001; Ong et al., 2011). In the AUC_g model, Level-1 included the repeated measures of AUC_g across three collection days as the independent outcome. Time was not included as a predictor at Level-1 in this model since it was accounted for in the calculation of AUC_g.

For both models, Level-2 captured between-individual variation in each testosterone measure as predicted by participant-level variables. Specifically, age, BMI, and the interaction between the two (i.e., age-by-BMI) were added as predictors at this level, wherein each variable was entered into the model in a stepwise manner to test the study hypotheses. Moreover, the present study was primarily interested in how participant-level variables influence testosterone variation between-individuals (i.e., instead of between-households); thus, the predictors at Level-2 were fixed at Level-3.

Finally, Level-3 estimated between-household differences in each model, but no predictor variables were included at this level.

Based on standard protocol (Shirtcliff & Essex, 2008), a baseline, partially unconditional model was first run in the waking/diurnal slope model. This model included hours since waking at Level-1 and was used to determine intraclass correlation coefficients (ICCs) in subsequent analyses. This technique allows for control of diurnal rhythm (Shirtcliff & Essex, 2008). However, fully unconditional models (i.e., without predictor variables at any level) were used to estimate ICC values for the AUC_g model. Likelihood ratio tests were run to compare model fit and test for random slope effects.

Model fit tests indicated that including clustering individuals within households (i.e., including Level-3 in the models) accounted for a negligible amount of variance in each model (i.e., $1.0 \times 10^{-6} – 6.76 \times 10^{-10}$) and did not significantly improve model fit for either model (χ^2 (2) = 0.00, $p = 1.00$). Level-3 was therefore dropped and two-level models (observations nested within individuals) were run for both waking/diurnal slope and AUC_g measures. In addition, likelihood ratio tests indicated that for both the waking/diurnal slope model (χ^2 (3) = 35.98, $p < 0.001$) and the AUC_g model (χ^2 (3) = 32.91, $p < 0.001$), adding the fixed effects (age, BMI, and an interaction term between the two) to the null models significantly improved the model. Conversely, treating time as a random variable at Level-2 of the waking/diurnal slope model (i.e., a random slopes model) did not significantly improve model fit (χ^2 (1) = 3.27, $p = 0.071$). Therefore, for both testosterone indices, two-level random intercept models (i.e., allowing waking/baseline testosterone levels to vary across individuals) were run including the fixed effects variables. Specifically, the following final models were run:

Testosterone Levels at Waking and the Diurnal Slope

Level-1: Within-Individuals

$\text{Testosterone}_{ij} = \beta_{0ij} + \beta_1(\text{HoursSinceWaking})_{ij} + e_{0ij}$

Level-2: Between-Individuals

$\beta_{0ij} = \beta_{0j} + \beta_2(\text{Age})_{ij} + \beta_3(\text{BMI})_{ij} + \beta_4(\text{Age*BMI})_{ij} + \mu_{0j}$

Level 2: $[\mu_{oij}] \sim N\ (0, \sigma^2_{u0})$

Level 1: $[e_{otij}] \sim N\ (0, \sigma^2_{e0})$

Area Under the Curve with Respect to the Ground (AUC_g)

Level-1: Within-Individuals

$\text{AUC}_{ij} = \beta_{0ij} + e_{0ij}$

Level-2: Between-Individuals

$\beta_{0ij} = \beta_{0j} + \beta_1(\text{Age})_{ij} + \beta_2(\text{BMI})_{ij} + \beta_3(\text{Age*BMI})_{ij} + \mu_{0j}$

Level 2: $[\mu_{oij}] \sim N\ (0, \sigma^2_{u0})$

Level 1: $[e_{otij}] \sim N\ (0, \sigma^2_{e0})$

Objective Two, Linear Regression Models: To further evaluate how measures of adiposity contribute to variation in Shuar average morning and evening testosterone levels, while accounting for the influence of relevant covariates, a series of linear regressions were also conducted. In each model age was entered in the first step, marital status and number of children were entered in the second step, and the three individual adiposity measures (BMI, percent body fat, waist circumference) were simultaneously entered or the composite score of all three (summed z-scores of each measure) was entered in the final step.

Objective Three, Linear Regression Models: Finally, to determine how SOL factors are associated with Shuar testosterone level variation (while accounting for the important covariates), an additional series of regressions was conducted. Linear regressions tested whether average morning or evening testosterone values were positively associated with MI level. In each of these models, age and BMI (both centered and untransformed) were entered in the first step, marital status and number of children were entered in the second step, the SOL index score (T-SOL, M-SOL, H-SOL or composite MI, each centered) was entered in the third step, and the interaction terms (age-BMI, age-SOL, BMI-SOL, and age-BMI-SOL) were entered in the final step.

Results

Descriptive Statistics

Table 4.2 presents descriptive statistics for participant age, marital status, number of children, BMI, percent body fat, waist circumference, SOL levels, and average morning, evening, and AUC_g testosterone levels from the original dataset (i.e., prior to multiple imputation analysis). For ease of interpretation, untransformed, raw values are presented. Most participants were married and had children. According to standard cutoffs (CDC, 2015), average BMI was healthy but close to overweight using American standards, but overweight according to modified cutoffs for use in smaller-bodied (i.e., Asian) populations (WHO, 2004). Given the small stature of the study population (Urlacher et al., 2016), the modified cutoffs for smaller-bodied populations are likely the most appropriate cutoffs to use for this sample. Average waist circumference was healthy according to both sets of cutoff values.

In general, both T-SOL and M-SOL average values were rather low, reflecting ownership of fewer than one third of the items on either list (i.e., a score of 1 reflects ownership of every item). However, this is unsurprising given that this average value was calculated across a diverse study population, representing individuals living in both very traditional and fairly market integrated settings. Finally, average morning testosterone levels were higher than average evening testosterone levels, as has been documented in other populations (Bribiescas, 2001).

	Mean (S.D.)
Age (years)	31.80 (14.66)
Number of children	3.40 (3.89)
Body mass index (kg/m^2)	24.19 (2.89)
Percent body fat (%)	17.74 (5.36)
Waist circumference (cm)	81.41 (12.14)
Adiposity composite z-score	0.17 (2.80)
T-SOL score	0.31 (0.22)
M-SOL score	0.22 (0.16)
H-SOL score	11.35 (3.25)
Composite M-SOL and H-SOL score	0.02 (1.67)
AM testosterone (pmol/L)	429.64 (177.15)
PM testosterone (pmol/L)	247.81 (99.43)
AUC$_g$ average across three days (pmol/L)	242887.52 (103164.90)
	Frequency (%)
Marital status, with partner	84 (61.3%)

Table 4.2. Description of the study population (n = 137) using untransformed variables.

Multilevel Growth Models of Diurnal Testosterone Rhythms

A two-level multilevel model was used to assess testosterone levels at waking and the diurnal slope of testosterone values throughout the day, with variance distributed to within-individual (Level-1) and between-individual (Level-2) components. The partially unconditional model including hours since waking as a fixed predictor at Level-1 was considered the null model, and was used to calculate the ICC value ($\rho = 0.5296$). In other words, 52.96% of the total variation in the model is accounted for by between individual (Level 2) differences. The addition of the fixed effect predictors reduced the ICC value ($\rho = 0.4678$), suggesting that the predictors added account for some of this between individual variation.

The results of the baseline at waking/diurnal slope model (with hours since waking) are presented in Table 4.3. Average waking testosterone levels were significantly different than zero ($\beta = 2.594$, $p < 0.001$). Moreover, hours since waking was a significant predictor of testosterone levels ($\beta = -0.020$, $p < 0.001$).

Fixed Effect	**Coefficient (SE)**	**t-value**	***p*-value**
Diurnal Testosterone Intercept	2.594 (0.017)	153.47	< 0.001
Hours since waking	-0.020 (0.001)	-20.18	< 0.001
Random Effect	**Estimate (SE)**	**95% Confidence Interval**	
Level-1	0.149 (0.012)	0.146-0.193	
Level-2	0.168 (0.005)	0.139-0.160	

Table 4.3. Two-level partially unconditional (null) model for testosterone waking/diurnal slope (using $\log_{10}$-transformed testosterone).

Individual (Level 2) variables were added to assess how these factors influenced testosterone levels; specifically, age, BMI, and an age-BMI interaction term were added. These results are presented in Table 4.4. Results indicate that both age ($\beta = 0.041$, $p < 0.001$) and BMI ($\beta = 0.076$, $p < 0.001$) were positively associated with testosterone levels. However, there was also a significant interaction between these two variables ($\beta =$

-0.002, $p < 0.001$). These main effects may therefore not be accurate and the interaction was examined using simple slopes analysis.

Fixed Effect	**Coefficient (SE)**	**t-value**	***p*-value**
Diurnal testosterone intercept	0.826 (0.228)	3.62	< 0.001
Hours since waking	-0.020 (0.001)	-19.57	< 0.001
Age	0.041 (0.007)	5.77	< 0.001
BMI	0.076 (0.010)	7.49	< 0.001
Age*BMI	-0.002 (0.0002)	-5.86	< 0.001
Random Effect	**Estimate (SE)**	**95% Confidence Interval**	
Level-1	0.147 (0.005)	0.137-0.157	
Level-2	0.129 (0.010)	0.111-0.151	

Table 4.4. Two-level fixed effects model for testosterone waking/diurnal slope (using $\log_{10}$-transformed testosterone).

To clarify the meaning of this significant interaction, simple slopes were estimated and graphed using conditional values of age (±1 SD from the mean age of 27.1 years) and BMI (±1 SD from the mean BMI of 23.5 kg/m^2). Participants below or above the mean age were considered as “younger” or “older”, respectively; likewise, participants with BMI levels below or above the mean BMI were classified as “lower BMI” and “higher BMI”, respectively. To further explore how different BMI cutoff values impact the interpretation of the interaction between age and BMI, simple effects analyses were also conducted using a BMI cutoff of 18.5 kg/m^2 instead of the mean value of 23.5 kg/m^2. The value of 18.5 kg/m^2 represents the low-end of the “normal/healthy”

BMI range according to modified cutoffs for use in smaller-bodied populations (as opposed to the slightly “overweight” average BMI value of 23.5 kg/m^2). This value was selected to examine whether using a relatively low (but healthy) BMI cutoff point might alter the interpretation of the interaction. However, the interpretation remained unchanged using this lower BMI value; the average BMI value was therefore used to remain consistent with previous studies (Holmbeck, 2002).

Given that aging has been linked with changes in BMI (Deurenberg et al., 1990), age was the assumed moderator. Regression models predicting testosterone levels were run with BMI (as a centered variable), the conditional high or low age terms (calculated by subtracting or adding the standard deviation of age from the age variable, a centered variable), and the interaction between the conditional age term and BMI. The regression results were used to generate two linear equations (one for younger ages and one for older ages), into which were entered the negative and positive standard deviation terms of BMI (a centered variable) to plot the regression lines (Figure 4.2). As shown in a plot of the lines, a clear divide between the age groups was less apparent among lower BMI participants, but younger individuals with higher BMI values exhibited higher testosterone levels than older individuals with higher BMI values (Figure 4.2).

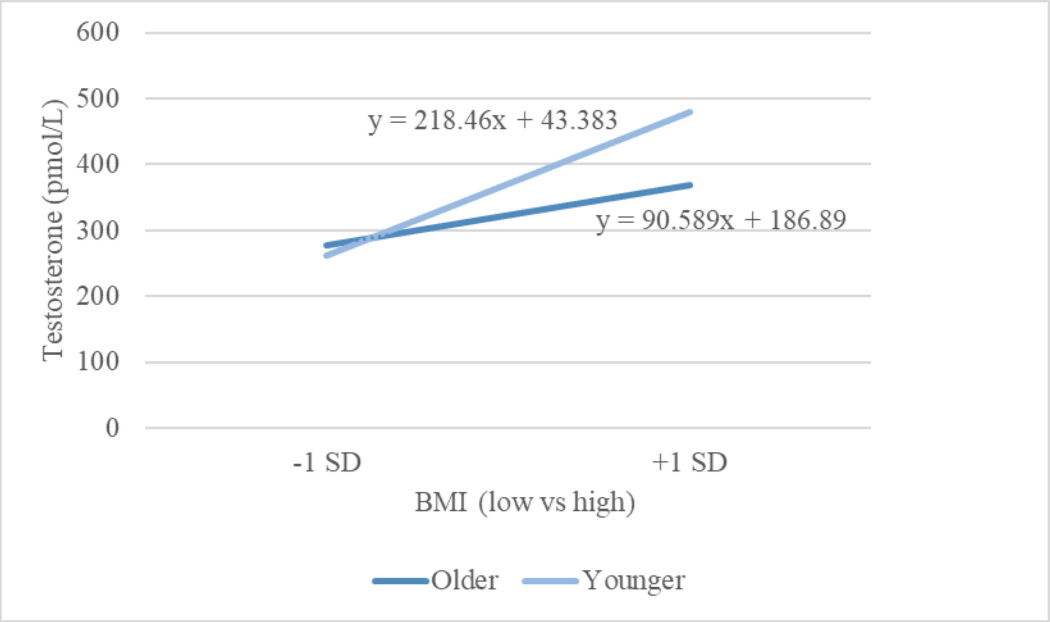


Figure 4.2. Regression lines for the interaction between BMI and testosterone levels (reported in raw, untransformed concentrations for ease of interpretation) as moderated by age (a two-way interaction). SD = standard deviation of BMI.

The interaction between age and BMI can also be visualized across age groups (Figure 4.3). Age groups were defined by decade of life, although men over the age of 50 were grouped together owing to the smaller number of older men in the sample. These results indicate that individuals with higher BMI values (at or above the mean of 23.5 kg/m^2) below the age of 50 exhibit higher waking testosterone levels than lower BMI individuals (below the mean of 23.5 kg/m^2), and that this difference between the two BMI groups is typically maintained as testosterone levels decrease throughout the day (with the exception of the 31-40 year old age group, where higher BMI men appear to have a steeper testosterone decline through the day than their lower BMI counterparts). However, this trend is reversed in men over the age of 50, and men with lower BMI values display higher waking testosterone values compared to higher BMI participants;

yet, these men exhibit a steeper decline in testosterone through the day, resulting in the two BMI groups exhibiting similar evening testosterone levels (Figure 4.3).

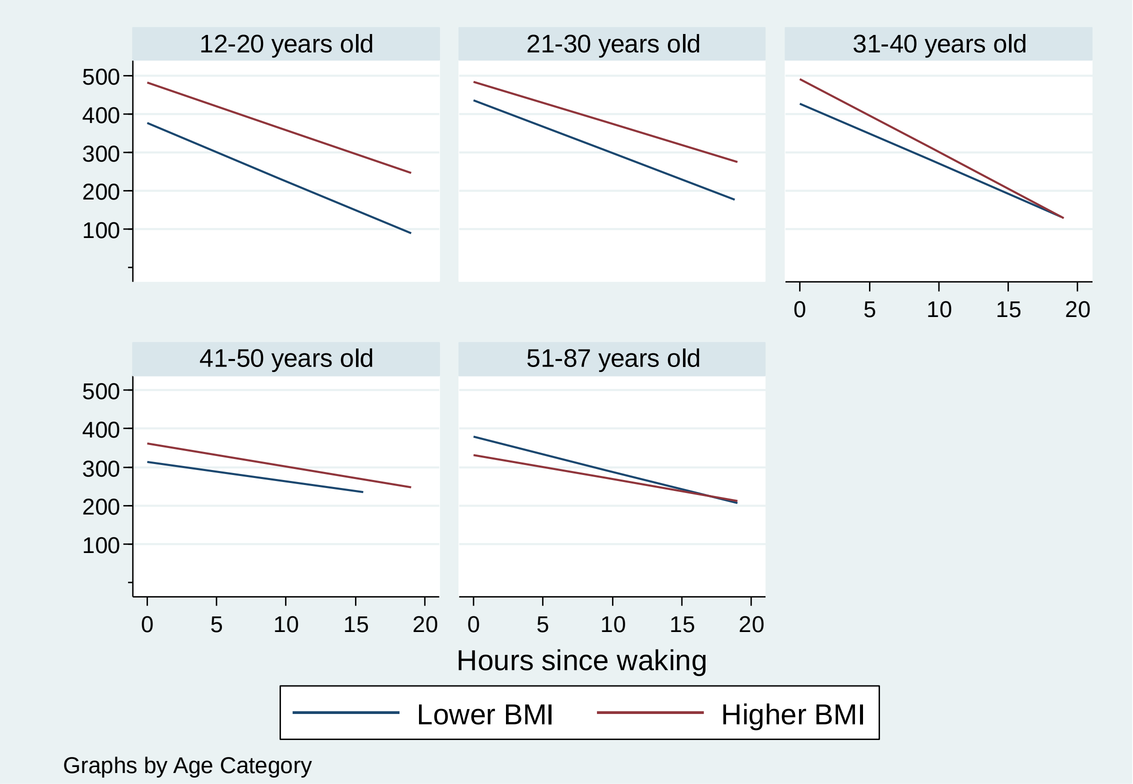


Figure 4.3. Regression lines for diurnal changes in testosterone levels (reported in raw, untransformed concentrations for ease of interpretation) across the day, by BMI (lower vs higher BMI values, with lower BMI representing values below the mean BMI value of 23.5 kg/m^2 and higher representing values at or above the mean) and age categories.

Multilevel Growth Models of AUC$_g$ Testosterone Values

Similar to the waking/diurnal slope analysis, a two-level multilevel model was used to assess AUC$_g$ values, with variance distributed to within-individual (Level-1) and between-individual (Level-2) parts. A fully unconditional model with no predictors was first run as the null model. This null model was used to calculate ICC values (ρ = 0.5724), suggesting 57.24% of the total variation in the model is accounted for by between individual (Level 2) differences. The addition of the fixed effect predictors reduced the ICC value (ρ = 0.5270), suggesting that adding these predictors accounted for some of the between individual variation. The results of the null AUC_g model are presented in Table 4.5. Average AUC_g levels were significantly different than zero ($\beta = 479.748$, $p < 0.001$).

Fixed Effect	**Coefficient (SE)**	**t-value**	***p*-value**
AUC_g Testosterone Intercept	479.748 (7.974)	60.16	< 0.001
Random Effect	**Estimate (SE)**	**95% Confidence Interval**	
Level-1	61.270 (3.906)	54.010-69.506	
Level-2	82.018 (6.733)	69.809-96.362	

Table 4.5. Two-level fully unconditional (null) model for AUC_g (using square root AUC_g testosterone values).

Individual (Level 2) variables were added to model to test individual differences in AUC_g measures (Table 4.6). This model suggests that both age ($\beta = 19.409$, $p < 0.001$) and BMI ($\beta = 33.932$, $p < 0.001$) were positively associated with testosterone levels. However, there was also a significant interaction between these two variables ($\beta = -0.824$, $p < 0.001$). The main effects of age and BMI may therefore not be accurate and the interaction between the two was examined using simple slopes analysis. Simple slopes were estimated and graphed using conditional values of age (±1 SD from the mean age of 28.0 years) and BMI (±1 SD from the mean BMI of 23.6 kg/m^2). Individuals below or above the mean age were considered as “younger” or “older”, respectively;

similarly, individuals with BMI levels below or above the mean BMI were classified as "lower BMI" and "higher BMI", respectively.

Fixed Effect	**Coefficient (SE)**	**t-value**	***p*-value**
AUC$_g$ Testosterone Intercept	-301.741 (138.157)	-2.18	= 0.030
Age	19.409 (3.891)	4.99	< 0.001
BMI	33.932 (6.148)	5.52	< 0.001
Age*BMI	-0.824 (0.161)	-5.12	< 0.001
Random Effect	**Estimate (SE)**	**95% Confidence Interval**	
Level-1	61.198 (3.823)	55.301-80.958	
Level-2	66.911 (6.485)	53.301-80.958	

Table 4.6. Two-level fixed effects model for AUC$_g$ (using square root transformed AUC$_g$ testosterone values).

Like the waking/diurnal slope model interaction analysis, age was assumed to be the moderator in regression models predicting AUC$_g$ with BMI (as a centered variable), conditional high or low age terms, and the interaction between the conditional term and BMI. These results were used to calculate two linear equations, into which were entered the negative and positive standard deviation value of BMI (a centered variable) to plot the lines (Figure 4.4). As shown in a plot of the lines, older individuals with lower BMI values exhibited higher AUC$_g$ values compared to younger individuals with lower BMI values, but this pattern was reversed among participants with higher BMI values (i.e., younger adults had higher AUC$_g$ values than older men) (Figure 4.4).

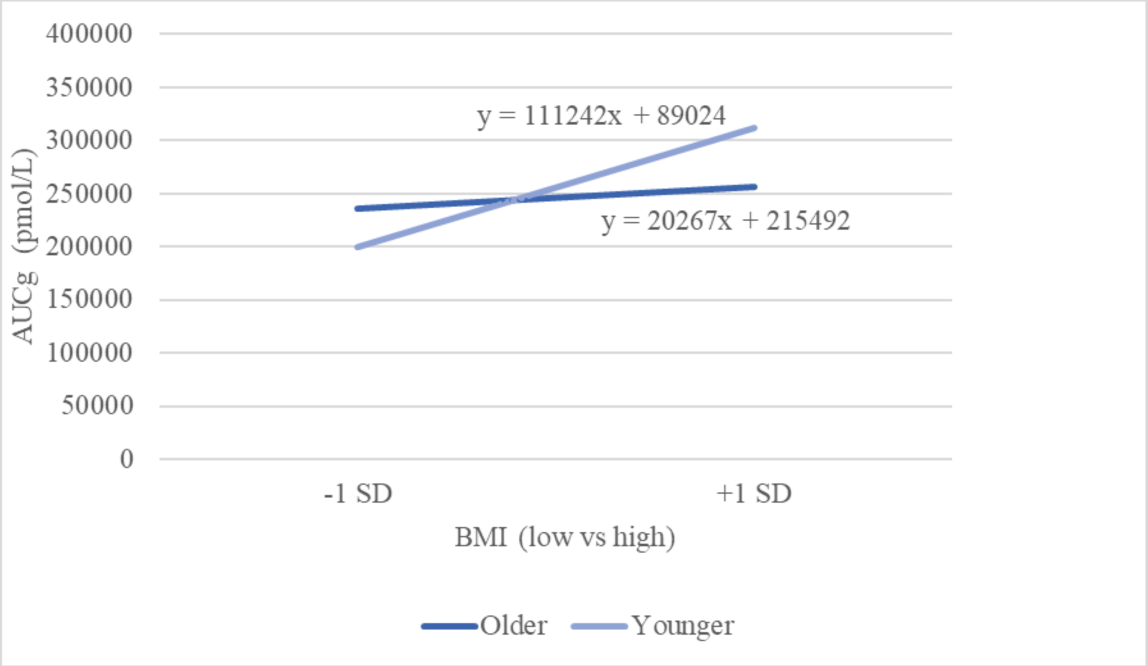


Figure 4.4. Regression lines for the interaction between BMI and AUC$_g$ values (reported in raw, untransformed concentrations for ease of interpretation) as moderated by age (a two-way interaction). SD = standard deviation of BMI.

This interaction between age and BMI can also be visualized across age categories (roughly grouped into decades) (Figure 4.5). It appears that below the age of 40 individuals with higher BMI values (at or above the mean of 23.6 kg/m^2) have higher AUC$_g$ values compared to lower BMI individuals (below the mean of 23.6 kg/m^2). However, this trend is reversed in men over the age of 40, and participants with lower BMI values display higher AUC$_g$ values compared to higher BMI men (Figure 4.5).

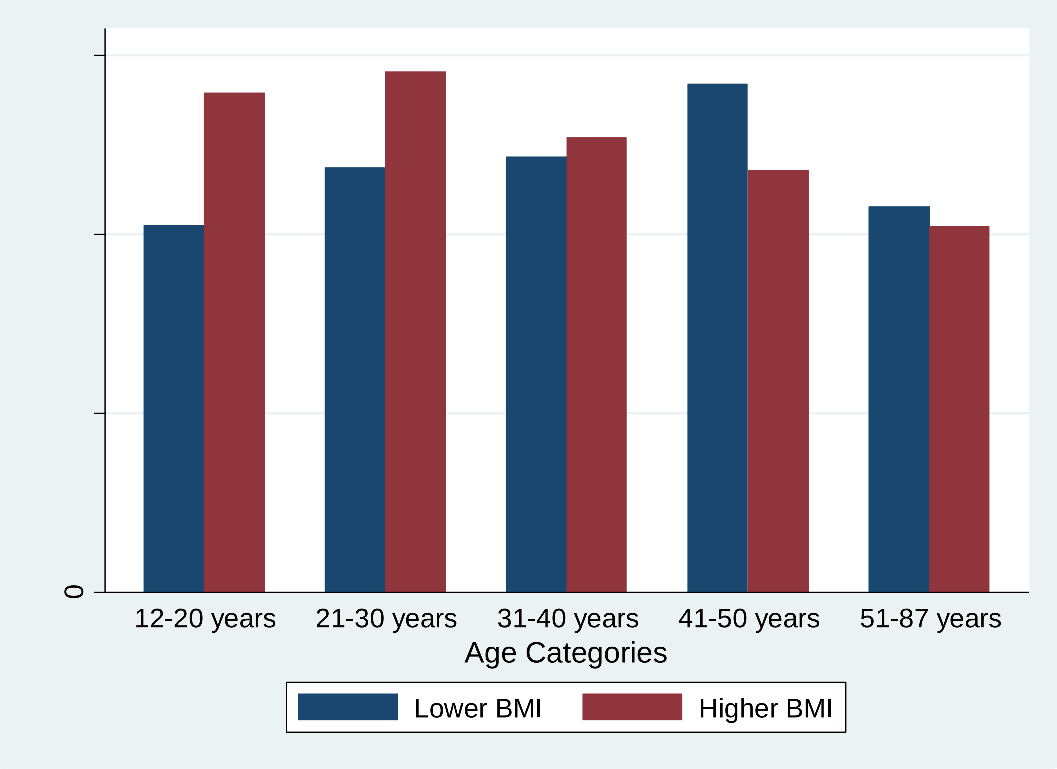


Figure 4.5. Average AUC_g values (reported in raw, untransformed concentrations for ease of interpretation) across age categories, by BMI (lower vs higher BMI values, with lower BMI representing values below the mean BMI value of 23.6 and higher representing values at or above the mean).

Associations between Average Testosterone Levels and Adiposity Measures

Additional analyses were conducted to examine associations between average morning or evening testosterone levels and other adiposity measures (Table 4.7). Interestingly, only BMI (a variable calculated by removing the contribution of age and percent body fat due to high levels of multicollinearity) was significantly associated with average morning (B = 1.058, $p < 0.001$) or average evening (B = 0.657, $p = 0.001$) testosterone levels. The other adiposity measures exhibited no significant relationship ($p > 0.05$). In addition, age was significantly associated with average evening testosterone in both the model including the individual adiposity measures (B = 0.039, $p = 0.029$) and the model including the composite adiposity measure (B = 0.041, $p = 0.028$), suggesting that average evening testosterone levels rise for each unit increase in participant age. Finally, number of children (as a log transformed variable) was inversely related to afternoon testosterone levels in the average evening testosterone model including the composite adiposity measure (B = -9.481, $p = 0.035$), indicating that men with more children had significantly lower mean evening testosterone levels.

	AM T (individual measures)	**PM T (individual measures)**	**AM T (composite z-score)**	**PM T (composite z-score)**
Constant	**20.27 (0.85)*****	**14.10 (0.64)*****	**21.12 (0.87)*****	**14.09 (0.65)*****
Age	0.004 (0.02)	**0.04 (0.02)***	0.01 (0.03)	**0.04 (0.02)***
Marital status	-0.80 (1.10)	-0.10 (0.84)	-0.13 (1.14)	0.13 (0.84)
Number of children	-7.73 (5.71)	-7.51 (4.44)	-10.68 (5.92)	**-9.48 (4.49)***
Percent body fat	0.02 (0.10)	-0.04 (0.07)	----	---
Body mass index	**1.06 (0.24)*****	**0.66 (0.20)****	---	---
Waist circumference	-0.32 (0.59)	0.07 (0.44)	---	---
Composite z-score	---	---	0.20 (0.23)	0.11 (0.18)

Table 4.7. Linear regressions for the prediction of average morning (AM) and evening (PM) testosterone levels from individual and composite adiposity measures. B coefficients with S.E. Comparisons are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Associations between Average Testosterone Levels and Style of Life Measures

Linear regressions were conducted to examine the association between average morning or evening testosterone level and participant MI level (as measured through SOL scores) (Table 4.8). No significant interactions were observed between the SOL factors and age or BMI. Likewise, none of the SOL variables (T-SOL, M-SOL, H-SOL, or composite SOL) were significantly associated with testosterone levels. The only significant findings were evident in the composite SOL variable models. Namely, BMI exhibited a significant positive association with variation in average morning testosterone levels (B = 1.193, $p = 0.016$); however, a significant association between age and BMI was observed (B = -0.024, $p = 0.041$). The main effect documented for BMI may therefore not be accurate.

Likewise, BMI was significantly associated with variation in average evening testosterone levels (B = 0.799, $p = 0.029$) and a significant association between age and BMI was observed in this model as well (B = -0.019, $p = 0.031$). In addition, number of children (a log transformed value) significantly contributed to variation in average evening testosterone levels (B = -9.248, $p = 0.047$). The interaction between age and BMI in both models was plotted, and the results supported those from the previous analyses. Specifically, among individuals with higher BMI values, younger men exhibited higher average morning and evening testosterone levels compared to older men, but this trend was reversed among lower BMI individuals (i.e., older men in this group had higher testosterone levels than younger men).

	AM T (T-SOL)	**PM T (T-SOL)**	**AM T (M-SOL)**	**PM T (MSOL)**	**AM T (H-SOL)**	**PM T (H-SOL)**	**AM T (CompSOL)**	**PM T (CompSOL)**
Constant	**20.497** **(2.004)*****	**14.361** **(1.545)*****	**22.595** **(1.709)*****	**15.956** **(1.274)*****	**26.116** **(3.417)*****	**16.927** **(2.546)*****	**21.293** **(1.060)*****	**15.121** **(0.795)*****
Age	-0.004 (0.054)	0.033 (0.043)	-0.077 (0.049)	-0.009 (0.037)	-0.170 (0.097)	-0.045 (0.073)	-0.027 (0.030)	0.014 (0.022)
BMI	1.695 (0.918)	0.954 (0.723)	0.475 (0.810)	0.158 (0.637)	0.879 (1.593)	0.104 (1.176)	**1.193** **(0.494)***	**0.799** **(0.346)***
Marital status	-0.887 (1.154)	-0.295 (0.881)	-1.006 (1.142)	-0.363 (0.867)	-1.022 (1.152)	-0.332 (0.882)	-0.795 (1.270)	0.160 (0.955)
Number of children	-7.276 (6.161)	-7.130 (4.746)	-6.761 (5.852)	-6.788 (4.437)	-8.513 (5.915)	-7.678 (4.536)	-9.601 (6.188)	**-9.248** **(4.660)***
SOL Measure	5.516 (13.579)	3.939 (10.552)	-16.914 (17.741)	-12.744 (13.699)	-0.458 (0.301)	-0.192 (0.229)	-1.106 (0.628)	-0.642 (0.474)
Age*BMI	-0.041 (0.025)	-0.022 (0.020)	-0.010 (0.020)	-0.006 (0.015)	-0.012 (0.039)	-0.004 (0.029)	**-0.024** **(0.012)***	**-0.019** **(0.009)***
Age*SOL	-0.165 (0.384)	-0.112 (0.306)	0.616 (0.515)	0.313 (0.403)	0.014 (0.009)	0.006 (0.007)	0.034 (0.018)	0.017 (0.014)
BMI*SOL	-4.236 (6.109)	-0.953 (4.781)	10.063 (8.357)	9.150 (6.843)	0.021 (0.123)	0.059 (0.095)	0.182 (0.289)	0.276 (0.221)
Age*BMI*SOL	0.140 (0.173)	0.024 (0.140)	-0.217 (0.224)	-0.190 (0.177)	-0.001 (0.003)	-0.001 (0.002)	-0.004 (0.008)	-0.006 (0.006)

Table 4.8. Linear regressions for the prediction of average morning (AM) and evening (PM) testosterone levels from individual characteristic and composite style of life measures (T-SOL, M-SOL, H-SOL, and composite SOL). B coefficients with S.E. Comparisons are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Discussion

These results indicate that complex patterns exist between testosterone and individual characteristics, especially age and BMI. As predicted, physical condition (as measured by BMI) was positively associated with testosterone levels. However, this relationship appears to be moderated by participant age, suggesting that testosterone profiles differ across the life course in response to changes in male reproductive strategies. For example, significantly lower testosterone levels were documented among fathers, supporting the premise that alterations in testosterone levels facilitate shifts in male reproductive priorities. Unexpectedly, no significant associations were documented between testosterone levels and participant MI (captured through SOL factors).

Diurnal Testosterone Patterns

As has been documented elsewhere (Bribiescas & Hill, 2010; Cooke et al., 1993; Montanini et al., 1988; Plymate et al., 1989; Simoni et al., 1988), Shuar testosterone levels peaked in the morning and decreased throughout the day. This has been attributed to the role testosterone plays in mobilizing energy for use throughout the day (Bribiescas & Hill, 2010). Furthermore, the multilevel models examining diurnal testosterone patterns documented significant differences in participant waking testosterone levels (i.e., regression line intercepts), suggesting that individuals begin at different starting points, thus influencing total daily testosterone production (i.e., AUC_g) and final evening testosterone values. In other words, individuals who exhibit higher morning levels are expected to produce more testosterone throughout the day and have higher evening levels compared to men who start at lower peak testosterone levels, and then decline from those lower initial levels.

Interestingly, adding random slope did not significantly improve the models, indicating that rate and degree of testosterone decline throughout the day from peak morning levels did not differ significantly between men in this sample. Previous research among the Shuar has documented that this population exhibits significantly lower testosterone levels compared to American men living in more industrialized settings (Gildner et al., 2016b). It is therefore possible that these lower levels of testosterone restrict the amount of daily variation possible. This is consistent with previous research, which has documented that morning levels in particular are suppressed in subsistence populations (Bribiescas, 1996), which would restrict the diurnal slope possible within these individuals.

Likewise, including a third model level to account variation at the household level did not improve model fit, suggesting that variation in testosterone patterns was not meaningfully influenced by participant household. This is not too surprising given that there was relatively little clustering present within households; for the 137 men in the sample, there were a total of 91 unique households. In addition, most household clustering was fathers and their sons. These individuals exhibit very different roles within the house and often spend their time in markedly different areas or buildings of the same household, thereby likely negating any discernable effect of a specific household setting on testosterone variation.

Age, BMI, and Testosterone Profile

Age exhibited a significant positive effect with evening testosterone levels in the adiposity regression models, suggesting that older men maintain higher testosterone levels at the end of the day. However, morning (peak) testosterone levels did not differ significantly by age. This finding does not support the hypothesis that older men would exhibit significantly lower testosterone levels (i.e., due to age-related decreased in testosterone production), as has been documented in higher income countries (Bribiescas, 2001, 2010). However, the sample included relatively few older men. Only 11.68% of participants were over the age of 50, while a relatively large proportion of the sample (26.3%) was under the age of 20. Further work is therefore needed to measure testosterone level variation at older ages among Shuar men.

This positive association between age and testosterone was also apparent in the multilevel models examining diurnal testosterone variation. However, these results indicate that the relationship between age and testosterone is not straightforward. Significant interactions between age and BMI were documented in the present study, indicating that age moderates the relationship between BMI and testosterone levels. Specifically, the positive association between BMI and testosterone levels appears to be driven by younger men, (i.e., younger men with higher BMI values have higher testosterone levels than older men). This supports previous findings and suggests that having sufficient energy reserves is required among lean populations to meet the energetic costs incurred by elevated testosterone levels in younger men (Alvarado, 2013; Bribiescas, 2001; Campbell et al., 2003, 2007; Gettler et al., 2010). In other words, in resource-limited conditions only young men with adequate energy reserves will be able to develop and maintain testosterone-linked traits, such as musculature.

This positive relationship between BMI and testosterone is also consistent with male life history patterns. Young men typically invest more heavily in reproductive effort compared to older individuals (Bribiescas, 2010; Jasienska et al., 2017). In particular, the physical traits associated with high testosterone levels have been hypothesized to play an important role in attracting a mate (Buss & Schmitt, 1993; Gangestad et al., 2010; Thornhill & Gangestad, 2003) and acquiring the resources needed to care for a family (Alvarado et al., 2015). Thus, increased energetic investment at this life stage is likely to increase male reproductive success, provided that a male has sufficient energy stores.

It therefore seems likely that males with greater energy reserves will increase their reproductive investment, resulting in elevated testosterone levels to support these efforts. Although not well tested, it seems likely that environmental conditions early in life influence the amount of resources a male invests in reproductive effort (including high testosterone levels). For example, evidence indicates that testosterone levels gradually decline with age (particularly after the age of 40) in well-fed sedentary men who exhibit higher testosterone levels during young adulthood (Harman et al., 2001). Still, most research suggests that immediate conditions, behavior, and nutrition more strongly influence testosterone production (Bishop et al., 1988; Spratt et al., 1988).

Further, it has been hypothesized that adequate adiposity levels also reflect excellent immune function, such that adiposity measures mediate the relationship between immune responses and male attractiveness (Rantala et al., 2012). Yet, it should be noted that this positive relationship between BMI and testosterone is expected to be context dependent, and limited to resource-constrained environments. At a certain point, excess adipose levels should exert the opposite effect, decreasing male attractiveness to potential mates (Singh, 1995) and also lowering testosterone levels through the aromatization due of androgens to estrogens within adipose tissues (Kley et al. 1980, 1981; Pritchard et al. 1998). Unhealthy adiposity levels (often linked with aging) also result in chronic inflammation, increasing the risk for a range of negative health outcomes (e.g., cardiovascular disease, type 2 diabetes, metabolic syndrome, and all-cause mortality) (Jenny et al., 2007; Pradhan et al., 2001; Ridker et al., 2003). Thus, high adiposity levels are associated with a range of health consequences.

It is therefore unsurprising that the results of the present study indicate that the association between BMI and age documented in younger men is reversed at older ages; such that lower BMI level, older men have higher testosterone levels than younger men (e.g., perhaps due to maintaining healthy diets and activity patterns). Again, this also fits with what is known about male aging patterns. Although reproductive decline in males is not as dramatic as female menopause, evidence indicates that energetic investment in male reproduction also decreases with age (Bribiescas, 2001, 2010). Male aging is associated with impaired semen volume, sperm motility, and sperm morphology, altered physiological profiles, and changes in body composition (Bribiescas, 2010; Kidd et al., 2001).

For example, due to complex factors including changes in metabolism (Fontana & Klein, 2007), the amount of adipose tissue typically increases while musculature decreases in older men (Bribiescas, 2010). These changes may subsequently decrease testosterone levels through increased aromatization of testosterone within adipose tissues

(Kley et al. 1980, 1981; Pritchard et al. 1998). In addition, concentrations of sex hormone binding globulin (SHBG) typically increase with age (Bribiescas, 2001, 2010), reducing the amount of bioavailable testosterone in older men. Finally, altered activity patterns at older ages (e.g., less participation in competitive events) may also be linked with lower testosterone levels (Bribiescas, 2001, 2009).

It is therefore possible that the inverse association between BMI and testosterone levels in older Shuar men reflects variation in aging patterns. For instance, lower BMI levels in older Shuar men may be indicative of more active older adults, who remain lean and have less adipose tissue available to aromatase androgens. Previous work supports this hypothesis, indicating the amount of testosterone decline a man experiences during aging is shaped by many factors, including activity patterns and overall health (Ellison et al., 2002; Uchida et al., 2006). Again, these patterns suggest that the testosterone level variation documented in the present study reflects changes in male reproductive investment across the lifespan.

Additional Adiposity Measures and Testosterone Levels

In addition to BMI, other adiposity measures were considered. Specifically, the relationship between BMI, percent body fat, WC, and a composite measure of these three values and average morning or evening testosterone levels were assessed. Interestingly, the only significant finding was between BMI and testosterone levels. A positive association was documented between this measure (an unstandardized residual, removing the contribution of age and percent body fat to BMI values) and both morning and evening testosterone values, a finding that seems to support the hypothesis that increased adiposity is required to meet the substantial energetic costs resulting from high testosterone levels (Bribiescas, 2001; Campbell et al., 2003).

However, the analysis examining the relationship between testosterone levels and adiposity measures also included WC, a measure of central adiposity strongly correlated with BMI values (Ardern et al., 2003; Janssen et al., 2002). In addition, the contribution of percent body fat was removed from this BMI value due to multicollinearity. It is therefore possible this BMI measure is reflecting levels of musculature, rather than adiposity. The inability to distinguish between muscle and adipose tissue is a limitation of the BMI measure (e.g., Nevill et al., 2006), necessitating additional body composition measures. The significant relationship documented here may consequently represent a positive association between muscle mass and testosterone levels.

An association between testosterone levels and musculature is consistent with previous working documenting a positive feedback loop between muscle mass and testosterone levels (Bribiescas et al., 2001). High testosterone levels support the development of muscle tissue (Udry, 1988, 1990), while increased muscle mass is typically linked with reduced adiposity (Bribiescas, 2001; Mauras et al.,1998), thereby decreasing the amount of testosterone aromatized to estrogen within adipose cells and maintaining elevated testosterone levels. Still, as was hypothesized for adiposity levels, a positive association between muscle mass and testosterone may also reflect participant energetic status, as energy availability is critical in the development of muscle mass (Bribiescas, 1996, 2001, 2010; Ellison, 2003; Muehlenbein & Bribiescas, 2005). Market integration level is therefore expected to be important drivers of this relationship and testosterone levels in general, given the key role SOL factors play in determining individual access to resources.

Style of Life Measures and Testosterone Levels

Unexpectedly, no significant associations were documented between any of the SOL factors and testosterone levels. However, the present study did not include any men living in market centers (i.e., the highly market-integrated town of Sucúa). The study included men from several distinctive communities, at different levels of MI. The most traditional community did not have any electricity, roads, schools, or a medical clinic at the time of data collection, while the most market integrated communities were easily accessible by road, had reliable electricity, several local schools, and outhouses with plumbing. Most communities fell within these two extremes, with wealthier households exhibiting more characteristics associated with MI (e.g., solar-power generated electricity, televisions, cell phones, cooking stoves, etc.).

Still, although the study sample was drawn from several distinctive communities, these participants all exhibited MI levels well below those typically observed in high-income nations. For example, a couple of men owned all the items found the T-SOL index (i.e., items associated with a less market integrated lifestyle), but no men owned all the items on the M-SOL scale (associated with a more market integrated lifestyle). The maximum number of items owned from the M-SOL index was 8 out of 12; and while there are more items on the M-SOL index, this is telling as many families in market towns exhibit higher levels of M-SOL item ownership.

Moreover, all men in the sample exhibited relatively similar activity patterns (i.e., daily work on the *finca*, large gardens which produce agricultural items for consumption or sale). These men also exhibited homogenous subsistence strategies, eating a predominantly low-quality diet heavily reliant on carbohydrates (Dufour, 1992; Harner, 1972; Pohle & Reinhardt, 2004). It is therefore unsurprising given these shared energetic factors that testosterone profiles were not discernably different across MI levels (as measured by SOL factors), since market integration appears to shape testosterone levels through altering diet and activity patterns (Bribiescas, 2010; Ellison, 2009).

For example, MI is typically thought to improve individual energetic status (i.e., through reducing workloads, improving health, and providing access to calorically rich food items), providing excess resources that can be converted into increased reproductive effort and higher testosterone levels (Bribiescas, 2010; Travison et al., 2007). Additional work is therefore required to examine Shuar testosterone patterns is more urban settings, where subsistence strategies and daily activities are more similar to those of Western populations. Inclusion of participants from these market centers could potentially alter the results of these analyses, leading to significant differences between SOL factors and testosterone levels.

Number of Children and Testosterone Levels

In addition, the adiposity and SOL regression analyses both documented a significant inverse association between number of children and evening testosterone levels. This supports previous findings (Gettler et al., 2011; Gray et al., 2002, 2009; Kuzawa et al., 2009), and suggests that fatherhood shifts energetic investment from efforts to attract a mate toward parenting (Gettler et al., 2011). Higher testosterone levels are most strongly associated with behaviors useful in acquiring a mate, but which may impair a man's ability to effectively care for his children (i.e., through supporting competitive or mating behavior) (Gray et al., 2002). The idea that behavior is strongly linked with altered testosterone levels in fathers is further supported by the fact that this negative association was only evident for evening testosterone levels. Evening testosterone levels are heavily influenced by the experiences and interactions a man has throughout the day (Gray et al., 2002); thus, it seems likely that time spent with children and parenting behavior during the day substantially decreases evening testosterone values. Further work is needed to test how the amount of time spent with children directly affects Shuar men testosterone variation.

Limitations

The present study has several limitations. First, most participants were missing data on at least one measure of interest, requiring the use of multiple imputation to produce a complete dataset. While this method of missing data analysis is common practice (Van der Heijden et al., 2006), it does not negate the fact that the actual data may differ from the values generated. Further work is therefore needed to compile a more complete dataset for future analyses. Second, the sample was composed of many young (i.e., teenage men) with relatively few older men (especially over the age of 50). Future data collection should therefore focus on increasing the number of older men included in the sample. Likewise, additional research should work to increase variation in participant SOL within the sample. Most of the men in the present study continue to rely heavily on traditional subsistence strategies and did not live within market centers; future samples should be drawn from men living in cities to better examine testosterone patterns across the entire range of Shuar MI levels.

Moreover, these analyses would benefit from the inclusion of longitudinal data. The cross-sectional study design here makes it difficult to disentangle the complex relationships evident between testosterone profiles and several key variables, especially age and BMI. For example, it is unclear how individual BMI changes might directly alter testosterone levels. Longitudinal data – such as repeat measures on the same participants over a period of months or even years – would help clarify which variables most strongly shape testosterone level variation. A final limitation is that the present study relied on relatively crude measures of body composition (BMI, percent body fat, and waist circumference). Although these measures are regularly used in clinical and epidemiological studies (Frisancho, 1990), additional work is needed to obtain high resolution body composition data, such as isotope dilution or skinfold measures. Still, despite these limitations, this study presents a unique examination of key factors influencing testosterone variation and male reproductive health in a natural fertility, subsistence population; thereby documenting previously undescribed hormone profiles among an indigenous group.

Bridge to Chapter V

The purpose of Chapter IV was to assess individual differences in diurnal testosterone rhythms among Shuar men, paying particular attention to participant age, measures of adiposity, and SOL factors. These data are useful in clarifying how testosterone levels vary across the life course and in populations experience rapid lifestyle change. The findings presented here suggest that complex relationships exist between BMI and testosterone levels, which are moderated by age. In addition, it seems that behavior throughout the day (i.e., interactions with children) shapes testosterone patterns. These findings cumulatively suggest that testosterone levels play an important role in male energetics and behavior. Chapter V builds upon these findings by testing how variation in testosterone profile is associated with masculine trait development and reproductive success; thereby examining how the types of reproductive investment supported by high testosterone may pay off by enhancing male fitness.

CHAPTER V

THE MERIT OF HONEST SIGNALS: ASSOCIATIONS BETWEEN MASCULINE PHENOTYPES, REPRODUCTIVE SUCCESS, AND PARASITE LOAD AMONG SHUAR MEN

This chapter contains unpublished, co-authored material and was prepared with assistance from J. Josh Snodgrass, Lawrence S. Sugiyama, Melissa A. Liebert, Samuel S. Urlacher, Joshua M. Schrock, Dorsa Amir, Christopher J. Harrington, Tara J. Cepon-Robins, and Felicia C. Madimenos. The author of this dissertation is responsible for the study design, collection and analysis of stool samples, data analyses, and all write up for this paper. Sugiyama, Liebert, Urlacher, Schrock, Amir, Harrington, Cepon-Robins, and Madimenos aided in data collection in the field. Sugiyama is the field site director and provided editorial assistance on this manuscript. Snodgrass is the academic adviser for this dissertation, provided editorial assistance, and is the director of the Global Health Biomarker Laboratory where all samples were analyzed.

Introduction

The assessment and processing of cues from the physical environment are critical psychological components necessary for the generation of many adaptive behaviors and responses. From finding nutritious food to choosing a healthy mate, physical traits serve as indicators enabling individuals to discern the best option in a specific context. These evolved preferences for certain characteristics over others motivate organisms to effectively respond to stimuli, and have evolved because (on average) they increased the reproductive fitness of their bearers in the past (e.g., Sugiyama, 2005, 2015). In other words, natural selection is expected to have resulted in suites of assessment and preference mechanisms that are activated in response to various environmental cues (Buss, 1993; Sugiyama, 2004, 2015; Symons, 1979, 1995; Tooby & Cosmides, 1992). These physiological mechanisms are expected to shape mate selection, such that men and women are expected to be attracted to particular physical and behavioral traits that were closely linked with ancestral mate value (i.e., the probable value another individual has for the assessor as a mate) (Sugiyama 2005, 2015).

Because some features associated with male and female mate value differ, men’s and women’s preferences are also sometimes expected to differ (Buss, 1993; Symons, 1979). For example, male mate value includes the ability and willingness to invest in a woman and her offspring, as well as physical traits associated with genetic quality, health, and physical formidability (e.g., Buss, 1993; Symons, 1979; Sugiyama, 2005, 2015). A number of physical cues hypothesized to be associated with human male mate value, and thus preferred by women, are associated with masculine phenotypic features of the face, body, and voice, developed under the influence of testosterone (e.g., DeBruine et al., 2006; Zilioli et al., 2014, 2015; Hodges-Simeon et al., 2014; Puts et al., 2010; Sugiyama, 2005, 2015 for review). However, mate selection does not always favor the proliferation of traits associated with health, survival, and longevity.

Charles Darwin identified the processes by which such traits evolve. He defined sexual selection as the proliferation of a trait that increases an organism’s reproductive success, either by allowing an individual to out-compete members of their own sex for mating access, or because it is preferred by members of the opposite sex in mates (Darwin, 1859, 1871). These traits may act to reduce survival through requiring substantial energetic investment or otherwise increasing mortality, such as making the individual an easier target for predators (Darwin, 1859, 1871; Puts, 2010). Although these characteristics might impair survival, Darwin proposed that if they increased net reproductive success by increasing the number of mating opportunities, they could nevertheless evolve (Darwin, 1859, 1871).

There are two general forms of sexual selection: intra- and inter-sexual selection. Intra-sexual selection is selection for traits that enhance one's ability to gain sexual access to members of the opposite sex via competition with same-sex competitors (e.g., male elephant seals physically competing for exclusive access to females) (Cox & Le Boeuf, 1977; Darwin, 1871; Freeman & Heron, 2007; Futuyma, 2009). In contrast, inter-sexual selection refers to selection for traits that increase fitness because they are preferred by members of the opposite sex. As a result, the trait spreads throughout the population and may become exaggerated over time, even if it has no survival value (e.g., male peacock feathers) (Darwin, 1871; Fisher, 1915, 1930; Freeman & Heron, 2007; Futuyma, 2009).

Evidence for both intra- and inter-sexual selection is apparent in humans. For example, men have substantially greater muscle mass and strength than do women and are able to accurately assess the relative strength of other men using body, vocal, or facial features; an ability thought indicate intra-sexual selection for male-male fighting prowess and intra-sexual competitiveness (Lassek & Gaulin, 2009; Puts, 2010; Sell et al., 2009). Both men and women also exhibit strong preferences for certain physical cues in prospective mates, indicating inter-sexual selection (e.g., waist-hip-ratio, facial symmetry, clear skin) (Grammer & Thornhill, 1994; Marlowe & Wetsman, 2001; Singh, 1993; Sugiyama, 2005, 2015). Signals of individual competitiveness (e.g., physical strength) and genetic quality are therefore hypothesized to play an important role in human reproduction, both in deterring competitors and attracting potential mates.

It is therefore important to distinguish between signals and cues used in mate choice. As defined by biologists, signals are features or behaviors that have evolved as indicators of some underlying quality; in addition, the information conveyed by signals alters the behavior of perceivers (Bradbury & Vehrencamp, 1998; Smith, 2003). Cues, on the other hand, have not evolved to carry an explicit meaning or convey specific information on behalf of the organism possessing that trait (Bradbury & Vehrencamp, 1998; Smith & Harper, 2003).

However, the differentiation between signal and cue is not always obvious. In some cases, the fact that variation in phenotypic features is linked to underlying fitness-related qualities can be the basis for inter-sexual selection, because these traits provide a cue to the relative mate value of different individuals, even though these cues have not evolved as signals for that information per se (Sugiyama 2005, 2015). In humans, these may include phenotypic traits that were under selection for biomechanical or reproductive efficiency, such as female gluteofemoral fat reserves to support fetal brain growth (Lassek & Gaulin, 2008; Sugiyama, 2015). Others may be correlates of reproductive value, such as skin tone and coloration associated with age, health, or particular nutrients in the human diet (e.g., Coetzee et al., 2012; Fink et al., 2006, 2012; Whitehead et al., 2012; Sugiyama, 2015 for review).

In contrast, other traits (e.g., the peacock tail, red face of the uakari monkey, or elaborate mating dances of the sandhill crane) appear to be costly traits that impair survivorship – the evolution of which Darwin saw as evidence of inter-sexual selection (e.g., Cronin, 1991). Several theories attempt to explain how such exorbitant traits could evolve (e.g, Fisher 1915, 1930; Zahavi & Zahavi, 1997). Zahavi and Zahavi (1997) pointed out that nature is full of communicative signals, but selection for these useful indicators might lead the concurrent evolution of false signals, ultimately resulting in the decline of signal efficacy and production.

Therefore, an important consideration in the evolution of sexually selected traits that evolved as signals is that they should honestly reflect individual quality. There should consequently be a cost associated with the development and maintenance of these phenotypic characteristics such that they incur a fitness price (in terms of mortality) that is less for higher quality individuals than lower quality individuals (e.g., Gangestad & Scheyd, 2005; Grafen, 1990; Johnstone, 1995; Kokko & Brooks, 2003; Sugiyama, 2015). In other words, these physical signals are “honest” because it is not in the interest of lower quality individuals to “cheat” and invest in a larger signal, as the resulting fitness related costs they would suffer would be greater than any fitness benefits gained (Gangestad & Scheyd, 2005; Grafen, 1990; Johnstone, 1995; Kokko & Brooks, 2003). Thus, the utility of these costly signals remains intact, they continue to convey important information to other individuals, and they are maintained by selection (as are the physiological factors that regulate their development and expression).

For instance, among males, it is thought that the androgen hormone testosterone is crucial for the development of costly phenotypic features (e.g., increased musculature) useful in acquiring a mate (Bribiescas 2001, 2009). As discussed in Chapter II of this dissertation, the development of these testosterone-linked traits may come at the expense of energetic investment in immune function. This has led to the hypothesis that they are honest signals of male quality, as only healthy males should be able to invest sufficiently in the maintenance of these costly traits (Grafen, 1990; Johnstone, 1995; Zahavi &

Zahavi, 1997). Testosterone is therefore thought to mediate life history trade-offs between reproductive effort and immunity in males of many species, including humans.

Females are therefore expected to select mates based on the production of costly and conspicuous testosterone-linked secondary sexual characteristics, because they reflect male ability to convert ecological resources into somatic characteristics without interruption from immune-related insults (Grossman, 1985; Klein, 2000, 2004). A large research literature is based on the idea that testosterone is related to development of masculine phenotypic features but compromises immune function (e.g., Folstad & Karter, 1992), and there is some evidence supporting the idea that females can reliably use these cues to assess male quality. For example, in many species testosterone levels appear to decrease in response to parasitic infection, and females appear to be highly sensitive to subtle variations in male secondary sexual characteristics and testosterone profile (Dunlap & Schall, 1995; Klein, 2000; Sheldon & Vehulst, 1996), indicating these cues may be important during mate selection.

In humans, masculine phenotypic features including size, strength, and facial masculinity are consequently thought to provide costly signals of immunocompetence that females use in mate choice, thus influencing male reproductive success (e.g., Buss & Schmitt, 1993; DeBruine et al., 2006; Folstad & Karter, 1992; Gangestad & Buss, 1993; Gangestad et al., 2010; Jones et al., 2012; Thornhill & Gangestad, 2003). In addition to muscle mass, testosterone has been linked development and/or maintenance with a variety of other phenotypic traits, often used to measure degree of individual masculinity (Gallup & Frederick, 2010; Sell et al., 2009). These include height, grip strength (a proxy of overall physical strength), chest compression (a measure of upper body strength), upper arm circumference (associated with amount of musculature), and 2D:4D digit ratio (ratio of the second finger digit to the fourth finger digit; an indicator of prenatal testosterone exposure) (Gallup & Frederick, 2010; Sell et al., 2009). These traits have been linked with testosterone levels, and are thought to reflect a male's ability to withstand health insults (including parasitic infection) (Frederick & Haselton, 2007; Gallup & Frederick, 2010; Gangestad & Buss, 1993; Hamilton & Zuk, 1982; Sugiyama 2005, 2015 for review).

Yet, as Sugiyama (2015) notes, recent research suggests that additional work is needed to determine whether masculine traits did indeed evolve as inter-sexually selected signals of male mate value, hence enhancing male attractiveness (e.g., Boothroyd et al., 2013; Puts, 2010, 2016; Scott et al., 2014). First, there are direct intra-sexual competitive benefits to testosterone-linked traits, suggesting intra-sexual selection may be the primary driver in shaping these traits (e.g., Puts 2010, 2016). Second, because high testosterone levels are linked with behavioral patterns such as probability of engaging in extra-pair copulation, mate desertion, and aggression (e.g., Booth et al., 1989; Gladue et al., 1989; Schmitt, 2015), the relative costs and benefits of partnering with high testosterone men will vary for women under different conditions (e.g., comparative value of long- vs. short-term mating, need for paternal investment, risks of violence by mate and other males, relative costs of desertion, and female reproductive status among others) (e.g., Schmidt, 2015; Sugiyama, 2015).

So, the hypothesized relationship between testosterone and male reproductive success could be primarily the result of greater mating success due to male-male competition, female choice or both. In other words, if testosterone-linked traits increase male mating opportunities – either through enhancing male competitiveness (with other males) or attractiveness (to females) – then males possessing these features should produce more offspring, thereby increasing their reproductive success.

Although a large research literature has examined women's mate preferences for masculine phenotypic traits, few human studies have tested the hypothesized relationships between masculine traits, health, and reproductive success in a natural fertility, subsistence-based (i.e., energy limited) population with a high infectious disease burden, conditions most relevant to those under which hypothesized testosterone-mediated costly signals evolved (Sugiyama, 2015; Thornhill & Gangestad, 2006). In particular, given a long period of coexistence, the rapid evolution of pathogens, and the long-term health effects resulting from infection, parasitic disease is hypothesized to have played an important role in human evolutionary processes, including sexual selection favoring traits indicative of parasite resistance. Still, the relationship between testosterone and immune function with regards to human parasitic infection remains poorly studied.

The present study directly tests whether testosterone linked phenotypic traits are associated with parasite infection status and age-related reproductive success among Shuar men of Amazonian Ecuador, a natural fertility, subsistence population experiencing high infectious/parasitic disease burdens. The following objectives and hypotheses are addressed:

- ***Objective One*** **- To investigate how masculine physical traits are related to parasite infection status and load.** If masculine traits are honest signals of immune function (Grafen, 1990; Johnstone, 1995; Zahavi & Zahavi, 1997), then we predict that there will be a negative association between these traits and parasite load.

  - **Hypothesis 1a: Individual masculine traits (height, grip strength, chest compression, upper arm circumference, 2D:4D digit ratio) will be negatively associated with parasite infection status and load.**
  - **Hypothesis 1b: A composite z-score of these five traits will be negatively associated with parasite infection status and load.**
- ***Objective Two*** **- To determine whether masculine characteristics are related to male reproductive success.**
  - **Hypothesis 2: Age-specific reproduction (e.g., number of living children) will be positively related to traits associated with high testosterone.** We hypothesize that traits associated with higher testosterone will increase mating opportunities (Buss & Schmitt, 1993; Gangestad et al., 2010; Thornhill & Gangestad, 2003), thus increasing male reproductive success.
- ***Objective Three*** **- To assess how parasite infection status and load are related to male reproductive success.**
  - **Hypothesis 2: Parasite infection status and load will be negatively related to age-specific reproduction.** We hypothesize that males who are resistant to parasitic infection invest more in long term reproductive effort (Grossman, 1985; Klein, 2000, 2004), and therefore have greater reproductive success.

Materials and Methods

Participants

This study was conducted as part of the Shuar Health and Life History Project (SHLHP). The Shuar are natural fertility indigenous population of ~100,000 people distributed in more than 400 communities in the neotropical eastern foothills of the Andes and the Amazonian lowlands of southeastern Ecuador in the provinces of Pastaza, Morona-Santiago, and Zamora-Chinchipe (Bennett et al., 2002; Rubenstein, 2001). Traditionally, Shuar lived in scattered households reliant on horticulture, fishing, hunting, and foraging (Harner, 1972; Karsten, 1935; Rubenstein, 2001). Today most Shuar continue to rely on traditional cultigens, supplemented to various degrees by hunting and fishing, and mixed agro-pastoral production (Liebert et al., 2013). Parasitic infection is common among Shuar, with approximately 65% of participants infected with at least one species of soil-transmitted helminth (parasitic worms) (Cepon-Robins et al., 2014; Gildner et al., 2016a).

Study Design and Sampling

This study employed a cross-sectional approach, with data collected over five field seasons (2012-14, 2016-17). Random sampling was not feasible across this large population, dispersed over relatively inaccessible areas, with sometimes divisive internal politics. Thus, through consultation with knowledgeable Shuar friends and political leaders, and the Dirigente de Salud of the Federación Interprovincial de Centros Shuar, a sample of communities were chosen based on their size, location, and agreement to collaborate on this research. Once communities agreed at an open community meeting that the SHLHP could conduct research in their community, all men were invited to participate in this study. The sample included 48 male volunteers from seven communities, aged 14 years - 66 years.

Informed consent was obtained from adult participants and parental verbal consent and child assent were obtained for individuals under 14 (the local age of consent). The University of Oregon Institutional Review Board approved this study, and the Federación Interprovincial de Centros Shuar and local leaders authorized research in participant communities.

Field and Laboratory Procedures

Stool collection and analysis

Fresh stool samples were collected using previously described methods (Cepon-Robins et al., 2014). A single Kato-Katz smear was prepared from each participant's fecal sample (by TEG or TJC) within an hour of sample collection (Katz et al., 1972); after 30-45 minutes, the smears were examined using 10x and 40x microscopy by a trained observer (either TEG or TJC) and species-specific eggs per gram (EPG; indicative of individual parasite load) of feces recorded. *Ascaris lumbricoides* and *Trichuris trichiura* infections were detected: 64.6% of participants were infected with at least one of these helminths. The present study therefore focuses on these two species.

Anthropometric measurements

A variety of phenotypic traits linked with testosterone levels in humans were collected to measure degree of individual masculinity. These included participant height, grip strength (a proxy of overall strength), chest compression (an indicator of upper body strength), upper arm circumference (associated with amount of musculature), and 2D:4D digit ratio (an indicator of prenatal testosterone exposure, with lower values representing higher rates of androgen exposure) (Gallup & Frederick, 2010; Sell et al., 2009).

These anthropometric measures were obtained using standard anthropometric measurement techniques (e.g., Lohman et al., 1988). Height was measured using a stadiometer (Seca Corporation 214, Hanover, MD). Grip strength was measured using a Jamar hand dynamometer (Bolingbrook, IL), with participants seated and their forearm resting on a surface at a right angle to their upper arm, close to their body, and then squeezing the device as hard as they could. This was repeated three times alternating between hands. The highest compression strength on each attempt was recorded to the nearest 0.25 kilogram. The Jamar hand dynamometer was then used to measure chest compression strength by having participants grip the two ends of the device in front of their chest with arms close to their body and press their hands towards each other as hard as possible. This process was repeated three times with maximum compression recorded for each attempt.

Measures of individual maximum grip strength (highest single compression obtained from all measures collected on both hands) and chest compression (highest single compression obtained from all measures collected) were then calculated.

Next, to control for the effects of body size on the maximum grip and chest compression, weight-standardized z-scores for grip strength were calculated and these values were subsequently included in the analyses. Flexed upper arm circumference was measured using a tape measure. In addition, the digit length of the second and fourth fingers were measured using electronic sliding calipers; these values were recorded to the nearest 0.01 millimeter and used to calculate 2D:4D ratio.

Finally, composite z-scores were calculated to facilitate the comparison of masculine phenotype between individuals. Prior to this calculation, participant 2D:4D ratio was reverse coded by subtracting each ratio value from the highest measure obtained in the sample, so that lower ratio values (considered more masculine) were given higher values. This was to ensure during composite variable creation all of the masculine measures were in the same direction (i.e., that higher values of each measure represented a more "masculine" phenotype). Z-scores for each anthropometric measure were computed and these five z-scores (height, maximum grip strength, maximum chest compression, arm circumference, and reverse 2D:4D ratio) were then summed for each participant, resulting in a final composite z-score.

Preliminary analysis of this dataset indicates that the composite masculine trait variable ($\rho = 0.347$, $p < 0.05$) and arm circumference ($\rho = 0.288$, $p < 0.05$) were positively correlated with individual average morning salivary testosterone levels. Similarly, the composite masculine trait variable ($\rho = 0.384$, $p < 0.01$), arm circumference ($\rho = 0.334$, $p < 0.05$), and height ($\rho = 0.349$, $p < 0.05$) were positively correlated with individual average salivary evening testosterone levels. This supports the idea that a significant relationship exists between salivary testosterone levels and traits indicative of testosterone levels present during development, suggesting these phenotypic traits are significantly correlated with testosterone levels as predicted and are therefore capturing aspects of male reproductive investment that may influence individual fitness.

Reproductive fitness

Participants completed a reproductive history questionnaire, reporting the number of children they have; this information was cross-checked by informants and existing SHLHP genealogical data. To determine male reproductive success while accounting for participant age, age-standardized z-scores for number of living children were calculated and these values were subsequently included in the analyses. Individual ages were determined by birthdates on government issued identification cards, and verified with informants and SHLHP genealogical data, as has been previously described elsewhere (Blackwell et al., 2011; Liebert et al., 2013).

Confounders

Past research shows that lifestyle factors are associated with parasite load among the Shuar (Cepon-Robbins et al., 2015; Gildner et al., 2016a). Participants completed a structured interview based on a Material Style of Life (SOL) Index developed by SHLHP for use with Shuar after extensive qualitative research and pre-testing (e.g., Liebert et al. 2013), modeled on examples used in other populations (Bindon et al., 1997; Leonard et al., 2002). This index provides a nuanced examination of individual market-integration level (defined as the degree to which an individual produces for and consumes from the market economy) (Liebert et al., 2013; Urlacher et al., 2016). The SOL index includes a list of items associated with degree of participation in the market-based or subsistence economy divided into three scales: Traditional Style of Life (T-SOL), Market-Integrated Style of Life (M-SOL), and Housing Style of Life (H-SOL).

T-SOL and M-SOL are calculated as a weighted sum of the total number of traditional (e.g., blowgun, hunting dog, fishing nets) or market-integrated (e.g., radio, car, television) index items a household owns. H-SOL is calculated as a weighted composite score based on different aspects of household type, size, access to water source, etc. (see Table 4.1). This approach is useful for capturing lifestyle variation with a few manageable variables (Liebert et al., 2013). These SOL measures were included in all models to account for the influence of lifestyle on parasite infection risk and male reproduction (i.e., number of children).

In addition, participants reported other factors known to influence parasite load. Specifically, participants were asked if they had taken medicine to treat parasitic infection in the past month. Participants age has been shown to influence infection risk (Dold & Holland, 2011; Kealey, 2010), and was therefore included in models examining parasite infection risk. Furthermore, individual BMI (kg/m^2) has also been shown to influence immune function, including the production of antibodies associated with parasitic infection (Blackwell et al., 2010). Participant BMI was therefore calculated and included in these models. Finally, variables thought to potentially influence male reproduction were included in this set of analyses. In addition to SOL factors, participant monthly income and education level (i.e., number of years in school) were included in these models to account for the influence of socio-economic factors on male reproductive success (Kruger, 2008).

Statistical Analyses

All analyses were conducted using SPSS version 25.0 (SPSS Inc. Chicago, Illinois). Results were considered significant at $p < 0.05$. Shapiro-Wilk tests were used to test for normality in all variables. As has been documented elsewhere, EPG variables were $\log_{10}$-transformed due to non-normal distributions (Cepon-Robins et al., 2014), this resulted in normally distributed variables with skew between ± 1. These transformed variables were subsequently used during analysis. In addition, given the negative skew and physical nature of the height measurement, a power transformation (of five) was used to normalize participant height. All other variables were normally distributed.

All variables were also examined for outliers (defined as $\underline{+}3$ SD above or below the mean). Two outliers were identified in participant 2D:4D ratios. However, preliminary analyses revealed non-significant differences in the results with outliers excluded. Thus, all 2D:4D measured were included in subsequent analyses to better account for naturally-occurring variation in this measurement. No other extreme outliers were detected and parametric tests of the transformed data were conducted to test the hypotheses.

A large proportion of individuals failed to report education (41.7%) or income level (35.4%). However, sensitivity analyses indicated that these variables were missing at random. Further, participant income and education level did not contribute significantly to any of the models and were therefore dropped from analysis. Preliminary analyses also indicated multicollinearity between participant age and BMI. An unstandardized residual was therefore calculated for BMI, removing the contribution of age to BMI. This residual BMI variable was subsequently included during analysis.

Additional diagnostic analyses revealed acceptable degrees of model linearity and heteroscedasticity.

Preliminary statistics

The prevalence values of participants infected with either *A. lumbricoides* or *T. trichiura* were calculated. Average values for all other variables of interest were also calculated. In addition, Pearson correlations were calculated to explore the relationship between the primary variables of interest.

Examination of the association between male physical traits and parasite infection

A logistic regression examined if male physical traits (a hypothesized signal of male health) were significantly associated with parasite infection status (a dichotomous variable; not infected vs. infected with at least one parasitic species). Moreover, a linear regression tested whether male physical traits were negatively associated with parasite load (either *A. lumbricoides* or *T. trichiura* EPG values). In each of these models, age and BMI were entered in the first step, medicine use (did not use vs. used) was entered in the second step, the SOL scores were entered in the third step, and the anthropometric masculine trait measures were entered simultaneously in the final step. An additional set of regressions was run, identical to the first but entering the composite masculine trait z-score in the final step in place of the individual masculine traits. In all models, participant medicine use did not contribute significantly to the regression models (i.e., did not improve to model fit) and was therefore dropped from analysis.

Examination of the association between male physical traits and age-specific reproduction

A set of linear regressions were run to determine whether male masculine traits significantly contributed to variation in age-specific reproduction. Income and education level were entered in the first step, the SOL factors were entered in the second step, and the anthropometric measures were entered simultaneously in the final step. A second regression was run, identical to the first but entering the composite masculine trait z-score in the final step in place of the individual masculine traits. In all models, participant income and education level did not contribute significantly to the regression models and were therefore dropped from analysis.

Examination of the association between parasite infection and age-specific reproduction

A final set of linear regressions were conducted to assess whether parasite infection status or parasite load significantly contributed to variation in age-specific reproduction. Income and education level were entered in the first step, the SOL factors were entered in the second step, and parasite infection status in the final step. An additional set of regressions was run, identical to the first but entering either *A. lumbricoides* or *T. trichiura* EPG values were entered in the final step. In all models, participant income and education level did not contribute significantly to the regression models and were therefore dropped from analysis.

Results

Descriptive Statistics

Table 5.1 presents frequencies and average values of all variables included in the regression models. Overall, 64.6% of participants were infected with at least one parasite species, 45.8% *A. lumbricoides* and 37.5% with *T. trichiura*. Mean untransformed EPG values were 3,334.47 and 64.34 eggs per gram for *A. lumbricoides* and *T. trichiura*, respectively. In addition, relatively high rates of fertility were observed; the mean number of living children was 4.4 for the sample.

	Mean (S.D.)
Number of living children	4.404 (0.693)
Age (years)	36.850 (2.305)
Body mass index (kg/m^2)	24.511 (0.427)
T-SOL score	0.355 (0.032)
M-SOL score	0.207 (0.020)
H-SOL score	10.936 (0.409)
Height (cm)	157.726 (1.000)
Grip strength (kg)	36.542 (1.158)
Chest compression (kg)	33.490 (1.326)
Flexed upper arm circumference (cm)	30.405 (0.469)
2D:4D digit ratio	0.925 (0.007)
Masculine trait composite z-score	-0.525 (0.389)
***Ascaris lumbricoides* EPG value**	3334.47 (1009.788)
***Trichuris trichiura* EPG value**	64.34 (24.905)
	Frequency (%)
Parasite infection status	31 (64.6%)

Table 5.1. Description of the study population (n = 48) using untransformed variables.

Pearson Correlations

Table 5.2 presents the correlations between the key variables. *Ascaris lumbricoides* EPG ($\log_{10}$-transformed) was positively correlated with the composite masculine trait score (r = 0.328, $p < 0.05$), maximum grip strength (r = 0.329, $p < 0.05$), and chest compression (r = 0.368, $p < 0.01$). *Trichuris trichiura* EPG values ($\log_{10}$-transformed) and infection status, meanwhile, were not significantly correlated to any of the masculine trait measures. Age-specific reproduction was not significantly correlated with any of the masculine traits or parasitic infection variables.

Male Physical Traits and Parasite Infection

Logistic regression analysis indicated that only age significantly contributed to the model predicting infection status (Table 5.3). Specifically, older men were significantly less likely to report infection (Exp(B) = 0.945, $p = 0.018$). None of the masculine trait variables significantly contributed to the infection status models (Table 5.3).

Conversely, masculine traits did significantly contribute to the model predicting *A. lumbricoides* EPG values. A positive relationship was observed, such that higher composite masculine z-scores were associated with higher *A. lumbricoides* loads (B = 0.681, $p = 0.009$) (Table 5.4). However, none of the individual masculine traits significantly contributed to variation in *A. lumbricoides* EPG values. Similarly, no significant relationships were observed between the masculine traits (individual or composite) and *T. trichiura* EPG values (Table 5.4).

	Ascaris EPG value	Trichuris EPG value	Infection Status	Number of Children	Composite Trait Score	2D:4D Ratio	Height	Arm Circum.	Max Grip Strength	Max Chest Compression
Ascaris EPG value	1	0.273	**0.678*****	0.147	**0.328***	0.074	0.252	0.022	**0.329***	**0.368***
Trichuris EPG value	0.273	1	**0.572*****	-0.090	0.107	0.007	0.091	-0.155	0.149	0.195
Infection Status	**0.678*****	**0.572*****	1	0.153	0.146	-0.058	0.084	-0.091	0.170	0.202
Number of Children	0.147	-0.090	0.153	1	0.070	-0.122	-0.081	-0.015	0.062	0.133
Composite Trait Score	**0.328***	0.107	0.146	0.070	1	0.082	**0.678*****	**0.626*****	**0.723*****	**0.695*****
2D:4D Ratio	0.074	0.007	-0.058	-0.122	0.082	1	0.182	0.228	0.184	0.283
Height	0.252	0.091	0.084	-0.081	**0.678*****	0.182	1	**0.477****	**0.318***	0.177
Arm Circum.	0.022	-0.155	-0.091	-0.015	**0.626*****	0.228	**0.477****	1	0.081	0.208
Max Grip Strength	**0.329***	0.149	0.170	0.062	**0.723*****	0.184	**0.318***	0.081	1	**0.672*****
Max Chest Compression	**0.368***	0.195	0.202	0.133	**0.695*****	0.283	0.177	0.208	**0.672*****	1

Table 5.2. Pearson correlations between parasite infection variables, age-specific number of children, composite masculine trait z-score, and individual masculine trait measures. Correlations are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

	Infection status (individual masculine traits)	**Infection status (composite masculine score)**
Age	0.957 (0.904-1.013)	**0.945 (0.901-0.990)***
BMI	1.096 (0.704-1.623)	0.934 (0.697-1.252)
H-SOL	1.085 (0.819-1.439)	1.109 (0.842-1.461)
M-SOL	0.298 (0.001-92.711)	0.613 (0.002-173.124)
T-SOL	1.698 (0.042-6.044)	0.652 (0.024-17.587)
Grip strength	0.577 (0.168-1.975)	---
Chest compression	2.637 (0.723-9.625)	---
Arm circumference	0.851 (0.575-1.260)	---
Height	1.000 (1.000-1.000)	---
2D:4D ratio	0.005 (0.000-79157.356)	---
Masculine trait z-score	---	1.186 (0.882-1.595)
Constant	7809.320	7.877

Table 5.3. Logistic regression for prediction of infection status from measures of masculine traits. OR values with 95% CIs. Infection is coded so that 1 = infected, 0 = not infected. Comparisons are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

	***A. lumbricoides* EPG value (individual masculine traits)**	***A. lumbricoides* EPG value (composite masculine score)**	***T. trichiura* EPG value (individual masculine traits)**	***T. trichiura* EPG value (composite masculine score)**
Constant	3.486 (15.661)	2.492 (1.616)	6.104 (12.241)	-0.042 (2.659)
Age	-0.046 (0.051)	-0.075 (0.040)	-0.040 (0.040)	**-0.077 (0.032)***
BMI	0.088 (0.367)	-0.198 (0.272)	0.251 (0.287)	-0.133 (0.220)
H-SOL	0.053 (0.253)	0.109 (0.242)	-0.118 (0.198)	-0.052 (0.195)
M-SOL	-2.128 (5.377)	-2.091 (5.132)	6.224 (4.203)	6.737 (4.139)
T-SOL	-2.042 (3.522)	-3.151 (3.060)	1.404 (2.753)	-0.491 (2.467)
Grip strength	-0.085 (1.065)	---	-1.064 (0.832)	---
Chest compression	1.585 (1.100)	---	1.509 (0.859)	---
Arm circumference	-0.191 (0.353)	---	-0.488 (0.276)	---
Height	6.494E-11 (0.000)	---	4.678E-11 (0.000)	---
2D:4D ratio	-2.907 (15.018)	---	2.870 (11.738)	---
Masculine trait z-score	---	**0.681 (0.248)****	---	0.169 (0.200)

Table 5.4. Linear regressions for the prediction of *Ascaris lumbricoides* and *Trichuris trichiura* from individual and composite masculine trait measures. B coefficients with S.E. Comparisons are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Masculine Traits and Age-Specific Reproduction

Supporting the findings from the correlation analyses, no significant relationships were observed between age-specific reproduction and male physical traits, for either the regression model including composite trait z-score or the model including the individual masculine traits (Table 5.5). However, in both models the style of life variables significantly contributed variation in male reproduction (Table 5.5). The housing SOL variable – measuring aspects of household construction – was positively related to number of children in both the composite trait z-score model (B = 0.151, *p* = 0.025) and the individual traits model (B = 0.142, *p* = 0.036). Likewise, the traditional SOL variable – representing ownership of traditional goods – was positively related to number of children in the both the composite trait z-score model (B = 1.990, *p* = 0.016) and the individual traits model (B = 2.337, *p* = 0.007).

	Age-specific number of children (individual masculine traits)	**Age-specific number of children (composite masculine score)**
Constant	3.511 (4.142)	**-1.901 (0.732)***
H-SOL	**0.142 (0.066)***	**0.151 (0.065)***
M-SOL	-2.264 (1.471)	-2.230 (1.455)
T-SOL	**2.337 (0.826)****	**1.990 (0.802)***
Grip strength	0.023 (0.226)	---
Chest compression	0.261 (0.226)	---
Arm circumference	7.753E-5 (0.058)	---
Height	-9.418E-12 (0.000)	---
2D:4D ratio	-4.844 (4.276)	---
Masculine trait z-score	---	0.040 (0.064)

Table 5.5. Linear regressions for the prediction of age-specific number of children from individual and composite masculine trait measures. B coefficients with S.E. Comparisons are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Parasite Infection and Age-Specific Reproduction

No significant relationships were observed between age-specific reproduction and parasite infection. Specifically, neither infection status, *A. lumbricoides* EPG values, or *T. trichiura* EPG values significantly contributed to variation in male reproduction (Table 5.6). However, once again, in all models housing SOL and traditional SOL exhibited a significantly contributed to variation in male reproduction (Table 5.6). The housing SOL variable was positively related to number of children in the infection model (B = 0.106, $p = 0.012$), *A. lumbricoides* EPG model (B = 0.108, $p = 0.010$), and the *T. trichiura* EPG model (B = 0.113, $p = 0.008$) Similarly, the traditional SOL variable exhibited a positive relationship to number of children in the infection model (B = 1.617, $p = 0.006$), *A. lumbricoides* EPG model (B = 1.656, $p = 0.005$) and the *T. trichiura* EPG model (B = 1.748, $p = 0.004$).

	Age-specific number of children (infection status)	**Age-specific number of children (*A. lumbricoides* EPG value)**	**Age-specific number of children (*T. trichiura* EPG value)**
Constant	**-1.577 (0.528)****	**-1.414 (0.523)****	**-1.613 (0.545)****
H-SOL	**0.106 (0.041)***	**0.108 (0.041)***	**0.113 (0.041)****
M-SOL	-1.403 (0.851)	-1.448 (0.849)	-1.450 (-0.853)
T-SOL	**1.617 (0.578)****	**1.656 (0.575)****	**1.748 (0.583)****
Parasite variable	0.268 (0.254)	0.032 (0.029)	-0.032 (0.038)

Table 5.6. Linear regressions for the prediction of age-specific number of children from three measures of parasitic disease (infection status, *Ascaris lumbricoides* EPG value, and *Trichuris trichiura* EPG value). B coefficients with S.E. Comparisons are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Discussion

This study provides a unique examination of associations between male physical traits thought to be important in male reproductive success and parasitic infection risk in a natural-fertility population experiencing a high infectious disease burden. Surprisingly, findings from the present study did not support any of the hypotheses.

Male Trade-Offs between Reproductive Effort and Immune Function

Contrary to Hypotheses 1a and 1b, a significant negative association was not observed between male physical traits and parasite infection. In fact, a significantly *positive* relationship was evident between the composite physical trait score and *A. lumbricoides* EPG values. This finding suggests that men with more developed masculine traits are not honestly signaling health to prospective mates, but rather face increased infection risk through favoring energetic investment in reproductive effort (as indicated through the development of these specific traits) over investment in immune function. While these relationships are not well examined in humans, this finding is consistent with results from previous research among non-human primates, including chimpanzees (Muehlenbein & Watts, 2010), and suggests the existence of a trade-off between male reproduction and immune function. It is likely this tradeoff between reproductive effort and immune function evolved because the reproductive benefits accrued outweighed any immune costs; however, it is unclear from the results of the present study what these reproductive benefits might be.

Men with these more defined masculine traits also do not have significantly more children compared to their age cohort. However, reproductive data are based on self and other reports. Further, these findings are based on a small sample that included a handful of young men not yet married or fathers. Still, young men represented a relatively small part of the sample (13 unmarried men and 14 without children), and were accounted for during analyses by using a reproduction variable standardized for participant age. In addition, the inclusion of young men in this sample was warranted as Shuar men in their late teens may marry and have children. Although this is not common, at least two of the men in the sample became fathers while still in their teens. Overall, Shuar men also tend to have children at younger ages than men living in higher-income populations; preliminary data analysis using the SHLHP database indicates that the average age at which a Shuar man becomes a father is 23 years old.

Therefore, while these results should be interpreted with significant caution, it appears an increased investment in reproductive effort in this sample does not enhance reproductive success as has been hypothesized (DeBruine et al., 2006; Gangestad & Buss, 1993; Jones et al., 2012). It is instead possible that other reproductive benefits are conferred. For example, it is possible that men with more exaggerated masculine traits are able to attract higher quality partners. Therefore, even if these men are not producing significantly more children compared to their competitors, they might still have higher long-term reproductive success through producing higher quality offspring that outcompete the children of other men for resources and high-quality mates. However, these benefits would only be evident after many years (or generations) of study.

Masculine Traits and Family Provisioning

It is also possible that the trade-off between male reproductive effort and immune function observed in the present study may benefit men through enhancing their ability to provide for their families. Although men with more developed masculine traits did not exhibit significantly higher numbers of children, these physical characteristics might allow these men to acquire more resources to support their families, thereby increasing their own reproductive success by ensuring the health and eventual reproduction of their children. For example, the masculine traits measured in this study are typically linked with individual strength (Gallup & Frederick, 2010; Sell et al., 2009), and should reflect an individual's ability to extract resources from the environment (Apicella, 2014; Gurven et al., 2009; Lancaster & Kaplan, 2009; Murdock & Provost, 1973). The ability to acquire resources is expected to be especially important in traditional subsistence populations (Alvarado et al., 2015; Apicella, 2014; Gurven et al., 2009; Lancaster & Kaplan, 2009; Murdock & Provost, 1973).

Male strength in particular is thought to be advantageous in foraging populations (e.g., the Hadza, Ache, and Tsimane), as men in these groups typically specialize in hunting, a difficult and risky activity that requires strength and endurance (Apicella, 2014; Gurven et al., 2009; Lancaster & Kaplan, 2009; Murdock & Provost, 1973). In these settings male work typically involves an increased reliance on physical strength, particularly episodic bursts of upper-body strength and force (Alvarado et al., 2015; Murdock & Provost, 1973; Wood & Eagly, 2002). Furthermore, while male parenting effort varies across societies (Gray & Anderson, 2010), evidence demonstrates that men generally increase their workload according to the number and age of their dependents (i.e., all household members reliant on this provisioning, not only the man's children) (Alvarado et al., 2015; Hooper, 2011; Lee et al., 2007; Wood & Marlowe, 2013).

This suggests that, within an evolutionary context, men must maintain physical strength and musculature to support their provisioning efforts. There is some evidence supporting the idea that male physical traits are more closely related to provisioning (Alvarado et al., 2015), and this could explain the findings in the present study. It is possible that the physical characteristics measured might be more strongly associated with activity and labor patterns than reproduction (as measured through number of living children). This idea could also help explain changes in testosterone levels over a man's reproductive lifespan.

While inter-individual comparisons suggest that higher testosterone levels are associated with more developed masculine traits, intra-individual analyses indicate that testosterone levels decrease during pair-bonding and fatherhood (e.g., among American, Filipino, and Hadza men) (Gettler et al., 2011; Gray et al., 2002; Kuzawa et al., 2009; Muller et al., 2009). However, reduced testosterone concentrations have not been documented among men who provide little direct paternal care (e.g., the Datoga) (Muller et al., 2009). Overall, this variation is thought to represent a physiological shift from mate-seeking behavior toward investment in a current partner and shared offspring in men who directly care for the children (Gettler et al., 2011; Gray et al., 2002; Kuzawa et al., 2009; Muller et al., 2009). Still, despite changes in testosterone levels, a father might maintain strength-related masculine traits through physical labor to care for his family.

The maintenance of masculine traits during fatherhood again suggests that men may be focusing on the quality of current offspring (i.e., through increased paternal investment and provisioning), rather than maximizing the quantity of offspring they produce. Past work among the Shuar does suggest that trade-offs exist between quantity and quality of offspring (Blackwell et al., 2009). In particular, trade-offs between offspring quantity and quality have been documented, but these patterns vary by region – likely due to differences in child growth costs and age-related value of juvenile male and female labor (Blackwell et al., 2009). It is therefore possible that in some circumstances Shuar men focus on investing in their existing family rather than in reproductive efforts to produce more children. Still, the amount of provisioning effort required and the number of children desired likely varies across different market integration levels.

Male Reproduction and Style of Life Factors

While masculine characteristics and parasitic infection were not significantly associated with age-specific number of children as hypothesized, SOL factors contributed to male reproduction in all models. Both the housing and traditional SOL variables were positively related to number of children, while the market integrated SOL variable was unrelated to age related reproduction. Ownership of more traditional subsistence items (indicative of a less market integrated household) is also associated with larger family sizes, and may reflect greater investment in hunting, fishing, and foraging the main traditional ways to augment an otherwise high carbohydrate diet. In these settings, more children can be desirable to assist with daily subsistence activities and chores (Bremner et al., 2009; Carr et al., 2006).

Similarly, high housing SOL scores (indicative of higher MI levels) are positively associated with male reproduction. In other groups high-calorie diets and reduced activity levels are associated with more market integrated settings (as indicated by higher H-SOL scores), and increase fertility rates among women (Ellison, 2009; Jasienska, 2000, 2001; Jasienska & Ellison, 2004; Panter-Brick et al., 1993), thereby also increasing the number of children men have. It is possible this is the case with the Shuar as well. It is also possible that number of children might influence household construction. For example, it is possible that men with many children invest in the construction of larger or additional houses to accommodate their bigger families. Finally, although rare, there are instances of polygyny in which a husband constructs a house for each of his wives and their children, thus contributing to this positive association between housing SOL and number of children. Longitudinal analyses are required to further explore these ideas about the relationships among SOL, diet, and reproduction among Shuar men.

Interestingly, the variable measuring the ownership of market items did not significantly contribute to any of the models. This is likely due to the uneven nature of MI. Although MI is occurring rapidly among the Shuar, this process is irregular across communities (Liebert et al., 2013; Urlacher et al., 2016). It is therefore possible that wealthier households in more isolated communities might own more market produced items (i.e., have higher market SOL scores), but lack access to family planning resources (resulting in higher rates of reproduction). This idea is supported by the fact that very little birth control use has been observed in any of the study communities until very recently, and access to these services remains limited. Furthermore, it is worth noting that factors linked with MI could be having unexpected effects on male reproduction, which might in part explain the lack of association between masculine traits and age-specific number of children in the present study.

For example, men at higher levels of MI in some households may now have fewer children due to prolonged absences due to travel for wage labor. This then may decouple reproduction from male physical traits, as cultural changes associated with MI may decrease the number of children desired by Shuar men and provide methods for controlling family size. Although not yet tested, the data do indicate that Shuar adults (both men and women) report that reducing family size is an important factor in achieving a "good life" (Liebert, unpublished data[1]). Future analyses should therefore test for how specific factors linked with MI (i.e., greater travel and medical care access) might influence patterns of male reproduction among the Shuar.

Limitations

The present study has several limitations. First, the small sample size limits the generalizability of these findings. Given the low power of these analyses ($1\text{-}\beta = 0.545$), these results should be interpreted with caution. Still, these same results were evident when non-parametric Spearman rank correlations were performed to account for the small sample. Second, data on male reproduction is based on self and other reports. Although significant attempt was made to cross-check reports with other informants, unless reported by participants, children sired with women not included in the larger SHLHP database would not be accounted for here. Given the large size of the Shuar population, this is certainly possible. Further, Shuar recognize single motherhood (especially among younger girls) as a problem, and women complain of male extra-pair

---

[1] Data collected as part of a questionnaire designed to develop cultural consonance models measuring how participants define the traditional Shuar value of *shiir waras* ("good life").

copulation when they are absent from their community. Moreover, in the larger SHLHP database, when women report that their children were the result of a brief union with an absent father, the men are most likely to come from other communities. Third, these data fail to capture the quality of a man's wife and children, factors which might contribute to his reproductive success beyond number of children produced. Additional work is therefore needed to clarify how male masculine traits might be linked to improving the quality of offspring through provisioning their families (i.e., assess whether men might be investing in offspring quality over quantity, and if these investment strategies vary between individuals and MI levels).

Likewise, longitudinal data are required to assess how the development of the masculine traits measured might vary over time (i.e., examining how changes in physical labor related to provisioning dependents might alter traits associated with strength). Finally, this study lacked data on contraception methods used (if any) for each participant. Despite these limitations, this study provides a novel test of how male health, signaling, and lifestyle patterns influence reproductive success in an indigenous population characterized by both high fertility rates and infectious disease burdens.

Transition to Chapter VI

Chapter V examined associations between masculine traits thought to increase male reproductive success, parasitic disease, and age-specific number of children. Results suggest that increased investment in the development and maintenance of these masculine characteristics may impair immune function and increase parasitic infection risk, at least for some helminth species. In addition, traditional and housing SOL factors appear to be positively associated with number of children, perhaps due to changing family planning strategies and living conditions.

Conversely, parasite load and masculine traits do not appear to contribute significantly to number of children. Still, these results indicate that a life history trade-off exists between male reproductive effort (as measured through masculine trait development) and immune function (assessed by parasite load). Additional work is required to determine whether this type of trade-off is also apparent between other measures of male investment in reproduction and immunity. Chapter VI further examines these proposed life history trade-offs through testing the Immunocompetence Handicap Hypothesis among the Shuar to determine whether current testosterone levels are associated with various measures of immune function.

CHAPTER VI

LIFE HISTORY TRADEOFFS BETWEEN MATING EFFORT AND IMMUNE FUNCTION: TESTING THE IMMUNOCOMPETENCE HANDICAP HYPOTHESIS AMONG SHUAR MEN OF AMAZONIAN ECUADOR

This chapter contains unpublished, co-authored material and was prepared with assistance from J. Josh Snodgrass, Lawrence S. Sugiyama, Melissa A. Liebert, Samuel S. Urlacher, Joshua M. Schrock, Tara J. Cepon-Robins, Dorsa Amir, Christopher J. Harrington, and Felicia C. Madimenos. The author of this dissertation is responsible for the study design, collection and analysis of stool samples, data analyses, and all write up for this paper. Sugiyama, Liebert, Urlacher, Schrock, Cepon-Robins, Amir, Harrington, and Madimenos aided in data collection in the field. Sugiyama is the field site director and provided editorial assistance on this manuscript. Snodgrass is the academic adviser for this dissertation, provided editorial assistance, and is the director of the Global Health Biomarker Laboratory where all samples were analyzed.

Introduction

Life History Theory is a theoretical framework often used to examine the differential allocation of energy and other resources – and the tradeoffs necessitated by resources being finite – to critical life functions, across organisms and distinctive environmental conditions (Hill, 1993; Hill & Kaplan, 1999; Nylin & Gotthard, 1998; Stearns, 1977, 1989). These tradeoffs are typically shaped by individual context-dependent biological processes, which are heavily influenced by hormonal signals. Hormones serve as chemical messengers in the body, initiating physiological changes in response to environmental conditions (Ellison, 2009; Hall & Guyton, 2011). Thus, certain hormone signals are hypothesized to reflect energetic constraints and consequently play an important role in shaping life history events and tradeoffs, such as influencing the timing of developmental events and reproductive status (Bribiescas, 2009; Ellison, 2009).

The androgen hormone testosterone plays a key role in the development of male characteristics during childhood and puberty, as well influencing energy allocation and the maintenance of the masculine traits during adulthood (e.g., energetically expensive traits like increased musculature) (Bribiescas, 2001, 2010). Furthermore, testosterone is hypothesized to have immunosuppressive or immunoregulatory effects based on evidence from animal studies (Adamo et al., 2001; Klein, 2000, 2004; Poulin, 1996; Zuk, 2009). For example, increased testosterone appears to reduce overall T cell count, decrease T and B cell production, and perhaps even increase the likelihood of immune cell apoptosis (Ansar et al., 1985; Campbell et al., 2001; Fish, 2008). In addition, studies using mouse models have demonstrated that castration improves overall immune activity, while subsequent injection with endogenous testosterone reverses these effects (Ansar et al., 1985).

Similar patterns have been documented among humans, with eunuchs exhibiting exceptional longevity compared to intact men (Gems, 2014; Hamilton & Mestler, 1969). It has been proposed that this pattern is the result of antagonistic pleiotropy acting on testosterone-linked male secondary sexual characteristics (i.e., sex-specific phenotypic traits not directly involved in the act of reproduction) (Gems, 2014). In other words, increased energetic investment in masculine traits and mating effort (directed by testosterone) occurs at the expense of immune function in men; increasing reproductive success early in life, but shortening the lifespan (Bribiescas, 2010).

Testosterone is therefore thought to mediate trade-offs between reproductive effort and immunity, and a large body of literature is based on this hypothesis. For example, the Immunocompetence Handicap Hypothesis (ICHH) contends that energetically expensive testosterone-linked traits provide costly signals of immunocompetence that females use in mate choice, thus influencing male reproductive success (e.g., DeBruine et al., 2006; Gangestad & Buss, 1993; Jones et al., 2012). Specifically, females are expected to prefer high quality mates with ‘superior genes’, and to be sensitive to cues of phenotypic condition indicative of this quality, including size, strength, and facial masculinity (Buss & Schmitt, 1993; Folstad & Karter, 1992; Gangestad et al., 2010; Thornhill & Gangestad, 2003). These traits have been linked with testosterone levels, and are thought to reflect a male’s ability to withstand health insults (including parasitic infection) (Frederick & Haselton, 2007; Gallup & Frederick, 2010; Gangestad & Buss, 1993; Hamilton & Zuk, 1982; Sugiyama, 2005).

Therefore, according to the ICHH, males face selective pressure to produce conspicuous secondary sexual characteristics linked with high testosterone levels, because these reflect resistance to infection and ability to maintain substantial energetic investment in reproductive effort, despite the immunosuppressive effects of testosterone (Grossman, 1985; Klein, 2000, 2004). Females are consequently hypothesized to use the quality of these masculine traits (indicative of underlying testosterone levels) as an honest signal of male genetic quality and immune function (Andersson & Simmons, 2006; Folstad & Karter, 1992; Little et al., 2010; Tybur & Gangestad, 2011). Thus, a direct relationship between testosterone level and male health should be observed and females should prefer males with higher testosterone levels. Cheaters should be prevented because their overall reproductive success will be detrimentally affected by high disease burdens resulting from the energetic costs associated with maladaptively elevated testosterone levels, which they are unable to afford (Roberts et al., 2004).

However, the ICHH also contends that the relationship between testosterone levels change over the course of an infection. At the onset of infection, a shift to lower levels of testosterone and an increased investment in immune function should be observed (Figure 6.1). Once the infection is cleared, testosterone levels should rise, marking a return to increased investment in mating effort (Folstad & Karter, 1992). High circulating testosterone levels are thus hypothesized to indicate the absence of infection. Although it is possible for males with exaggerated secondary sexual characteristics to temporarily experience lower testosterone levels during periods of intense infection, according to this model, males with more developed masculine characteristics should generally exhibit increased resistance to illness compared to other males (thereby permitting the continual energetic investment required to support high testosterone levels and develop these exaggerated traits in the first place). Females should therefore be able to use testosterone-linked masculine traits during mate selection to ascertain heritable immunocompetence and associated phenotypic quality (Stoehr & Kokko, 2006).

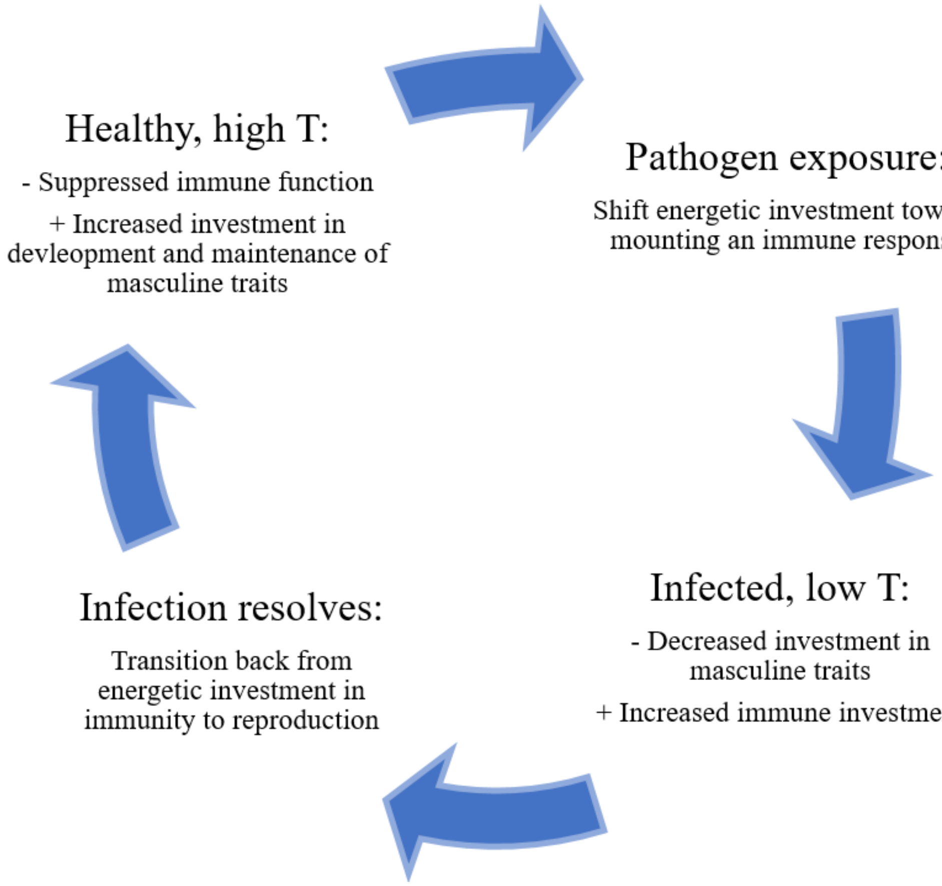


Figure 6.1. Changes in male testosterone levels during the course of an infection, as outlined by the Immunocompetence Handicap Hypothesis.

There is some empirical evidence to support the ICHH in animal models (Klein, 2000; Sheldon & Vehulst, 1996). In many species (e.g., fence lizards, mice, several species of birds), females appear to be highly sensitive to subtle variations in male secondary sexual characteristics and testosterone levels, perhaps due to visual, auditory, olfactory, or behavioral cues (Dunlap & Schall, 1995; Klein, 2000; Sheldon & Vehulst, 1996). Still, to date, few studies have tested these associations in humans, and the little research conducted has produced mixed results. For instance, recent work testing the association between testosterone levels and immune function among indigenous Tsimane of Bolivia documented complex relationships (Trumble et al., 2016). In this population, higher testosterone levels downregulated more costly aspects of immune function (e.g., T-cell mitogen induced cytokine production) as predicted by the ICHH, but had no effect on less costly forms of immune activation (e.g., B-cell induced cytokine production), suggesting that the influence of testosterone varies based on immune response type (Trumble et al., 2016). It may therefore be more accurate to label the effects of testosterone as immunomodulatory, rather than immunosuppressive.

Still, there is evidence from human studies that men experience decreased testosterone levels during periods of illness or somatic injury (Dym & Orenstein, 1990; Muehlenbein et al., 2010; Spratt et al., 1993; Spratt, 2001). These findings suggest that testosterone levels in adult men may be suppressed from their “normal levels” (i.e., the typical level for a certain individual within a specific social and environmental context) during periods of illness. This suppression it thought to divert resources away from reproductive effort (e.g., the maintenance of secondary sexual characteristics), thereby ensuring adequate energetic investment in immune function until the infection is resolved, at which time a return to earlier testosterone levels is expected (Muehlenbein et al., 2010; Muehlenbein & Bribiescas, 2005). However, additional work is needed to test the ICHH in human populations. The ICHH is not well tested in natural fertility, subsistence-based populations with a high infectious disease burden (including parasitic disease), conditions most relevant to those under which testosterone-mediated costly signals evolved (Thornhill & Gangestad, 2006).

It is also important to consider the type of immune responses driving the evolution of male traits signaling good health. Parasitic infection may be a particularly important measure here given the long coevolutionary history humans share with parasites, and the costs of infection and immune defense (Hurtado et al., 2008; Jackson et al., 2009). Given this long period of coexistence and the rapid evolution of pathogens, parasitic disease is hypothesized to have played an important role in human evolutionary processes, including sexual selection favoring traits indicative of parasite resistance as has been demonstrated among other species (e.g., lower parasite loads among brightly colored male satin bowerbirds or uakari monkeys) (Ayres, 1996; Doucet & Montgomerie, 2003; Lasry & Sheridan, 1965). Still, the relationship between testosterone and immune function with regards to human parasitic infection remains poorly studied.

The present study directly tests the ICHH among the Shuar, an indigenous population living in the lowland region of Amazonian Ecuador, with the following objectives:

- ***Objective One* - To test associations between testosterone levels and parasite load.**
    - **Hypothesis 1: Testosterone levels will be inversely associated with parasite load.** Testosterone levels are expected to be suppressed during periods of immune activation (Muehlenbein et al., 2010; Muehlenbein & Bribiescas, 2005), and should there be negatively related to parasite load (which reflects current infection status).
- ***Objective Two* - To determine the relationship between testosterone and key immune parameters.**
    - **Hypothesis 2: Testosterone levels will be inversely associated with three markers of immune function.** Circulating testosterone levels are thought to be immunosuppressive (Adamo et al., 2001; Klein, 2000, 2004; Poulin, 1996; Zuk,

2009), and are therefore expected to be negatively associated with immune response as measured by (i) C-reactive protein (CRP; an acute inflammatory marker produced within days of infection), (ii) Immunoglobulin G (IgG; a marker of acquired humoral immunity against bacteria and viruses acting over a period of many months), and (iii) Immunoglobulin E (IgE; an indicator of macro-parasitic infection acting over a period of several years).

Materials and Methods

Participants

This study was conducted as part of the Shuar Health and Life History Project (SHLHP; http://www.bonesandbehavior.org/shuar/). The Shuar are natural fertility indigenous population of ~100,000 people distributed in more than 400 communities in the neotropical eastern foothills of the Andes and the Amazonian lowlands of southeastern Ecuador in the provinces of Pastaza, Morona-Santiago, and Zamora-Chinchipe (Bennett et al., 2002; Rubenstein, 2001). Traditionally, Shuar lived in scattered households reliant on horticulture, fishing, hunting, and foraging (Harner, 1984; Karsten, 1935; Rubenstein, 2001). However, Shuar are currently undergoing rapid, yet uneven market integration (MI; the degree to which individuals consume from and produce for a market economy).

Today, most Shuar continue to depend heavily on traditional subsistence activities, although Shuar communities closer to market centers may also rely on agro-pastoral production for market sale and various sources of wage labor (Liebert et al., 2013). Lifestyle patterns among the Shuar vary in relation to MI, and this variation in turn influences health outcomes (including growth patterns and chronic disease risk) (Blackwell et al., 2009; Liebert et al., 2013; Urlacher et al., 2016). Still, across all levels of MI, previous research indicates that parasitic infection is common among Shuar, with approximately 65% of participants infected with at least one species of soil-transmitted helminth (parasitic worms) (Cepon-Robins et al., 2014; Gildner et al., 2016a).

Study Design and Sampling

This study employed a cross-sectional approach, with data collected over five field seasons (2012-14, 2016-17). Random sampling was not feasible across this large population, dispersed over relatively inaccessible areas, with sometimes divisive internal politics. Thus, through consultation with knowledgeable Shuar friends and political leaders, and the Dirigente de Salud of the Federación Interprovincial de Centros Shuar, a sample of communities were chosen based on their size, location, and agreement to collaborate on this research. Once communities agreed at an open community meeting that the SHLHP could conduct research in their community, all men were invited to participate in this study.

The sample included 136 male volunteers from 11 communities, aged 12 - 67 years old. The age of 12 was selected to capture those entering puberty at a younger age, thereby capturing the full range of testosterone profiles across key developmental reproductive transitions. Informed consent was obtained from adult participants and parental verbal consent and child assent were obtained for individuals under 14 (the local age of consent). The University of Oregon Institutional Review Board approved this study, and the Federación Interprovincial de Centros Shuar and local leaders authorized research in participant communities.

Field and Laboratory Procedures

Salivary testosterone collection and analysis

Participants were asked to provide saliva samples via passive drool, wherein they pooled saliva in their mouth and then gently “drooled” directly into a 2.0 mL vial. Due to diurnal fluctuation (daily changes in testosterone levels), at least 1 mL saliva was collected before 9 am and after 4 pm for three consecutive mornings and evenings to establish an individual average for both morning (AM) and evening (PM) testosterone levels. Samples were stored in a portable -20°C field freezer until transfer to Quito, where they were shipped on dry ice via courier service to the Global Health Biomarker Lab at the University of Oregon for analysis.

One researcher (TEG) analyzed all 773 testosterone samples using commercially available enzyme immunoassay (ELISA) kits from Salimetrics (Kit #1- 2402; State College, PA), which have been well-validated to quantitatively assess salivary testosterone. Salivary testosterone levels are useful measures of biologically active testosterone concentrations. In blood, only 1-10% of testosterone is in a biologically active form; most testosterone is bound to serum proteins, such as sex hormone-binding globulin (Wang et al., 1981). Conversely, the majority of testosterone in saliva is not protein-bound and therefore active; in addition, the serum-saliva correlation for testosterone is very high for males (Wang et al., 1981).

Here, saliva samples were assayed in duplicate and those that did not meet the coefficient of variation (CV) and/or absolute value difference criterion were re-analyzed (Kim et al., 2015). Interassay coefficients of variation were 8.36% and 20.75% for high and low control samples, respectively; resulting in an overall interassay coefficient of 14.55%. The intraassay coefficient of variation was 8.44%. Analysis indicated that 1.68% of testosterone concentrations were below the lower limit of detection; for statistical analyses, these low values were substituted with the average lower limit of detection calculated (133.39 pmol/L).

Stool collection and analysis

Fresh stool samples were collected using previously described methods (Cepon-Robins et al., 2014). A single Kato-Katz smear was prepared from each participant's fecal sample (by TEG or TJC) within an hour of sample collection (Katz et al., 1972); after 30-45 minutes, the smears were examined using 10x and 40x microscopy by trained observers (either TEG or TJC) and species-specific eggs per gram (EPG; indicative of individual parasite load) of feces recorded. Infection prevalence was calculated based on the proportion of participants with helminth ova in their stool. 46.3% of participants were infected with *Ascaris lumbricoides* and 28.4% with *Trichuris trichiura.* The present study therefore focuses on these two species.

Immune markers

Testosterone is thought to negatively influence several components of the immune system, thereby increasing risk of viral, bacterial, and parasitic infection (Klein, 2008).

Thus, to better explore the relationship between testosterone and different branches of the immune system, several immune parameters were measured from dried blood spots (DBS). Three to five drops of whole blood were collected on filter paper from a single finger prick following standard minimally invasive collection methods (McDade et al., 2007). Samples were dried 4 hours, and stored in a -20°C portable freezer until transport on dry ice via courier service to the Global Health Biomarker Lab at the University of Oregon for analysis.

One researcher (TEG) measured concentrations of C-reactive protein (CRP; an acute inflammatory marker), Immunoglobulin G (IgG; a common antibody and marker of acquired humoral immunity), and Immunoglobulin E (IgE; an indicator of macro-parasitic infection) from DBS samples using validated enzyme-linked immunosorbent assay (ELISA) analyses, following established methods (e.g., Urlacher, 2016). Specifically, CRP concentrations were determined using a high-sensitivity DBS assay (McDade et al., 2004) modified for new coating and detection antibodies (coating antibody = M86005M, streptavidin-HRP = V8Z21-2712, and biotinylated antibody = M01319B; Meridian Life Sciences: Boca Raton, FL) (Blackwell et al., 2010). Concentrations of IgG were evaluated using a commercially available assay kit designed to detect human IgG levels in serum, plasma, milk, urine, feces, or saliva (Cat. No. E80-104; Bethyl Labs: Montgomery, TX). Finally, IgE concentrations were determined using a commercially available assay kit designed to detect human IgE levels in serum, plasma, urine, feces, or saliva (Cat. No. E80-108; Bethyl Labs: Montgomery, TX) (Blackwell et al., 2010; Tanner & McDade, 2007).

These three measures were selected because they reflect different forms of immune system activity and because they represent distinct timescales of immune function, an approach adopted in other Shuar immune function studies (Urlacher et al., 2018). Specifically, elevated CRP levels are linked with immediate, short-term immune responses apparent in the days following the initial immune insult (e.g., Pepys & Hirschfield, 2003). Increased IgG levels are associated with longer lasting immune responses, lasting on the order of months (e.g, Abbas et al., 2014). Finally, elevated IgE concentrations are typically indicative of chronic physiological responses lasting years (from stimulation to return to baseline concentration) (e.g., Kidon et al., 2005). Including these various measures therefore provides a unique opportunity to assess how testosterone levels may influence various types of immune function across different time scales.

Samples were assayed in duplicate and those that did not meet the coefficient of variation (CV) and/or absolute value difference criterion were re-analyzed (Kim et al., 2015). Inter- and intra-assay coefficients were also calculated for each immune marker. For the IgE samples, interassay coefficients of variation were 15.67%, 16.49%, and 21.67% for the high, medium, and low control samples, respectively. The intraassay coefficient of variation was 2.82%. Analysis indicated that none of IgE mean concentrations were below the lower limit of detection. For IgG, interassay coefficients of variation were 15.28% and 21.53% for the high and low control samples, respectively. The intraassay coefficient of variation was 2.31%. Analysis indicated that none of IgG concentrations were below the lower limit of detection. Finally, for CRP, interassay coefficients of variation were 35.38% and 44.89% for the high and low control samples, respectively. The intraassay coefficient of variation was 2.61%. Analysis indicated that 4.49% of CRP mean concentration values were below the lower limit of detection; these low values were substituted with the average lower limit of detection calculated (0.051 mg/L).

It should be noted that these analyses occurred over a two-year period and different controls were used at different time points; this likely accounts for the high interassay coefficients. In addition, IgE and CRP controls were calculated from DBS samples, resulting in between-plate variability not only from daily differences in lab conditions (e.g., temperature), but also variability between individual blood drops (e.g., size and time spent drying) and between sample punches from the same drop (Lenk et al., 2015). Overall, these different sources of variation increase the size of the interassay coefficients. Still, the intraassay coefficients were very low for each assay.

Confounders

Variables known to influence testosterone levels, infection risk, or immune profile were controlled for during analysis. Evidence indicates that both testosterone levels (Bribiescas et al., 2010) and parasitic infection risk (Dold & Holland, 2011; Kealey, 2010) may decrease with age; individual age was consequently included in all models. Moreover, a variable accounting for the interaction between testosterone and age was included in all models to account for the possible effects of this interaction on immune function. Ages were determined by birthdates on government issued identification cards, and cross-checked by informants and existing SHLHP genealogical data, as has been previously described elsewhere (Blackwell et al., 2011; Liebert et al., 2013).

Individual body mass index (BMI; kg/m^2) has also been shown to influence immune function, including the production of antibodies (i.e., IgE) associated with parasitic infection (Blackwell et al., 2010). Participant height was therefore measured using a stadiometer (Seca Corporation 214, Hanover, MD) and a Tanita scale (model BF680W) was used to obtain participant weight. These height and weight measures were then used to calculate BMI and this measure was included in all models. Participants also reported past medicine use (i.e., anti-helminth medications) within the past month.

In addition, lifestyle change has been shown to influence infection risk through altering disease transmission pathways (Godoy et al., 2000, 2005; Patz et al., 2000). Socioeconomic factors and lifestyle patterns have also been linked with variation in testosterone levels (Bentley, 1993; Bribiescas, 1996, 2010; Ellison & Panter-Brick, 1996). Participants therefore completed a structured interview based on a Material Style of Life (SOL) Index developed by SHLHP for use with Shuar after extensive qualitative research and pre-testing (e.g., Liebert et al. 2013), modeled on examples used in other populations (Bindon et al., 1997; Leonard et al., 2002). The SOL index includes a list of items associated with degree of participation in the market-based or subsistence economy divided into three scales: Traditional Style of Life (T-SOL), Market-Integrated Style of Life (M-SOL), and Housing Style of Life (H-SOL).

Traditional-SOL and M-SOL are calculated as a weighted sum of the total number of traditional (e.g., blowgun, hunting dog, fishing nets) or market-integrated (e.g., radio, car, television) index items a household owns. H-SOL is calculated as a weighted composite score based on different aspects of household type, size, access to water source, etc. This approach is useful for capturing lifestyle variation with a few manageable variables (Liebert et al., 2013; Urlacher et al., 2016). Preliminary analyses indicated that M-SOL and H-SOL exhibited a significant positive correlation, therefore a composite score was created by calculating the z-scores of M-SOL and H-SOL and adding these scores together to better capture cumulative factors associated with higher levels of MI (i.e., ownership of market produced items and aspects of household construction linked with higher MI).

Statistical Analyses

All analyses were conducted using SPSS version 25 (SPSS Inc., Chicago, Illinois). Prior to analysis, data were examined for outliers, defined as ±3 SD above or below the mean. Outliers were evident only for the helminth EPG variables. Because of the high degree of variation (i.e., overdispersion) evident in the EPG data, extreme outliers were not excluded from the sample as this would have removed data points of special interest (i.e., particularly heavy parasite loads). Additionally, Shapiro-Wilk tests were used to test for normality in all variables. Helminth EPG variables were $\log_{10}$-transformed due to non-normal distributions. This resulted in normally distributed variables with skew between ± 1. These transformed data were subsequently used during analysis. Likewise, due to a positive skew in the distribution of testosterone values, all indices were transformed using a square root transformation. The transformed variables were normal, with a skew between ± 1; these transformed measures were used in all analyses.

Concentrations of IgE were $\log_{10}$-transformed prior to analysis to normalize the data. IgG levels were normally distributed, and were therefore not transformed. In addition, to increase interpretability, CRP DBS measures were converted to serum blood equivalents using an equation derived from 40 matched serum:DBS samples (CRPserum = e^[1.2*lnCRPDBS + 0.3405]; $r^2 = 0.87$). Following a standard approach (McDade et al., 2005; Urlacher et al., 2018), serum-equivalent CRP concentration were dichotomized for use during analysis. Specifically, a binary variable was created to indicate the presence (CRP ≥ 1 mg/L) or absence (CRP < 1 mg/L) of an acute inflammatory immune response (McDade et al., 2005). Previous work among the Shuar indicates this cut-off value (CRP ≥ 1 mg/L) accurately identifies acute (i.e., from short-termed immune responses), rather than chronic, inflammation (McDade et al., 2012; Urlacher et al., 2018).

Finally, the SOL indices, age, and BMI were all normally distributed and therefore were not transformed. However, preliminary analyses indicated multicollinearity between participant age and BMI. An unstandardized residual was therefore calculated for BMI, removing the contribution of age to BMI. Similarly, multicollinearity was apparent between AM and PM testosterone levels. An unstandardized residual was calculated for PM testosterone, removing the contribution of AM testosterone to PM testosterone. These residual variables were subsequently included during analysis. Additional diagnostic analyses revealed acceptable degrees of model linearity and heteroscedasticity.

Parametric tests of the transformed data were conducted to test the hypotheses. Specifically, logistic and linear regression analyses were used to test the hypothesis while also assessing the relative contribution of covariates to variation in parasite load or antibody levels. However, a large proportion of participants (64%) were missing at least one variable. For example, 30.1% of participants were missing information on medication use; yet, this variable did not contribute significantly to any of the models and exclusion of this measure did not significantly change any results (likely because only 2% of participants reported taking any medication in the last month). This variable was therefore dropped from analysis. Little's Missing Completely as Random (MCAR) Test (Little, 1988) was also run to assess whether these data were missing at random. Accounting for all variables included in the final models, results indicate that the data are missing completely at random both for the models assessing parasite load ($p = 0.429$) and the models assessing antibody profiles ($p = 0.688$).

Still, separate variance t-tests conducted to test how each variable influenced missingness in each other variable did document significant relationships, suggesting that other variables may be predicting some of the missingness evident in the dataset. In particular, age significantly predicted missingness in parasite load, BMI, CRP and IgE concentrations ($p \leq 0.01$). Similarly, T-SOL significantly predicted missingness in parasite load, BMI, evening average testosterone level, and CRP, IgG, and IgE concentrations ($p < 0.05$). Multiple imputation was consequently used to create a complete dataset by replacing each missing value with a set of probable values, resulting in valid statistical inferences that properly reflect the existing uncertainty caused by missing values (Rubin 1978, 1986). This technique is commonly used to handle missing data in similar data sets (Van der Heijden et al., 2006). The complete, pooled dataset resulting from multiple imputation was used during analysis, and these results were compared to those from the original (incomplete) data set.

Preliminary statistics

The number of participants infected with either *A. lumbricoides* or *T. trichiura* was calculated. Average values for all other variables included in the models were also determined. Furthermore, Pearson correlations were calculated to examine the relationship between the primary variables of interest.

Examination of the association between testosterone levels and parasite infection

A logistic regression examined if morning or evening testosterone levels were significantly associated with parasite infection status (a dichotomous variable; not infected vs. infected with at least one parasitic species). In addition, a linear regression tested whether morning or evening testosterone levels were negatively associated with parasite load (either *A. lumbricoides* or *T. trichiura* EPG values). In each of these models, age and BMI were entered in the first step, the SOL variables (T-SOL and the composite SOL measure combining M-SOL and H-SOL) were entered together in the second step, the testosterone level measures were entered simultaneously in the third step, and the testosterone-age interaction terms for both morning and evening testosterone were entered in the final step.

Examination of the association between testosterone levels and antibody concentrations

A logistic regression examined if morning or evening testosterone levels were significantly associated with CRP level (a dichotomous variable; low levels vs. acute inflammation). In addition, a series of linear regressions tested whether morning or evening testosterone levels were negatively associated with IgG or IgE concentrations. In each of these models, age and BMI were entered in the first step, the SOL measures (T-SOL and the composite SOL measure combining M-SOL and H-SOL) were entered in the second step, the testosterone variables were entered in the third step, and the testosterone-age interaction terms were entered in the final step.

Results

Descriptive Statistics

Table 6.1 presents frequencies and average values of all variables included in the regression models. Most participants were infected with at least one soil-transmitted helminth. Overall, 60.0% of participants were infected with at least one parasite species, with 46.3% of participants infected with *A. lumbricoides* and 28.4% with *T. trichiura*. Mean untransformed EPG values were 3,004.80 and 59.62 eggs per gram for *A. lumbricoides* and *T. trichiura*, respectively. In addition, as has been documented elsewhere (Bribiescas & Hill, 2010), average morning testosterone levels were higher than average evening levels: at 429.64 pmol/L and 247.81 pmol/L, respectively (untransformed data).

Correlations were also conducted to examine associations between key variables in the pooled multiple imputation data set (Table 6.2). These preliminary analyses indicated that morning testosterone levels were positively correlated with BMI ($p < 0.05$), while evening testosterone levels were negatively correlated with CRP status and positively correlated with age ($p < 0.05$). Further, CRP was positively correlated with M-SOL ($p < 0.01$). Concertation of IgG was positively correlated with IgE concentration ($p < 0.05$); likewise, parasite infection status was positively correlated with IgE ($p < 0.05$).

Trichuris trichiura EPG value was negatively correlated with age ($p < 0.01$). Finally, M-SOL and H-SOL were positively correlated ($p < 0.01$), hence the composite SOL measure combining these two indices was used during analysis.

	Mean (S.D.)
Age (years)	31.830 (14.713)
Body mass index (kg/m^2)	24.189 (2.894)
H-SOL score	11.345 (3.261)
M-SOL score	0.224 (0.157)
T-SOL score	0.313 (0.222)
Average morning testosterone level (pmol/L)	429.642 (177.146)
Average evening testosterone level (pmol/L)	247.809 (99.435)
***Ascaris lumbricoides* EPG value**	3,004.800 (6243.492)
***Trichuris trichiura* EPG value**	59.620 (196.569)
Immunoglobulin-G concentration (g/L)	2.341 (5.810)
Immunoglobulin-E concentration (ng/ml)	7,051.235 (6,039.500)
	Frequency (%)
Parasite infection diagnosis	57 (60.0%)
Acutely elevated C-reactive protein	28 (31.8%)

Table 6.1. Description of the study population using original data (untransformed variables prior to multiple imputation untransformed variables).

	Age	BMI	H-SOL	M-SOL	T-SOL	AM T	PM T	Infection	Ascaris EPG	Trichuris EPG	CRP status	IgG conc.	IgE conc.
Age	1	**0.598***	-0.098	-0.047	-0.035	0.010	**0.196***	**-0.285****	-0.176	**-0.326****	-0.025	0.185	0.031
BMI	**0.598*****	1	-0.070	0.130	-0.024	**0.236***	0.159	-0.065	-0.104	-0.104	**0.242***	**-0.261***	**-0.252***
H-SOL	-0.098	-0.070	1	**0.389*****	-0.137	-0.094	-0.088	0.082	0.047	0.031	0.089	-0.022	-0.153
M-SOL	-0.047	0.130	**0.389*****	1	0.038	-0.030	-0.135	-0.067	-0.045	0.082	**0.410*****	-0.033	-0.210
T-SOL	-0.035	-0.024	-0.137	0.038	1	-0.007	-0.013	0.105	0.053	0.160	0.086	-0.049	0.067
AM T	0.010	**0.236***	-0.094	-0.030	-0.007	1	**0.796*****	0.041	0.085	-0.041	-0.058	-0.114	-0.079
PM T	**0.196***	0.159	-0.088	-0.135	-0.013	**0.796*****	1	0.058	0.037	0.069	-0.183	-0.017	-0.079
Infection	**-0.285****	-0.065	0.082	-0.067	0.105	0.041	0.058	1	**0.756*****	**0.463*****	0.008	0.067	**0.274***
Ascaris EPG	-0.176	-0.104	0.047	-0.045	0.053	0.085	0.037	**0.756*****	1	0.187	-0.015	0.118	0.198
Trichuris EPG	**-0.326****	-0.104	0.031	0.082	0.160	-0.041	0.069	**0.463*****	0.187	1	0.032	0.018	0.202
CRP status	-0.025	**0.242***	0.089	**0.410*****	0.086	-0.058	-0.183	0.008	-0.015	0.032	1	0.113	-0.033
IgG conc.	0.185	**-0.261***	-0.022	-0.033	-0.049	-0.114	-0.017	0.067	0.118	0.018	0.113	1	**0.368****
IgE conc.	0.031	**-0.252***	-0.153	-0.210	0.067	-0.079	-0.079	**0.274***	0.198	0.202	-0.033	**0.368****	1

Table 6.2. Pearson correlations between age, BMI, style of life factors, testosterone levels, parasite infection variables, and antibody markers. Correlations are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Associations between Testosterone Levels and Parasitic Infection

The overall findings for this series of regressions did not differ between the original data set and the complete data set generated from the pooled multiple imputation analyses; the results from the multiple imputation data set are presented here. No significant associations were observed between parasite infection status and any of the variables in the model (Table 6.3). Likewise, no significant associations were apparent in the model examining the relationship between testosterone levels and *A. lumbricoides* EPG values (Table 6.4, Figure 6.2). However, significant findings were apparent in the *T. trichiura* EPG model (Table 6.4, Figure 6.3). No significant interactions were observed between testosterone level and age, so the main effects of the variables in the model can be determined. Age exhibited a significant inverse relationship with *T. trichiura* EPG values (B = -0.083, $p = 0.001$), indicating *T. trichiura* infection intensity is highest at younger ages. Finally, afternoon testosterone levels were positively related to *T. trichiura* EPG values (B = 0.558, $p = 0.004$), suggesting that high average testosterone levels at the end of the day are linked with heavy *T. trichiura* loads.

	Parasite infection status	**C-Reactive Protein classification**
Age	0.969 (0.929-1.011)	1.014 (0.976-1.054)
BMI	0.945 (0.774-1.154)	1.280 (0.986-1.662)
Composite SOL	1.001 (0.754-1.330)	**1.447 (1.030-2.032)***
T-SOL	1.955 (0.175-21.783)	2.670 (0.285-25.056)
Average AM testosterone	1.023 (0.923-1.135)	0.937 (0.826-1.063)
Average PM testosterone	1.129 (0.849-1.503)	**0.681 (0.481-0.965)***
Constant	2.125 (0.127-35.430)	0.649 (0.032-13.119)

Table 6.3. Logistic regression for prediction of infection status and C-Reactive Protein classification from average morning or evening testosterone levels. Odds ratio values with 95% confidence intervals. Infection is coded so that 1 = infected, 0 = not infected. C-reactive protein is coded so that 1 = acute inflammation, 0 = low inflammation. Comparisons are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

	***Ascaris lumbricoides* EPG value**	***Trichuris trichiura* EPG value**	**IgG concentration**	**IgE concentration**
Constant	-2.188 (2.912)	-1.070 (1.763)	**2.462 (0.417)*****	**3.787 (0.204)*****
Age	-0.027 (0.038)	**-0.083 (0.024)****	0.005 (0.005)	-0.030 (0.018)
BMI	-0.196 (0.213)	-0.129 (0.145)	-0.052 (0.040)	0.001 (0.003)
Composite SOL	-0.119 (0.302)	0.231 (0.201)	-0.014 (0.046)	**-0.047 (0.020)***
T-SOL	0.444 (2.483)	1.806 (1.396)	-0.130 (0.308)	0.075 (0.154)
AM T level	0.088 (0.113)	0.013 (0.068)	-0.012 (0.018)	-0.005 (0.009)
PM T level	-0.095 (0.298)	**0.558 (0.193)****	0.018 (0.048)	-0.027 (0.022)

Table 6.4. Linear regressions for the prediction of parasite eggs per gram (EPG) values and immunoglobulin (Ig) concentrations from average morning (AM) and evening (PM) testosterone levels. Beta coefficients with S.E. Comparisons are statistically significant at: *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Figure 6.2. Regression-derived standardized predicted values of *Ascaris lumbricoides* log-transformed EPG for both morning (AM) and afternoon (PM) square root transformed testosterone levels.

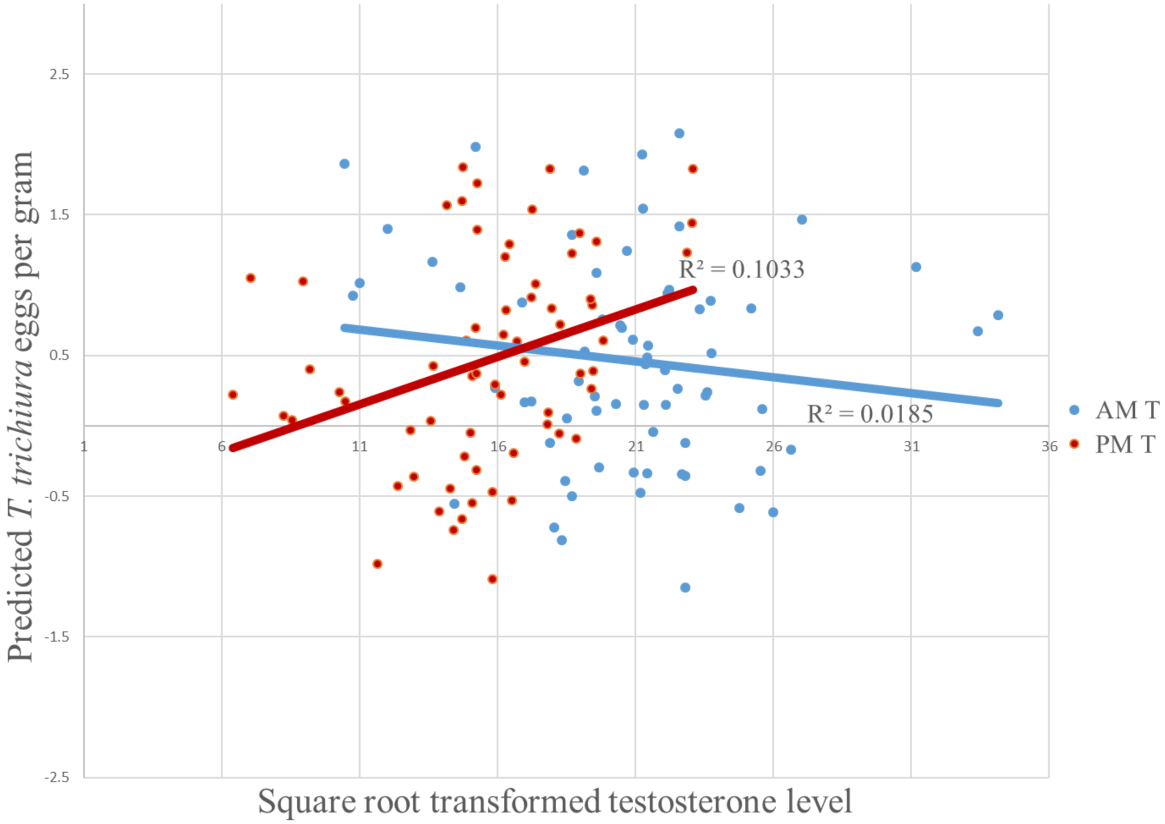


Figure 6.3. Regression-derived standardized predicted values of *Trichuris trichiura* log-transformed EPG for both morning (AM) and afternoon (PM) square root transformed testosterone levels.

Associations between Testosterone Levels and Antibody Profiles

The results of this set of analyses were the same in the original data set and the complete data set generated from the pooled multiple imputation analyses; the results from the multiple imputation data set are presented. Significant findings were apparent in the CRP logistic regression model (Table 6.3). No significant interactions were observed between testosterone level and age, so the main effects of the variables can be determined. Composite SOL score exhibited a significant positive association with CRP classification (Exp(B) = 1.447, $p = 0.033$), suggesting that more market integrated individuals are more likely to have CRP levels indicative of acute inflammation.

Afternoon testosterone levels displayed a significant relationship with CRP (Exp(B) = 0.681, *p* = 0.031), such that for each unit increase in afternoon testosterone the odds of being classified with high CRP levels decreased. No significant associations were evident in IgG concentration and testosterone level model (Table 6.4). The only significant association observed in the IgE concentration model was between composite SOL score and IgE levels (Table 6.4). Specifically, composite SOL exhibited a significant negative association with IgE (B = -0.047, *p* = 0.021), suggesting that more market integrated individuals have lower IgE concentrations.

Discussion

Findings from the present study do not indicate that testosterone levels and parasite load are inversely related, as hypothesized. However, these results did provide partial support for the second hypothesis, indicating that higher testosterone levels may impair specific aspects of immune function. Overall, this study suggests testosterone exhibits a complex relationship with different infection types and distinctive immune system components.

General Infection and Immune Patterns

Testosterone levels were significantly correlated with a number of variables. For example, participant BMI was positively correlated with morning testosterone, suggesting that high energy reserves (i.e., increased adiposity) are required to meet the energetic requirements linked with high testosterone levels within an energy-constrained environment (Bribiescas, 2001; Campbell et al., 2003). Evening testosterone was also positively correlated with age, and while this at first appears to contradict the hypothesis that testosterone levels decrease with age (Bribiescas et al., 2010), it should be noted that a large portion of the study sample (26.5%) were teenagers (under the age of 20) that had likely not yet reached their peak lifetime testosterone production levels. This positive association between age and average evening testosterone level may therefore be reversed with inclusion of more older men. Finally, evening testosterone was inversely correlated with CRP classification, indicating that men with higher testosterone levels were less likely to exhibit CRP levels indicative of acute inflammation.

The parasite infection variables displayed fewer significant correlations. Infection status exhibited a significant positive correlation with IgE levels; this is unsurprising, as IgE is a key antibody in immune responses targeting parasites (e.g., Jarrett & Bazin, 1974). Moreover, *T. trichiura* EPG values were negatively correlated with age, supporting previous results and suggesting that parasite load decreases with age in areas of endemic infection as individuals exposed to continual reinfection develop partial immunity (Dold & Holland, 2011; Gildner et al., 2016a; Kealey, 2010).

Significant correlations were also evident between the covariates. High CRP levels were positively correlated with M-SOL. This suggests that higher inflammation levels are apparent in more market integrated individuals, perhaps due to increased immune challenges resulting from factors associated with MI. For example, MI has been linked with elevated infection risk due to greater population densities, travel between communities, and more contact with domestic animals (e.g., Armelagos et al., 1991). In addition, IgE and IgG were positively correlated, supporting previous findings that these two antibodies act in conjunction to fight certain types of infection (King & Nutman, 1993). Finally, M-SOL and H-SOL were positively correlated. This is to be expected, given that higher values of both measures are indicative of increased MI levels (Liebert et al., 2013).

Testosterone and Parasitic Infection Risk

No significant associations were observed in the regression models assessing whether testosterone levels significantly predicted parasite infection status (while accounting for the influence of covariates). However, only two common helminth species were included in these analyses. Evidence demonstrates that different parasite species respond to (and may directly alter) hormone levels in dissimilar ways (Dunlap & Schall, 1995; Escobedo et al., 2010). It is therefore possible that a significant association between testosterone levels and parasitic infection status might have been evident with the inclusion of additional parasite species endemic to Ecuador (e.g., Onchocerciasis, Paragonimiasis, Chagas disease, and more sensitive measures of hookworm) (Hotez, 2016; Hotez et al., 2008).

Likewise, testosterone levels did not significantly contribute to variation in *A. lumbricoides* EPG values. This is consistent with previous results which failed to document a significant association between testosterone levels and *A. lumbricoides* load in a smaller sample of Shuar men (Gildner et al., 2016b), and may be due to especially high rates of *A. lumbricoides* infection in this population. *Ascaris lumbricoides* is characterized by prolific egg production and infection at relatively low levels of exposure compared to other helminth species (Bethony et al., 2006). Thus, the uniformly high rates of *A. lumbricoides* infection documented here may be the result of high infectivity and ovum production.

A significant positive relationship was documented between evening testosterone levels and *T. trichiura* EPG values. These findings do not indicate that higher testosterone levels are related to lack of infection, as proposed by the ICHH (Folstad & Karter, 1992). However, this association does support the idea that a trade-off exists between male investment in testosterone production and immune function, such that males invest in high testosterone levels at the expense of immune function and consequently have both elevated testosterone levels and higher loads of certain parasitic species. The present study therefore provides a direct test of the ICHH – in a human population experiencing endemic parasitic infection – that has implications for our understanding of how energetic investment in reproductive effort shapes male health and infection risk.

The association between testosterone levels and parasite load documented here suggests that high testosterone males do not have a more effective immune system, as the ICHH argues, rather they simply cope with the negative effects of parasitic infection (Muehlenbein & Watts, 2010). The existence of this tradeoff between investment in reproductive effort (as measured by testosterone levels) and immune function (indicated by parasite load) implies that the reproductive benefits accrued through energetic investment in reproductive effort outweigh any resulting immune function costs. There is some evidence of this in non-human primates (e.g., chimpanzees), which have documented higher parasite loads in dominant males with higher testosterone levels

(Muehlenbein & Watts, 2010); yet, these dominant males have access to more mates as a result, thus enhancing their reproductive success.

Interestingly, a significant association between testosterone concentration and parasite load was only evident for evening testosterone. Evening (but not morning) testosterone has been linked to measures such as dominance rank in non-human primates (Muller & Wrangham, 2004). This is likely due to the influence of daily events in shaping evening testosterone levels. Testosterone levels are diurnal and tend to peak in the morning – likely to mobilize energy for use during the day – and decline throughout the afternoon (Bribiescas & Hill, 2010). However, research suggests that interactions and experiences throughout the day influence the degree and rate of this decline (Muller & Wrangham, 2004). Dominant males may exhibit more aggressive or competitive behaviors which increase testosterone levels, and therefore experience less testosterone decline throughout the day. Consequently, these sustained high testosterone levels may have negative health effects and increase *T. trichiura* infection risk.

Testosterone and Antibody Profiles

The regression analyses demonstrate that testosterone levels and each covariate included in the model did not significantly contribute to variation in IgG concentrations. This suggests that testosterone levels may not strongly influence adaptive humoral immune responses to viruses or bacteria. While these relationships are not well studied, these findings do support some previous research that found no significant association between testosterone levels and IgG concentrations (Sullivan & Hann, 1989). Similarly, testosterone levels were not significantly associated with IgE levels, suggesting that testosterone may not directly influence long-term anti-parasite adaptive immune responses. Although unexpected according to the ICHH, these results support previous research which has failed to document a significant association between testosterone and IgE levels (Worm & Henz, 1997). Still, a significant inverse relationship was observed between participant composite SOL score and IgE concentration, implying that more market integrated individuals exhibit lower IgE scores. This is consistent with previous work among the Shuar, and could be the result of Shuar at higher MI levels having better access to medical care, including treatment for helminths (Blackwell et al., 2010).

Conversely, the final regression model documented a positive association was evident between composite SOL score and CRP levels, suggesting that lifestyle changes linked with market integration are associated with acute inflammation. This finding aligns with previous research among the Shuar, which suggest that individuals in more market integrated communities have higher levels of current infection (Gildner et al., 2016a), thereby likely resulting in acutely elevated CRP levels. In contrast, the Shuar appear to exhibit relatively low rates of chronic low-grade CRP elevation compared to more market integrated, Western populations (McDade, 2012; McDade et al., 2012). This pattern of acute CRP elevation (i.e., during innate immune system activation in response to infection) has also been documented among other indigenous groups. For example, indigenous Tsimane of Bolivia have higher CRP levels (at least in early life) compared to a U.S. sample (Blackwell et al., 2011; Gurven et al., 2008).

In addition to documenting a significant relationship between MI and CRP, the present study also found a significant association between testosterone levels and CRP; namely, an inverse association was apparent between average evening testosterone levels and CRP classification, such that men with higher evening testosterone levels were less likely to exhibit high CRP levels indicative of acute inflammation. These results support the ICHH and suggest that testosterone may impair at least certain aspects of immune function. This documented relationship also aligns with evidence indicating that testosterone levels directly impact CRP production (Kalinchenko et al., 2010; Kapoor et al., 2007). Specifically, testosterone supplementation has been linked with subsequent declines in CRP, supporting the hypothesis that testosterone has immediate suppressive effects on certain aspects of immune activity.

The inverse relationship between testosterone and CRP levels documented here is perhaps unsurprising, given that these measures reflect biologically active testosterone levels and current infection. This association therefore supports the temporal changes in testosterone levels in response to infection proposed by the ICHH (Folstad & Karter, 1992). In particular, this model contends that testosterone levels decrease once an individual is infected, thus facilitating an increased investment in immune function over the reproductive efforts maintained by high testosterone levels (Folstad & Karter, 1992). Testosterone levels should then rise back to baseline levels once the infection is cleared, marking increased investment in mating effort over immunity (Folstad & Karter, 1992). Although cross-sectional, the results documented here indirectly support this timeline, suggesting that testosterone levels are low when immediate responses to infection are active.

In addition, it is logical that current testosterone levels would influence acute immune function, but not be associated with immune measures reflecting activation over longer time periods. Hormones serve as chemical signals of immediate environmental conditions (Ellison, 2009), and therefore often fluctuate over relatively short time periods to ensure individual physiological responses align with current circumstances. Given these regular hormonal fluctuations, it seems unlikely that testosterone would consistently shape antibody concentrations associated with long-lasting immune function. Still, more work is needed to examine these associations in the same individual longitudinally.

Limitations

The present study has several limitations. Additional work is required to collect longitudinal data on the same participants to assess how testosterone shapes disease risk and is altered in response to initial infection and over the course of an illness. In addition, more data is needed from older men to determine how the relationship between testosterone levels and male health might change through the life course. This study also only included two helminth species, the addition of other helminth species or different classes of parasites is needed to better determine how testosterone profiles impact various aspects of disease risk.

Similarly, only three antibody concentrations were included during analysis, the inclusion of other immune markers would further elucidate the impact of testosterone on various immune responses. Finally, future analyses should account for the influence of diet and activity patterns – additional factors linked with individual energetic status – on both immune function and testosterone production. Still, despite these limitations, this study provides a unique examination of the relationships between testosterone levels and various measure of immune function; associations which remain poorly tested in human samples, especially subsistent populations characterized by high infectious disease burdens.

Transition to Chapter VII

This study examined the association between testosterone profile and immune function in an unstudied setting, contributing unique data toward an improved understanding of the energetic tradeoffs apparent between energetic investment in reproductive effort (as indicated by high testosterone levels) and immune function (as measured by helminth infection patterns and various antibody concentrations). Results suggest that higher testosterone levels may increase infection intensity for some parasite species, perhaps by suppressing the production of immune pathways associated with immediate, acute immune responses. These results have implications for the identification of physiological factors that impact male disease risk and prognosis, thereby helping to inform future disease intervention and treatment programs. This study provides important insights into human life history tradeoffs and makes a significant contribution to our understanding of male reproduction and health. In the next and concluding chapter, important connections between central theoretical ideas and the main findings from this dissertation will be discussed. Future research plans will also be presented.

CHAPTER VII

CONCLUSIONS AND FUTURE DIRECTIONS

This dissertation tests hypothesized life history tradeoffs between male reproductive effort and immune function. These relationships are examined using a range of variables linked with male reproductive investment (testosterone and masculine phenotypic characteristics) and several key measures of immune function (parasite load, two antibody concentrations, and an inflammatory protein biomarker). This research further examines male reproduction in relation to lifestyle variation in a population (the Shuar) undergoing rapid market integration (MI), thereby providing novel information on how this process influences male reproductive health. As a central goal of this dissertation, these data are also used to test the Immunocompetence Handicap Hypothesis (ICHH). The ICHH is a model proposed to explain aspects of sexual selection and male disease patterns, yet poorly tested in human populations.

To review, the ICHH argues that testosterone is immunosuppressive, resulting in a life history tradeoff between male reproductive effort and immune function (Folstad & Karter, 1992). Testosterone-linked masculine traits are therefore hypothesized to honestly signal mate quality to females, as only non-parasitized males with good immune function should be able to maintain high testosterone levels (Folstad & Karter, 1992). In addition, evidence indicates that testosterone profiles are influenced by additional lifestyle factors, including activity patterns, nutrition, parenthood, and age (Bribiescas, 2001, 2010; Gettler et al., 2011). These relationships imply that testosterone levels vary across populations and individuals as a result of specific environmental and social factors.

In this dissertation I use a life history framework to explore these hypothesized associations among the Shuar of Amazonian Ecuador. I focus on how individual factors shape testosterone variation and male health in a population characterized by a high infectious disease burden. Importantly, all Shuar share a recent genetic and cultural history, providing a unique opportunity to examine the effects of recent lifestyle changes on hormone levels and health outcomes. As a natural fertility population experiencing high parasite loads, the Shuar experience conditions relevant to those under which the hypothesized life history tradeoffs and testosterone-mediated costly signals would have evolved, ideal for testing these hypotheses.

Synthetic Overview

Whether testosterone plays a significant role in immune response is hotly debated (Klein, 2008; Poulin, 1996; Zuk, 2009), though very few attempts have attempted to document a direct link between hormone levels and immune activity in human populations. This dissertation therefore makes a significant contribution to work examining the proposed immunosuppressive effects of circulating testosterone and the hypothesized testosterone-mediated tradeoff between mating effort and immune function. The results of this work also contribute novel data on the role of masculine traits in shaping male reproductive success and female mate choice.

This dissertation combines methodology from several disciplines to examine evolutionary links among ecological variation, sexual selection, behavioral endocrinology, and human biology – fields sharing a fundamental basis in evolutionary theory. Synthesizing related hypotheses from these interrelated branches of evolutionary research is analytically powerful; thus, this project adds unique analyses and data toward the theoretical unification in the emerging field of human evolutionary biology, focusing in particular on the role that evolved life history tradeoffs play in shaping male reproduction and health. Overall, this dissertation has three overarching research goals and associated hypotheses, discussed in the following sections.

Research Goal 1

The first overarching goal of this dissertation was to assess testosterone level variation among Shuar men and specifically looks at how individual characteristics and sociodemographic factors influence testosterone profiles. This goal was addressed in Chapter IV, testing whether salivary testosterone levels are positively correlated with household MI level and adiposity, but negatively correlated with age. The data collected to investigate these associations provide a unique opportunity to examine whether physiological patterns documented in high income countries are also evident among less market integrated populations facing nutritional challenges and a high disease burden.

Importantly, this study examined testosterone variation across multiple scales (i.e., communities, households, and individuals), providing insights into how different environmental and social settings might affect testosterone levels. In particular, the examination of testosterone patterns in relation to participant age, body composition, and MI-level provided important information on how individual differences shape testosterone levels. The influence of physical measures and lifestyle factors on the production of flexible testosterone profiles (i.e., hormone levels well-suited to a specific environment) has important implications for subsequent behavioral patterns and health outcomes.

In particular, this study demonstrates that individual age and BMI interactions shape testosterone levels in complex ways, such that the relationship between body composition and testosterone profile appears to vary throughout the life course. These findings are consistent with patterns predicted within a Life History Theory framework. Individual energetic requirements and resource investment strategies are expected to change throughout different life history stages. For example, biological changes linked with aging typically alter body composition patterns and impair somatic functions (i.e., immune function and wound healing) (Brown, 2010; Roberts & Rosenberg, 2006). These cumulative changes are expected to shift energy usage and life history tradeoff patterns in predictable ways. Evidence clearly demonstrates that humans invest significantly more time and energy into reproductive efforts during young adulthood, but this investment declines during senescence in both sexes (Jasienska et al., 2017).

In addition, metabolism generally slows after young adulthood and adiposity levels may increase; this is likely due to several factors, including changes in basal metabolic rate (BMR), reduced activity levels, and changes in digestion (i.e., lower levels of fat oxidation following meals) (Roberts & Rosenberg, 2006). It is therefore unsurprising that age and BMI would interact to influence other biological processes, including testosterone production, as was documented in this research. Again, these complex patterns illustrate the flexibility of human physiology in responding to both environmental and somatic changes throughout the lifespan. This plasticity ultimately produces hormone profiles sensitive to ecological and social conditions, thereby shaping individual life history transitions and tradeoffs in a context-dependent manner and enhancing individual fitness.

Interestingly, no significant associations were apparent between testosterone concentrations and style of life variables designed to measure individual MI level. Still, it is likely that these nonsignificant findings are due to the lack of highly market integrated men included in the study. While associations between MI and other variables (e.g., number of children) were detected in this dissertation, age-related testosterone declines have only been documented in wealthy nations at the highest levels of MI. The participants included in the sample were largely still reliant on traditional subsistence strategies, and did not display activity and nutrition patterns commonly observed in more urbanized areas. Further work is needed to collect additional data to capture the full range of Shuar male lifestyle patters.

Research Goal 2

The second overarching goal of this dissertation was to determine if individual reproductive success is significantly influenced by signals of developmental testosterone and parasite load. Chapter V addressed this goal, testing whether age-specific reproduction (measured as number of living children) is positively related to indicators of developmental testosterone (height, grip strength, chest compression, upper arm circumference, 2D:4D digit ratio). This chapter also investigated the hypothesis that masculine features reflect increased resistance to parasitic infection, and parasite load is therefore negatively associated with indicators of developmental testosterone.

The results of this study failed to support any of these hypotheses. No evidence was found to support the premise that increased resource investment in energetically expensive masculine traits would “pay off” by increasing male reproductive fitness (i.e., number of children). While this study found no support for this hypothesis, it should be noted that the sample was relatively small and the questionnaire items used may not have accurately captured male reproductive success (i.e., measured age-specific reproduction in a sample of mostly younger men, rather than capturing completed reproductive success among only older men no longer having children).

Marriage and large families are extremely common among the Shuar, but many men travel for work and may seek extramarital mating opportunities. There was at least one instance of a participant of this study having a child outside of marriage, which he did not report with the rest of his children. It is therefore possible that other men also failed to report fathering children from extramarital relationships. Given that some evidence suggests women prefer more masculine men for short-term sexual relationships (especially close to ovulation) (Penton-Voak et al., 1999), it is possible that men with more developed masculine traits are in fact producing more children as hypothesized. However, due to inaccurate reporting, we are not able to capture that pattern with the given data.

Similarly, these results failed to support the hypothesis that masculine traits honestly signal good health; instead, a significant positive association was observed between a composite score of masculine traits and *Ascaris lumbricoides* infection load. This suggests that masculine traits may not serve as honest signals of male health and parasite resistance, as hypothesized by the ICHH (Folstad & Karter, 1992). Instead, this finding could support other evolutionary models, such as the Disposable Soma Theory. This model contends that preserving physical health is intrinsically costly, and resources used for reproduction reduce what is available to be invested in somatic maintenance (Kirkwood, 1979). This type of tradeoff should be especially apparent in energy-constrained, high-pathogen environments, such as those inhabited by the Shuar.

It is also possible that the reproductive benefits (e.g., increased mating opportunities) gained through energetic investment in secondary sexual characteristics and high testosterone levels outweigh any immune costs (e.g., higher infection rates). Previous work in non-human primates supports this idea, indicating that infection risk is of secondary importance relative to selection pressures acting on mating success (Nunn & Altizer, 2004). Specifically, evidence indicates that non-human primates face substantial sexually transmitted disease risks from more frequent sexual encounters (Nunn et al., 2000; Nunn & Altizer, 2004). Yet, these primates do not reduce their reproductive effort to decrease infection risk; rather, the reproductive benefits of this promiscuous behavior appear to outweigh any longevity costs resulting from sexually transmitted infections (Nunn et al., 2000; Nunn & Altizer, 2004).

This continued investment in reproductive effort despite elevated infection risks has important implications for hypotheses regarding mate choice (e.g., the ability of females to use male traits as honest signals of mate quality). In other words, while these results do not support the hypothesis that masculine traits signal disease resistance (as proposed by the ICHH), it is still possible that these men may be signaling an increased ability to tolerate infection (i.e., with fewer severe health outcomes). Overall, this ability to tolerate infections should allow males to maintain reproductive effort and enhance reproductive success, even at the expense of immune function. Interestingly, the results from the analyses investigating Research Goal 3 found similar patterns, suggesting that increased investment in reproductive effort (i.e., through maintaining high testosterone levels) may downregulate certain aspects of immune function and increase risk for some infections. Together these results suggest the relationship between male reproduction and immune function is multifaceted, and that the hypothesized uniform effects of testosterone on immune function outlined by the ICHH are overly simplistic.

Research Goal 3

The third and final overarching goal of this dissertation was to test if testosterone is negatively associated with immune function. This goal was the focus of Chapter VI, which tests the hypothesis that salivary testosterone levels are immunosuppressive and therefore negatively associated with immune response as measured by two antibodies (IgG, IgE), an acute inflammatory biomarker (CRP), and parasite load. In other words, these analyses tested the ICHH within a human population. The results suggest that testosterone levels are inversely associated with CRP levels as hypothesized by the ICHH and documented in other studies (Lassek and Gaulin, 2009; Tang et al., 2007). However, no significant associations were evident between testosterone levels and either IgG or IgE. Finally, a positive relationship between testosterone levels and *Trichuris trichiura* infection load was documented. In conjunction with the positive association documented between the composite score of masculine traits and *Ascaris lumbricoides* infection load in the analyses for Research Goal 2, these findings suggest that a complex relationship exists between male investment in reproductive effort (as measured through masculine trait development and testosterone levels) and immune function.

The mixed results apparent between testosterone levels and the various immune markers indicates that testosterone is not uniformly immunosuppressive, but rather immunomodulatory (Prall & Muehlenbein, 2014; Trumble et al., 2016). These mixed results imply that testosterone is not purely immunosuppressive and inversely related to infection status, as outlined by the ICHH. Rather, testosterone levels appear to differentially impact distinctive branches of immune function, as has been proposed by other studies (Prall & Muehlenbein, 2014; Trumble et al., 2016). For instance, testosterone appears to be positively associated with some immune responses, such as changes in salivary secretory immunoglobulin A (SIgA) (Gettler et al., 2014b). Conversely, testosterone activity has also been linked with increased risk for certain parasitic infections (i.e., malaria parasitemia) (Muehlenbein et al., 2005).

Additional evidence suggests that endogenous testosterone levels act to downregulate costlier forms of immune activation, but do not significantly influence less costly forms of immune activation (Trumble et al., 2016). This is consistent with the relationships between testosterone and immune markers documented in this study. Specifically, testosterone levels were inversely related to CRP levels, suggesting testosterone may suppress this costly form of immunity. Evidence indicates that CRP immune activation costs are much higher than the energetic costs associated with longer acting antibodies, such as IgG and IgE (Bonneaud et al., 2003; Derting & Compton, 2003; Lee, 2006; Leshchinsky & Klasing, 2001; McDade et al., 2016; Wolowczuk et al., 2008). It is therefore unsurprising that testosterone would exhibit a significant inverse relationship with CRP (but not with IgG or IgE), as suppressing this very costly immune process would hypothetically increase the amount of energetic resources available for testosterone-linked investment in reproductive effort.

Still, these results suggest that increased investment in reproductive effort comes at price. The positive relationship documented between testosterone level and *T. trichiura* implies that high testosterone men do experience increased risks for certain types of infection. In other words, these men are not honestly signaling good health (i.e., disease resistance) as the ICHH contends. However, it also possible men capable of sustaining high testosterone levels are also better able to cope with parasitic infections (i.e., never developing severe symptoms and continuing to maintain reproductive efforts), and are therefore still signaling a kind of good health to prospective mates. Previous research suggests that clearance of parasite infection is not always optimal (Behnke et al., 1992; Sheldon & Verhulst, 1996), especially in areas where disease is endemic and reinfection likely; thus, coping with infection may represent a better immune strategy in some cases.

In an ideal world all infections would be cleared through host resistance mechanisms (i.e., detection, neutralization, and destruction or expulsion of pathogens); however, these processes typically require a substantial energetic investment and may result in collateral tissue damage (i.e., immunopathology) (Medzhitov et al., 2012). Immunopathology is generally related to the duration and degree of the immune response in a dose-dependent manner, resulting in a tradeoff between immunity and immunopathology (Medzhitov et al., 2012). Hosts are therefore expected to tolerate infections when the energetic costs of immune activity and the negative side effects associated with these responses are greater than any reproductive or survival benefits accrued through clearing the infection (Behnke et al., 1992; Medzhitov et al., 2012; Sheldon & Verhulst, 1996).

Host tolerance involves a range of physiological mechanisms, all largely concerned with suppressing damaging immune activity (Medzhitov et al., 2012). Although not yet verified, it is possible that testosterone may play a role in this process. Many types of immune cells (e.g., cells in the thymus, spleen, and bone marrow) have hormone receptors, including those specific to sex steroids like testosterone, indicating immune cell activity is sensitive to sex hormone levels (Grossman, 1985; Klein, 2000). However, the role of hormones in shaping host disease tolerance is not well tested. Further work is needed to test the possible role testosterone plays in determining disease tolerance in lieu of clearance; this information could provide insight into the positive relationship between testosterone levels and *T. trichiura* infection load documented in this dissertation.

These data could also help explain the inverse relationship documented between testosterone levels and CRP concentrations. Inflammatory pathways (such as those mediated by CRP) represent a common form of immunopathology if chronically activated, resulting in substantial host tissue damage (Medzhitov et al., 2012); thus, suppressed CRP levels through the effects of testosterone may promote tolerance in place of ongoing (potentially harmful) immune activity in some instances. This hypothesized pathway is consistent with previous research which has demonstrated that exogenous testosterone administration decreases CRP levels and other inflammatory markers (Haider et al., 2010; Kalinchenko et al., 2010). It is therefore possible that the immune effects documented in the present study may actually be beneficial to the host, not immunosuppressive as originally hypothesized.

Future Directions

The results presented in this dissertation suggest several avenues for future research that will further clarify our understanding of the complex relationships between male investment in reproductive effort, different aspects of immune function, and lifestyle change. These research opportunities are explored in the following sections.

Male Variation Throughout the Life Course

A major limitation of this research is that the sample of Shuar men included did not fully reflect the full range of living conditions and age groups present in this population. Collecting data from a truly representative sample of Shuar men is difficult. Shuar men frequently travel for work and do not often keep regular schedules, making it difficult to collect a full three days of saliva samples, in addition to the other anthropometric and immune markers. Moreover, most communities contain relatively few older adults (i.e., grandparents). Still, focused future data collection could expand the current sample. The study hypotheses could then be re-examined using a sample which includes more older men (i.e., over the age of 50) to better determine how aging impacts testosterone variation in this population (i.e., whether testosterone levels decline in older men as has been documented in other populations). Similarly, samples need to be collected from Shuar men living in urban market centers to clarify how MI might alter testosterone profiles (i.e., whether more market integrated men exhibit higher testosterone levels as hypothesized).

While few studies have examined links between MI and testosterone levels, the lack of associations between MI measures and testosterone variation in this dissertation is consistent with one previous study, which found that testosterone levels are still relatively low in communities adjacent to urban centers compared to men living within the cities themselves (Gray et al., 2006). Thus, it seems likely that the inclusion of city-living Shuar during analysis could produce significant associations between MI and testosterone levels. Not only will these data elucidate hormonal variation in the study population, but this information will improve our understanding of how lifestyle change impacts male health. These data are especially important given the rapid MI occurring among the Shuar, but also many other indigenous populations worldwide.

In addition, future research should also test how testosterone might act to influence male health at different developmental stages. It would be especially interesting to investigate whether testosterone significantly influences immune function during puberty, when testosterone production rises substantially and energetic investment in reproductive function increases (Bribiescas, 2009). It is therefore likely that this shift in resource investment could impair other somatic function, including immune activity, although this is not well tested in energy-constrained environments.

Longitudinal Data Collection

Another potential avenue for future study is the collection of longitudinal testosterone data; specifically, the collection of repeat samples from the same individuals over the course of months or years (as opposed to days). Longitudinal data would enable the establishment of causal relationships between testosterone variation and individual factors; testing, for example, whether parasite load influences testosterone levels or whether testosterone levels appear to drive individual disease risk over time. These data would also facilitate a more nuanced examination of testosterone variation in response to life events. For example, it would be possible to determine the magnitude testosterone levels change in response to big life events – such as pair-bonding or fatherhood – as well as measure how quickly these changes occur and how long they persist. In addition, these data would help clarify the complex relation between testosterone and BMI documented in this dissertation by tracking how individual testosterone patterns are impacted by changes in body composition.

Male Reproductive Strategies

In addition to the collection of longitudinal data, further work is needed to identify different reproductive strategies men may be using, and how these strategies are associated with physiological factors (including testosterone levels). For example, the classic reproductive tradeoff of mate or offspring quality versus quantity should be considered. Although measures of masculine traits were not significantly associated with reproductive success in this study (i.e., offspring quantity, measured as age-specific number of living children), it is possible more masculine men are experiencing increased reproductive fitness through acquiring higher quality mates and/or producing higher quality offspring.

Along with measures of male physiological and physical characteristics, future research should therefore also assess partner and offspring quality (i.e., measurements of facial symmetry, body composition, current infection status, and health histories). Likewise, male efforts to enhance offspring quality should also be measured, including fine-grained measures of father-child interactions and male provisioning activities (i.e., hunting or other forms of food production). This information is needed to identify the various mating strategies men use across different environmental and social contexts, and well as determine the apparent success of these approaches.

High Resolution Measures of Male Health and Energetics

In addition to fine-grained measures of male reproductive strategies, high resolution measures of male health and energetic status are needed. For instance, a complex association between testosterone and participant BMI was documented in this dissertation, but is unclear what exactly BMI is capturing in this lean population. This crude measure does not distinguish well between individual muscle mass and adipose tissue; it is therefore possible this measure is reflecting musculature instead of fatness.

To address this issue, future research should obtain higher resolution body composition data (i.e., a more complete dataset of skinfold measures and/or isotope dilution analysis). These measurements would clarify how specific aspects of body composition contribute to variation in hormones like testosterone. Likewise, additional immune markers are needed to better test how testosterone influences various facets of immune function.

This dissertation only considered two parasite species and three different immune parameters; the inclusion of other classes of parasites and antibody types could therefore illuminate the effects of testosterone on previously untested immune responses. In particular, examining associations between testosterone and: (i) measures of constitutive innate immune defenses (e.g., Natural Killer [NK] cell activity; Lee, 2006); (ii) constitutive innate humoral immunity (e.g., natural antibodies-mediated complement activation and red blood cell agglutination; Matson et al., 2005); (iii) induced cell-mediated (Th1) responses (e.g., Interleukin-2 [IL-2], Tumor Necrosis Factor [TNF], and Interferon gamma [IFN-y]; Klein, 2004); and, (iv) biomarkers associated with both innate and adaptive immunity (e.g., IL-10; Trinchieri, 2007). The inclusion of these other immune branches in the present dataset would help clarify the nuanced associations evident between testosterone and various types of immunity.

Fine-grained measures of male energy usage are also needed. Male reproductive function appears to be relatively buffered from acute energetic stressors compared to women (Ellison, 2003); however, male energetics still likely shape long-term investment in certain aspects of male reproductive effort (i.e., attracting and acquiring mates) (Bribiescas, 2001; Ellison, 2003). Yet additional work is needed to clarify to what extend male energy status drives reproductive success and male health. Moreover, the multifaceted relationships documented in this dissertation between testosterone, BMI, *T. trichiura* load, and the inflammatory marker CRP are likely all influenced by male energetics and activity patterns.

For instance, an alternative hypothesis to the ICHH suggests that it is individual energetic budgets that most strongly influence disease risk. This hypothesis contends that some males have inherently larger energetic budgets, allowing them to invest sufficiently in both immune function and the development of ornamentation (Roney, 2009), thereby offsetting any potential energetic tradeoffs between immune function and reproductive effort. Additional work is therefore needed to parse out these interactions by collecting more fine-grained data on male nutrition and exercise/activity patterns, as well as baseline metabolic function (e.g., BMR measures). Furthermore, factors which influence body composition and infection exposure could be measured using accelerometers, GPS data, detailed 24-hour dietary recalls, and doubly labeled water.

There is also surprising little information available quantifying the energetic impact of human infection (i.e., caloric costs). What little work that has been done indicates that immune activity significantly increases BMR and Resting Metabolic Rate (RMR) (Lochmiller and Deerenberg, 2000; Muehlenbein et al., 2010; Roe & Kinney, 1965), while chronic infections may deplete energetic reserves (e.g., adipose tissue) (Lochmiller and Deerenberg, 2000). However, little work has been done testing energy use patterns during naturally occurring infections (i.e., illness not artificially induced within a laboratory setting), or investigating how the energetic costs on infection vary across distinctive populations, cultures, and environmental conditions.

The influence of key hormones (like testosterone) is also important to consider in these types of studies, Given the key role testosterone plays in male energetics (Bhasin et al., 2003; Bribiescas, 2001), fine-grained measures of infection costs could help researchers identify cutoff points for when male reproductive investment is relatively buffered from immune activity costs, versus when infection is expected to markedly alter hormone profiles. Thus, these types of studies would provide a much-needed contribution to research investigating male life history tradeoffs.

Hormonal Complexity and Associations

Finally, while many studies examine the effects of a single hormone, it is important to realize hormones do not act in isolation. For example, it has been proposed that if sex hormones do act as physiological mediators of tradeoffs between reproductive effort and immunity, then their influence should involve interactions with other endocrine signals known to modulate immune activity (i.e., glucocorticoids or leptin) (McDade, 2003). Future research testing the influence of testosterone on immune function would therefore benefit from considering how this hormone interacts with other important aspects of the endocrine system. For example, one especially interesting relationship that should be examined is the association between testosterone and the stress hormone cortisol in relation to immune function. Psychological studies suggest these hormones interact to influence male behavior and dominance (Mehta & Josephs, 2010), but less is known about how these interactions might shape immune activity.

Furthermore, some have suggested a modified version of the ICHH, accounting for interactions between testosterone and cortisol in shaping male attractiveness (Moore et al., 2011). This so-called Stress-Linked Immunocompetence Handicap Hypothesis (SL-ICHH) contends that interactions between cortisol and testosterone impact the development of masculine traits (Moore et al., 2011). There is some empirical evidence for this model, indicating that women strongly prefer men with certain testosterone-cortisol level combinations, suggesting these hormonal interactions may influence male reproductive success (Moore et al., 2011). Still, additional work is needed to determine whether the relationship between testosterone and cortisol alters disease risk, as has been documented among some non-human primates (Muehlenbein & Watts, 2010).

In addition, more work is needed to examine the functional significance of testosterone variation and activity in women. While some recent studies have examined how testosterone influences behavior (e.g., aggression) in both men and women (e.g., Carré et al., 2013; Casto & Edwards, 2016), little work has been done examining how testosterone levels influence infection risk and immune function among women. While total testosterone levels are generally lower in women than men (Hall & Guyton, 2011), previous work does indicate that testosterone levels in women change in response to social stimuli (e.g., Denson et al., 2012; Zilioli et al., 2014), as has been documented in men. Yet, more research is needed examining how testosterone variation influences female reproduction and immune function.

Concluding Remarks

This dissertation provides important insights into the complex associations between male physiological processes and immune activity. While the results presented here largely do not support the ICHH, they do demonstrate a tradeoff between energetic investment in male reproductive effort and some aspects of immune function. However, these findings also suggest that the negative health effects of male reproductive investment are not uniform across all individuals and contexts. In particular, the actions of the hormone testosterone appear to be sensitive to physical condition, likely resulting in reproductive and immune outcomes that are appropriate for a specific context (i.e., accounting for individual energetic parameters and disease exposure). Thus, testosterone activity can be thought of as flexible, rapidly responding to changes in male energetics (i.e., body composition variation) and disease exposure (i.e., endemic parasite infection risk).

It should also be noted that while the primary objective of this dissertation was to test the hypothesized associations between a regulator of human mating effort (testosterone) and immune function, these relationships were examined within the constraints of a single population. As such, this dissertation research was not designed to offer a definitive answer on existing tradeoffs between male mating effort and immune function across all ecological contexts. Still, this research tested hypothesized relationships between testosterone and testosterone-associated traits, biological markers of immune investment, and a specific evolutionarily relevant health outcome (parasite load) in a more evolutionary relevant ecological context than has been tested to date.

This project contributes novel information to the growing cross-cultural dataset required to holistically examine these multifaceted relationships. Like all studies examining human reproductive fitness (Jasienska et al., 2004; Peters et al., 2008; Singh, 1993; Streeter & McBurney, 2003), these results can be used with data from other sources to tease apart the myriad factors that influence human reproductive success. Indeed, every aspect of this dissertation research highlights the complex nature of interactions between physical characteristics, physiological processes, immune activity, and lifestyle change. It is therefore important to continue to test these associations across diverse populations, only then will we be able to fully understand the evolution of human life history patterns, male reproduction, and the manifold factors shaping male health.

REFERENCES CITED

Aagaard-Hansen, J., & Chaignat, C. L. (2010). Neglected tropical diseases: equity and social determinants. Equity, social determinants and public health programmes, 135.

Abbas, A. K., Lichtman, A. H., & Pillai, S. (2014). Basic immunology: functions and disorders of the immune system. Elsevier Health Sciences.

Abraido-Lanza, A. F., Chao, M. T., & Florez, K. R. (2005). Do healthy behaviors decline with greater acculturation?: Implications for the Latino mortality paradox. Social science & medicine, 61(6), 1243-1255.

Acosta, A. (2001). “El Falso Dilema de la Dolarizacion.” Nueva Sociedad 172, Marzo-Abril, 66-84.

Adam, E. K., & Gunnar, M. R. (2001). Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. Psychoneuroendocrinology, 26(2), 189-208.

Adam, E. K. (2006). Transactions among adolescent trait and state emotion and diurnal and momentary cortisol activity in naturalistic settings. Psychoneuroendocrinology, 31(5), 664-679.

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology, 34(10), 1423-1436.

Adam Smith Institute. (2014). Dollarisation in Ecuador. Retrieved from http://www.adamsmith.org/blog/international/dollarisation-in-ecuador.

Adamo, S. A., Jensen, M., & Younger, M. (2001). Changes in lifetime immunocompetence in male and female Gryllus texensis: trade-offs between immunity and reproduction. Animal Behaviour, 62(3), 417-425.

Ahmed, S. A., Penhale, W. J., & Talal, N. (1985). Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. The American journal of pathology, 121(3), 531.

Ahaotu, I., Ogueke, C. C., Owuamanam, C. I., Ahaotu, N. N., & Nwosu, J. N. (2011). Protein improvement in gari by the use of pure cultures of microorganisms involved in the natural fermentation process. Pakistan Journal of Biological Sciences, 14(20), 933.

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. Cell, 124(4), 783-801.

Alexander, J., Irving, K., Snider, H., & Satoskar, A. (2010). Sex Hormones and Regulation of Host Responses Against Parasites. In Sex Hormones and Immunity to Infection (pp. 147-186). Springer Berlin Heidelberg.

Altizer, S., Harvell, D., & Friedle, E. (2003). Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology & Evolution, 18(11), 589-596.

Alvarado, L. C. (2013). Do evolutionary life-history trade-offs influence prostate cancer risk? a review of population variation in testosterone levels and prostate cancer disparities. Evolutionary applications, 6(1), 117-133.

Alvarado, L. C., Muller, M. N., Thompson, M. E., Klimek, M., Nenko, I., & Jasienska, G. (2014, March). Seasonal fluctuation of men's testosterone levels and body composition in rural Poland. In American Journal of Physical Anthropology (Vol. 153, pp. 65-66).

Alvarado, L. C., Muller, M. N., Emery Thompson, M., Klimek, M., Nenko, I., & Jasienska, G. (2015). The paternal provisioning hypothesis: effects of workload and testosterone production on men's musculature. American journal of physical anthropology, 158(1), 19-35.

Aminov, R. I. (2010). A brief history of the antibiotic era: lessons learned and challenges for the future. Frontiers in microbiology, 1, 134.

Andersson, M., & Simmons, L. W. (2006). Sexual selection and mate choice. Trends in ecology & evolution, 21(6), 296-302.

Apicella, C. L. (2014). Upper-body strength predicts hunting reputation and reproductive success in Hadza hunter–gatherers. Evolution and Human Behavior, 35(6), 508-518.

Archer, J. (2006). Testosterone and human aggression: an evaluation of the challenge hypothesis. Neuroscience & Biobehavioral Reviews, 30(3), 319-345.

Ardern, C. I., Katzmarzyk, P. T., Janssen, I., & Ross, R. (2003). Discrimination of health risk by combined body mass index and waist circumference. Obesity, 11(1), 135-142.

Armelagos, G. J., & Cohen, M. N. (Eds.). (1984). Paleopathology at the Origins of Agriculture (pp. 235-269). Orlando (FL): Academic Press.

Armelagos, G. J., Goodman, A. H., & Jacobs, K. H. (1991). The origins of agriculture: Population growth during a period of declining health. Population and environment, 13(1), 9-22.

Ayres, J. M. C. (1996). Uakaris and Amazonian flooded forest. Unpubloshed PhD thesis, University of Cambridge.

Baker, P. T., Hanna, J. M., & Baker, T. S. (1986). The changing Samoans: behavior and health in transition (Vol. 5). Oxford University Press, USA.

Bagatell, C. J., & Bremner, W. J. (1990). Sperm counts and reproductive hormones in male marathoners and lean controls. Fertility and sterility, 53(4), 688-692.

Barberia, J. M., Giner, J., & Cortes-Gallegos, V. (1973). Diurnal variations of plasma testosterone in men. Steroids, 22(5), 615-626.

Barrett, R., Kuzawa, C. W., McDade, T., & Armelagos, G. J. (1998). Emerging and re-emerging infectious diseases: the third epidemiologic transition. Annual review of anthropology, 27(1), 247-271.

Behnke, J. M., Cabaj, W., & Wakelin, D. (1992). Susceptibility of adult Heligmosomoides polygyrus to intestinal inflammatory responses induced by heterologous infection. International journal for parasitology, 22(1), 75-86.

Bentley, G. R., Harrigan, A. M., Campbell, B., & Ellison, P. T. (1993). Seasonal effects on salivary testosterone levels among Lese males of the Ituri Forest, Zaire. American Journal of Human Biology, 5(6), 711-717.

Bennett, B. C. (1992). Hallucinogenic plants of the Shuar and related indigenous groups in Amazonian Ecuador and Peru. Brittonia, 44(4), 483-493.

Bennett, B. C. (2002). Forest products and traditional peoples: Economic, biological, and cultural considerations. In Natural Resources Forum (Vol. 26, No. 4, pp. 293-301). Blackwell Publishing Ltd.

Bennett, B. C., Baker, M. A., & Andrade, P. G. (2002). Ethnobotany of the Shuar of eastern Ecuador. New York Botanical Garden.

Bennett, E.L., & Robinson, J.G. (2000). Hunting for sustainability: the start of a synthesis. In Hunting for Sustainability in Tropical Forests (eds J.G. Robinson & E.L. Bennett), pp. 499–519. Columbia University Press, New York, USA.

Berlin, B., & Berlin, E. A. (1983). Adaptation and ethnozoological classification: Theoretical implications of animal resources and diet of the Aguaruna and Huambisa. In Adaptive responses of native Amazonians (pp. 301-325).

Berlin, E. A., & Markell, E. K. (1977). An assessment of the nutritional and health status of an Aguaruna Jivaro community, Amazonas, Peru. Ecology of Food and Nutrition, 6(2), 69-81.

Bernhardt, P. C., Dabbs Jr, J. M., Fielden, J. A., & Lutter, C. D. (1998). Testosterone changes during vicarious experiences of winning and losing among fans at sporting events. Physiology & Behavior, 65(1), 59-62.

Berry, J. W., Trimble, J. E., Olmedo, E. L. (1986). Assessment of acculturation. In: Lonner, W., Berry, J. W., (eds). Field methods in cross-cultural research. Thousand Oaks, CA: Sage Publications. p 298-324.

Berry, J. W., & Kim, U. (1988). Acculturation and mental health. In: Dasen, P., Berry, J. W., Sartorious, N., (eds). Health and cross-cultural psychological: Towards applications. Thousand Oaks, CA: Sage Publications. p 207-236.

Bethony, J., Brooker, S., Albonico, M., Geiger, S. M., Loukas, A., Diemert, D., & Hotez, P. J. (2006). Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. The Lancet, 367(9521), 1521-1532.

Bhasin, S. (2003). Effects of testosterone administration on fat distribution, insulin sensitivity, and atherosclerosis progression. Clinical infectious diseases, 37(Supplement_2), S142-S149.

Bindon, J. R. (1995). Polynesian responses to modernization: Overweight and obesity in the South Pacific. Social aspects of obesity, 227-251.

Bindon, J. R., Knight, A., Dressler, W. W., & Crews, D. E. (1997). Social context and psychosocial influences on blood pressure among American Samoans. American journal of physical anthropology, 103(1), 7-18.

Biolo, G., Toigo, G., Ciocchi, B., Situlin, R., Iscra, F., Gullo, A., & Guarnieri, G. (1997). Metabolic response to injury and sepsis: changes in protein metabolism. Nutrition, 13(9), 52-57.

Bishop, D. T., Meikle, A. W., Slattery, M. L., Stringham, J. D., Ford, M. H., West, D. W., ... & Rao, D. C. (1988). The effect of nutritional factors on sex hormone levels in male twins. Genetic epidemiology, 5(1), 43-59.

Blackwell, A. D. (2009). Life history trade-offs in growth and immune function: The behavioral and immunological ecology of the Shuar of Amazonian Ecuador, an indigenous population in the midst of rapid economic and ecological change. University of Oregon.

Blackwell, A. D., Pryor, G., Pozo, J., Tiwia, W., & Sugiyama, L. S. (2009). Growth and market integration in Amazonia: a comparison of growth indicators between Shuar, Shiwiar, and nonindigenous school children. American Journal of Human Biology, 21(2), 161-171.

Blackwell, A. D., Snodgrass, J. J., Madimenos, F. C., & Sugiyama, L. S. (2010). Life history, immune function, and intestinal helminths: Trade-offs among immunoglobulin E, C-reactive protein, and growth in an Amazonian population. American Journal of Human Biology, 22(6), 836-848.

Blackwell, A. D., Gurven, M. D., Sugiyama, L. S., Madimenos, F. C., Liebert, M. A., Martin, M. A., ... & Snodgrass, J. J. (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 US NHANES. PLoS Negl Trop Dis, 5(6), e1218.

Bodley, J. H. (2014). Victims of progress. Rowman & Littlefield.

Bogin, B., Varea, C., Hermanussen, M., & Scheffler, C. (2018). Human life course biology: A centennial perspective of scholarship on the human pattern of physical growth and its place in human biocultural evolution. American journal of physical anthropology, 165(4), 834-854.

Boni, M. F., & Feldman, M. W. (2005). Evolution of antibiotic resistance by human and bacterial niche construction. Evolution, 59(3), 477-491.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., & Sorci, G. (2003). Assessing the cost of mounting an immune response. The American Naturalist, 161(3), 367-379.

Boonnop, K., Wanapat, M., Nontaso, N., & Wanapat, S. (2009). Enriching nutritive value of cassava root by yeast fermentation. Scientia Agricola, 66(5), 629-633.

Booth, A., Shelley, G., Mazur, A., Tharp, G., & Kittok, R. (1989). Testosterone, and winning and losing in human competition. Hormones and behavior, 23(4), 556-571.

Boothroyd, L. G., Scott, I., Gray, A. W., Coombes, C. I., & Pound, N. (2013). Male facial masculinity as a cue to health outcomes. Evolutionary psychology, 11(5), 147470491301100508.

Bouman, A., Heineman, M. J., & Faas, M. M. (2005). Sex hormones and the immune response in humans. Human Reproduction Update, 11(4), 411-423.

Bradbury, J. W., & Vehrencamp, S. L. Principles of animal communication. 1998. Sunderland, Massachusetts: Sinauer Google Scholar.

Bremner, J., Bilsborrow, R., Feldacker, C., & Holt, F. L. (2009). Fertility beyond the frontier: indigenous women, fertility, and reproductive practices in the Ecuadorian Amazon. Population and Environment, 30(3), 93-113.

Bribiescas, R. G. (1996). Testosterone levels among Aché hunter-gatherer men: A functional interpretation of population variation among adult males. Human Nature (Hawthorne, N.Y.), 7(2), 163–88.

Bribiescas, R. G. (2001). Reproductive ecology and life history of the human male. American journal of physical anthropology, 116(S33), 148-176.

Bribiescas, R. G. (2009). Men: Evolutionary and life history. Harvard University Press.

Bribiescas, R. G. (2010). An evolutionary and life history perspective on human male reproductive senescence. Annals of the New York Academy of Sciences,1204(1), 54-64.

Bribiescas, R. G., & Hill, K. R. (2010). Circadian variation in salivary testosterone across age classes in Ache Amerindian males of Paraguay. American Journal of Human Biology, 22(2), 216-220.

Brinkmann, U. K. (1994). Economic development and tropical disease. Annals of the New York Academy of Sciences, 740(1), 303-311.

Brown, D. E. (2015). Life Span: Aging and Senescence. In Human Biological Diversity (pp. 197-216). Routledge.

Brown, G. D., Fincher, C. L., & Walasek, L. (2016). Personality, parasites, political attitudes, and cooperation: a model of how infection prevalence influences openness and social group formation. Topics in cognitive science, 8(1), 98-117.

Buchanan, K. L., Catchpole, C. K., Lewis, J. W., & Lodge, A. (1999). Song as an indicator of parasitism in the sedge warbler. Animal behaviour, 57(2), 307-314.

Buitrón, D., Hurtig. A., San Sebastián, M. (2004). Nutritional status of Naporuna children under five in the Amazon region of Ecuador. Revista Panamericana Salud Publica, 15(3), 151–159.

Bundy, D. A. P. (1988). Population ecology of intestinal helminth infections in human communities. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 321(1207), 405-420.

Burnet, F. M. (1951). Viruses. Scientific American, 184(5), 43-51.

Burnet, F. M. (1962). Natural history of infectious disease (3rd edition). Cambridge University Press.

Buss, D. M., & Schmitt, D. P. (1993). Sexual strategies theory: an evolutionary perspective on human mating. Psychological review, 100(2), 204.

Campbell, B.C., Lukas, W. D., & Campbell, K. L. (2001). Reproductive ecology of male immune function and gonadal function. In: Ellison, P. T. (Ed.). Reproductive ecology and human evolution. Transaction Publishers.

Campbell, B., O'Rourke, M. T., & Lipson, S. F. (2003). Salivary testosterone and body composition among Ariaal males. American Journal of Human Biology, 15(5), 697-708.

Campbell, B. C., Gray, P. B., Eisenberg, D. T., Ellison, P., & Sorenson, M. D. (2007). Androgen receptor CAG repeats and body composition among Ariaal men. International journal of andrology, 32(2), 140-148.

Carr, D. L., Pan, W. K., & Bilsborrow, R. E. (2006). Declining fertility on the frontier: The Ecuadorian Amazon. Population and Environment, 28(1), 17.

Carré, J. M., Campbell, J. A., Lozoya, E., Goetz, S. M., & Welker, K. M. (2013). Changes in testosterone mediate the effect of winning on subsequent aggressive behaviour. Psychoneuroendocrinology, 38(10), 2034-2041.

Casto, K. V., & Edwards, D. A. (2016). Testosterone and reconciliation among women: after-competition testosterone predicts prosocial attitudes towards opponents. Adaptive Human Behavior and Physiology, 2(3), 220-233.

Cavender, A., & Albán, M. (1998). Compulsory medical service in Ecuador: the physician's perspective. Social science & medicine, 47(12), 1937-1946.

Centers for Disease Control and Prevention [CDC]. 2011. Neglected Tropical Diseases. Retrieved from http:// http://www.cdc.gov/globalhealth/ntd/

Centers for Disease Control and Prevention [CDC]. 2015. Body Mass Index (BMI). Retrieved from https://www.cdc.gov/healthyweight/assessing/bmi/index.html

Cepon-Robins, T. J., Liebert, M. A., Gildner, T. E., Urlacher, S. S., Colehour, A. M., Snodgrass, J. J., ... & Sugiyama, L. S. (2014). Soil-transmitted helminth prevalence and infection intensity among geographically and economically distinct Shuar communities in the Ecuadorian Amazon. Journal of Parasitology, 100(5), 598-607.

Chaplin, D. D. (2006). 1. Overview of the human immune response. Journal of allergy and clinical immunology, 117(2), S430-S435.

Chen, L., Juon, H. S., & Lee, S. (2012). Acculturation and BMI among Chinese, Korean and Vietnamese adults. Journal of community health, 37(3), 539-546.

Chemes, H. E. (2001). Infancy is not a quiescent period of testicular development. International journal of andrology, 24(1), 2-7.

Christiansen, K. H. (1991). Serum and saliva sex hormone levels in! Kung San men. American journal of physical anthropology, 86(1), 37-44.

Cockburn, T. A. (1971). Infectious diseases in ancient populations. Current Anthropology, 12(1), 45-62.

CODENPE (Consejo de Desarrollo de las Nacionalidades y Pueblos del Ecuador). (2012). Nacionalidad Indígena Shuar.

Coetzee, V., Faerber, S. J., Greeff, J. M., Lefevre, C. E., Re, D. E., & Perrett, D. I. (2012). African perceptions of female attractiveness. PloS one, 7(10), e48116.

Cohen, M. N., & Crane-Kramer, G. M. M. (Eds.). (2007). Ancient health: skeletal indicators of agricultural and economic intensification. University Press of Florida.

Colehour, A. M., Meadow, J. F., Liebert, M. A., Cepon-Robins, T. J., Gildner, T. E., Urlacher, S. S., ... & Sugiyama, L. S. (2014). Local domestication of lactic acid bacteria via cassava beer fermentation. PeerJ, 2, e479.

Cooke, R. R., McIntosh, J. E. A., & McIntosh, R. P. (1993). Circadian variation in serum free and non-SHBG-bound testosterone in normal men: measurements, and simulation using a mass action model. Clinical endocrinology, 39(2), 163-171.

Cox, C. R., & Le Boeuf, B. J. (1977). Female incitation of male competition: a mechanism in sexual selection. The American Naturalist, 111(978), 317-335.

Cronin, H. (1991). The ant and the peacock: altruism and sexual selection from Darwin to to-day.

Darwin, C. (1859). On the origin of the species by natural selection.

Darwin, C. (1871). The descent of man and selection in relation to sex (Vol. 1). Murray.

Davis, S., & Partridge, W. (1994). Promoting the development of indigenous peoples in Latin America. Finance and Development, 31(31), 38-40.

Davis, J., Gray, C., & Bilsborrow, R. (2015). Delayed Fertility Transition among Indigenous Women: A Case Study in the Ecuadoran Amazon. International perspectives on sexual and reproductive health, 41(1), 1.

De Onis, M., Monteiro, C., Akré, J., & Clugston, G. (1993). The worldwide magnitude of protein-energy malnutrition: an overview from the WHO Global Database on Child Growth. Bulletin of the World health Organization, 71(6), 703-712.

De Velasco, J. (1841). Historia del reino de Quito en la América meridional: Impr. del gobierno.

De Zegher, F., Devlieger, H., & Veldhuis, J. D. (1992). Pulsatile and sexually dimorphic secretion of luteinizing hormone in the human infant on the day of birth. Pediatric research, 32(5), 605.

DeBruine, L. M., Jones, B. C., Little, A. C., Boothroyd, L. G., Perrett, D. I., Penton-Voak, I. S., ... & Tiddeman, B. P. (2006). Correlated preferences for facial masculinity and ideal or actual partner's masculinity. Proceedings of the Royal Society of London B: Biological Sciences, 273(1592), 1355-1360.

Demas, G. E., Chefer, V., Talan, M. I., & Nelson, R. J. (1997). Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 273(5), R1631-R1637.

Demas, G., & Nelson, R. (Eds.). (2012). Ecoimmunology. Oxford University Press.

Dennett, G., Connell, J., Allen, B. J., Basu, A., Clarke, W. C., Jenkins, C., ... & Schatzkin, A. (1988). Acculturation and Health in the Highlands of Papua New Guinea: Dissent on Diversity, Diets, and Development [and Comments and Reply]. Current Anthropology, 29(2), 273-299.

Denson, T. F., Mehta, P. H., & Tan, D. H. (2013). Endogenous testosterone and cortisol jointly influence reactive aggression in women. Psychoneuroendocrinology, 38(3), 416-424.

Derting, T. L., & Compton, S. (2003). Immune response, not immune maintenance, is energetically costly in wild white-footed mice (Peromyscus leucopus). Physiological and Biochemical Zoology, 76(5), 744-752.

Descola, P. (1994). In the society of nature: A native ecology in Amazonia. Cambridge: Cambridge University Press.

Descola, P. (1996). The spears of twilight: life and death in the Amazon jungle. London: Harper Collins Publishers.

Deurenberg, P., van der Kooij, K., Evers, P., & Hulshof, T. (1990). Assessment of body composition by bioelectrical impedance in a population aged greater than 60 y. The American journal of clinical nutrition, 51(1), 3-6.

Dilworth, L. L., Brown, K. J., & Asemota, H. N. (2013). Bioavailability of essential minerals from staples commonly consumed in the tropics. Journal of Food Research, 2(2), 83.

Dold, C., & Holland, C. V. (2011). Ascaris and ascariasis. Microbes and infection, 13(7), 632-637.

Doucet, S. M., & Montgomerie, R. (2003). Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. Behavioral Ecology, 14(4), 503-509.

Drafta, D., Schindler, A. E., Stroe, E., & Neacşu, E. (1982). Age-related changes of plasma steroids in normal adult males. Journal of steroid biochemistry, 17(6), 683-687.

Dressler, W. W., Mata, A., Chavez, A., & Viteri, F. E. (1987). Arterial blood pressure and individual modernization in a Mexican community. Social Science & Medicine, 24(8), 679-687.

Dressler, W. W. (1988). Social consistency and psychological distress. Journal of Health and Social Behavior, 79-91.

Dressler, W. W. (1999). Modernization, stress, and blood pressure: new directions in research. Human Biology, 583-605.

Dufour, D. L. (1992). Nutritional ecology in the tropical rain forests of Amazonia. American Journal of Human Biology, 4(2), 197-207.

Dunkel, L., & Huhtaniemi, I. (1990). Testicular responsiveness to hCG during infancy measured by salivary testosterone. Acta endocrinologica, 123(6), 633-636.

Dunlap, K. D., & Schall, J. J. (1995). Hormonal alterations and reproductive inhibition in male fence lizards (Sceloporus occidentalis) infected with the malarial parasite Plasmodium mexicanum. Physiological Zoology, 68(4), 608-621.

Dym, M., & Orenstein, J. (1990). Structure of the male reproductive tract in AIDS patients. In: Alexander, N. J., Gabelnick, H. L., Spieler, J. M. (ed). Heterosexual transmission of AIDS. New York: Alan R. Liss. p181–196.

Echeverría, R. (2002). Sistema Nacional de Salud. Quito, Ecuador:Ministerio de Salud Publica.

Elia, M. (1992). Organ and tissue contribution to metabolic rate. Energy Metabolism, Tissue Determinants and Cellular Corollaries, 61-80.

Elias, M. (1981). Serum cortisol, testosterone, and testosterone-binding globulin responses to competitive fighting in human males. Aggressive Behavior, 7(3), 215-224.

Ellison, P. T., Panter-Brick, C., Lipson, S. F., & O'Rourke, M. T. (1993). The ecological context of human ovarian function. Human Reproduction, 8(12), 2248-2258.

Ellison, P. T., & Panter-Brick, C. (1996). Salivary testosterone levels among Tamang and Kami males of central Nepal. Human Biology, 955-965.

Ellison, P. T. (2001). Introduction. In: Ellison, P. T. (Ed.). Reproductive ecology and human evolution. Transaction Publishers.

Ellison, P. T., Bribiescas, R. G., Bentley, G. R., Campbell, B. C., Lipson, S. F., Panter-Brick, C., & Hill, K. (2002). Population variation in age-related decline in male salivary testosterone. Human Reproduction, 17(12), 3251-3253.

Ellison, P. T. (2003). Energetics and reproductive effort. American Journal of Human Biology, 15(3), 342-351.

Ellison, P. T. (2008). Energetics, reproductive ecology, and human evolution. Paleoanthropology 2008, 172–200.

Ellison, P. T. (2009). Social relationships and reproductive ecology. In: Ellison, P. T., & Gray, P. B. (Eds.). Endocrinology of social relationships. Harvard University Press.

Eraud, C., Duriez, O., Chastel, O., & Faivre, B. (2005). The energetic cost of humoral immunity in the Collared Dove, Streptopelia decaocto: is the magnitude sufficient to force energy-based trade-offs?. Functional Ecology, 19(1), 110-118.

Escobedo, G., De León-Nava, M. A., & Morales-Montor, J. (2010). Sex differences in parasitic infections: beyond the dogma of female-biased resistance. In Sex Hormones and Immunity to Infection (pp. 187-204). Springer Berlin Heidelberg.

Ewald, P. W. (1983). Host-parasite relations, vectors, and the evolution of disease severity. Annual Review of Ecology and Systematics, 14. 465-485.

Ewald, P. W. (1995). The evolution of virulence: A unifying link between parasitology and ecology Journal of Parasitology, 81, 659-669.

Ewald, P. W. (2002). Plague time: the new germ theory of disease (pp. 282-p). New York: Anchor Books.

Fauci, A. S. (2001). Infectious diseases: considerations for the 21st century. Clinical Infectious Diseases, 32(5), 675-685.

Ferraro, K. F., Thorpe Jr, R. J., & Wilkinson, J. A. (2003). The life course of severe obesity: does childhood overweight matter?. The Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 58(2), S110-S119.

Fincher, C. L., Thornhill, R., Murray, D. R., & Schaller, M. (2008). Pathogen prevalence predicts human cross-cultural variability in individualism/collectivism. Proceedings of the Royal Society of London B: Biological Sciences, 275(1640), 1279-1285.

Fincher, C. L., & Thornhill, R. (2008). A parasite-driven wedge: infectious diseases may explain language and other biodiversity. Oikos, 117(9), 1289-1297.

Fink, B., Grammer, K., & Matts, P. J. (2006). Visible skin color distribution plays a role in the perception of age, attractiveness, and health in female faces. Evolution and Human Behavior, 27(6), 433-442.

Fink, B., Bunse, L., Matts, P. J., & D’Emiliano, D. (2012). Visible skin colouration predicts perception of male facial age, health and attractiveness. International Journal of Cosmetic Science, 34(4), 307-310.

Fish, E. N. (2008). The X-files in immunity: sex-based differences predispose immune responses. Nature Reviews Immunology, 8(9), 737-744.

Fisher, R. A. (1915). The evolution of sexual preference. Eugenics Review, 7,115-123.

Fisher, R. A. (1930). The Genetical Theory of Natural Selection. Clarendon Press, Oxford, UK.

Fitton, L. J. (2000). Helminthiasis and culture change among the Cofán of Ecuador. American Journal of Human Biology: The Official Journal of the Human Biology Association, 12(4), 465-477.

Folstad, I., & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. The American Naturalist, 139(3), 603-622.

Fontana, L., & Klein, S. (2007). Aging, adiposity, and calorie restriction. Jama, 297(9), 986-994.

Frederick, D. A., & Haselton, M. G. (2007). Why is muscularity sexy? Tests of the fitness indicator hypothesis. Personality and Social Psychology Bulletin, 33(8), 1167-1183.

Freeman, S., & Herron, J. C. (2007). Evolutionary analysis (No. QH 366.2. F73 2007). Upper Saddle River, New Jersey: Pearson Prentice Hall.

Frisancho, A. R. (1990). Anthropometric standards for the assessment of growth and nutritional status. University of Michigan Press.

Furman, D., Hejblum, B. P., Simon, N., Jojic, V., Dekker, C. L., Thiébaut, R., ... & Davis, M. M. (2014). Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proceedings of the National Academy of Sciences, 111(2), 869-874.

Futuyma, D. J. (2009). Natural selection and adaptation. Evolution. Massachusetts, USA: Sinauer Associates, Inc. Sunderland, 279-301.

Gage, K. L., & Kosoy, M. Y. (2005). Natural history of plague: perspectives from more than a century of research. Annu. Rev. Entomol., 50, 505-528.

Gallup Jr, G. G., & Frederick, D. A. (2010). The science of sex appeal: An evolutionary perspective. Review of General Psychology, 14(3), 240.

Gangestad, S. W., & Buss, D. M. (1993). Pathogen prevalence and human mate preferences. Evolution and Human Behavior, 14(2), 89-96.

Gangestad, S. W., & Thornhill, R. (2003). Fluctuating asymmetry, developmental instability, and fitness: Toward model-based interpretation. Developmental instability: Causes and consequences, 62-80.

Gangestad, S. W., & Scheyd, G. J. (2005). The evolution of human physical attractiveness. Annu. Rev. Anthropol., 34, 523-548.

Gangestad, S. W., Thornhill, R., & Garver-Apgar, C. E. (2010). Men's facial masculinity predicts changes in their female partners' sexual interests across the ovulatory cycle, whereas men's intelligence does not. Evolution and Human Behavior, 31(6), 412-424.

GBD. (2016). Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–20215: a systematic analysis for the Global Burden of Disease Study 2015. Lancet, 388(10053), 1603–1658.

Gems, D. (2014). Evolution of sexually dimorphic longevity in humans. Aging (Albany NY), 6(2), 84.

Gerique, A. (2010). Biodiversity as a resource: plant use and land use among the Shuar, Saraguros, and Mestizos in tropical rainforest areas of southern Ecuador. Institute of Geography. Friedrich-Alexander Universität, Erlangen-Nürnberg, 429.

Gettler, L. T., Agustin, S. S., & Kuzawa, C. W. (2010). Testosterone, physical activity, and somatic outcomes among Filipino males. American journal of physical anthropology, 142(4), 590-599.

Gettler, L. T., McDade, T. W., Feranil, A. B., & Kuzawa, C. W. (2011). Longitudinal evidence that fatherhood decreases testosterone in human males. Proceedings of the National Academy of Sciences, 108(39), 16194-16199.

Gettler, L. T., McDade, T. W., Agustin, S. S., Feranil, A. B., & Kuzawa, C. W. (2013). Do testosterone declines during the transition to marriage and fatherhood relate to men's sexual behavior? Evidence from the Philippines. Hormones and Behavior, 64(5), 755-763.

Gettler, L. T. (2014a). Applying socioendocrinology to evolutionary models: fatherhood and physiology. Evolutionary Anthropology: Issues, News, and Reviews, 23(4), 146-160.

Gettler, L. T., McDade, T. W., Agustin, S. S., Feranil, A. B., & Kuzawa, C. W. (2014b). Testosterone, immune function, and life history transitions in Filipino males (Homo sapiens). International Journal of Primatology, 35(3-4), 787-804.

Gettler, L. T. (2016). Becoming DADS: considering the role of cultural context and developmental plasticity for paternal socioendocrinology. Current Anthropology, 57(S13), S38-S51.

Gildner, T. E., Cepon-Robins, T. J., Liebert, M. A., Urlacher, S. S., Snodgrass, J. J., Madimenos, F. C., Sugiyama, L. S. (2016a). Regional variation in Ascaris lumbricoides and Trichuris trichiura infections by age cohort and sex: effects of market integration among the indigenous Shuar of Amazonian Ecuador. Journal of Physiological Anthropology, 35(1), 28.

Gildner, T. E., Liebert, M. A., Bribiescas, R. G., Sugiyama, L. S., Urlacher, S. S., Cepon-Robins, T. J., Madimenos, F. C., and Snodgrass, J. J. (2016b). Diurnal testosterone variation among indigenous Shuar men from Amazonian Ecuador.

Poster presentation at the annual meeting of the American Association of Physical Anthropology, Atlanta, GA.

Gladue, B. A., Boechler, M., & McCaul, K. D. (1989). Hormonal response to competition in human males. Aggressive behavior, 15(6), 409-422.

Gluckman, P. D., Hanson, M. A., & Beedle, A. S. (2007). Early life events and their consequences for later disease: a life history and evolutionary perspective. American Journal of Human Biology, 19(1), 1-19.

Godoy, R., & Cardenas, M. (2000). Markets and the health of indigenous people: A methodological contribution. Human Organization, 59(1), 117-124.

Godoy, R. (2001). Indians, rain forests, and markets: theory, methods, and analysis. New York: Columbia University Press.

Godoy, R., Reyes-García, V., Huanca, T., Leonard, W. R., Vadez, V., Valdés-Galicia, C., & Zhao, D. (2005). Why do subsistence-level people join the market economy? Testing hypotheses of push and pull determinants in Bolivian Amazonia. Journal of Anthropological Research, 61(2), 157-178.

Gohdes, D. (1995). Diabetes in North American Indians and Alaska Natives. Atlantic, 43, 87.

Gould, D. C., & Petty, R. (2000). The male menopause: does it exist?: for: some men need investigation and testosterone treatment. Western Journal of Medicine, 173(2), 76.

Gracey, M., King, M. (2009). Indigenous health part 1: determinants and disease patterns. The Lancet, 374, 65–75

Grafen, A. (1990). Biological signals as handicaps. Journal of theoretical biology, 144(4), 517-546.

Grammer, K., & Thornhill, R. (1994). Human (Homo sapiens) facial attractiveness and sexual selection: the role of symmetry and averageness. Journal of comparative psychology, 108(3), 233.

Graves, T. D., Graves, N. B. (1984). Stress and health: Modernization in a traditional Polynesian society. Medical Anthropology, 3, 23-60.

Gray, A., Feldman, H. A., McKinlay, J. B., & Longcope, C. (1991). Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. The Journal of Clinical Endocrinology & Metabolism, 73(5), 1016-1025.

Gray, P. B., Kahlenberg, S. M., Barrett, E. S., Lipson, S. F., & Ellison, P. T. (2002). Marriage and fatherhood are associated with lower testosterone in males. Evolution and Human Behavior, 23(3), 193-201.

Gray, P. B., Kruger, A., Huisman, H. W., Wissing, M. P., & Vorster, H. H. (2006). Predictors of South African male testosterone levels: the THUSA study. American Journal of Human Biology, 18(1), 123-132.

Gray, P. B., & Campbell, B. C. (2009). Human male testosterone, pair bonding and fatherhood. Endocrinology of social relationships, 270-293.

Gray, P. B., & Anderson, K. G. (2010). Fatherhood: Evolution and human paternal behavior. Harvard University Press.

Gray, C. L., Bozigar, M., & Bilsborrow, R. E. (2015). Declining use of wild resources by indigenous peoples of the Ecuadorian Amazon. Biological conservation, 182, 270-277.

Grenoble, L. A., & Whaley, L. J. (2006). Saving languages: An introduction to language revitalization. Cambridge University Press.

Grossman, C. J. (1985). Interactions between the gonadal steroids and the immune system. Science, 227(4684), 257-261.

Guégan, J. F., Thomas, F., Hochberg, M. E., de Meeûs, T., & Renaud, F. (2001). Disease diversity and human fertility. Evolution, 55(7), 1308-1314.

Gurven, M., Kaplan, H., Winking, J., Finch, C., & Crimmins, E. M. (2008). Aging and inflammation in two epidemiological worlds. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 63(2), 196-199.

Gurven, M., Hill, K., Hames, R., Kameda, T., McDermott, R., Lupo, K., ... & Hill, K. (2009). Why do men hunt? A reevaluation of “man the hunter” and the sexual division of labor. Current Anthropology, 50(1), 51-74.

Gurven, M., Costa, M., Trumble, B., Stieglitz, J., Beheim, B., Rodriguez, D. E., ... & Kaplan, H. (2016). Health costs of reproduction are minimal despite high fertility, mortality and subsistence lifestyle. Scientific reports, 6, 30056.

Guzman, J. R., Jurado, H. M., & Kron, M. A. (1995). Infectious disease in Ecuador. Journal of travel medicine, 2(2), 89-95.

Haider, A., Gooren, L. J. G., Padungtod, P., & Saad, F. (2010). Improvement of the metabolic syndrome and of non-alcoholic liver steatosis upon treatment of hypogonadal elderly men with parenteral testosterone undecanoate. Experimental and Clinical Endocrinology & Diabetes, 118(03), 167-171.

Hall, J. E., & Guyton, A. C. (2011). Textbook of medical physiology. 12th ed. Saunders. Chapters 74, 75, 80, 81, & 82.

Hamilton, J. B., & Mestler, G. E. (1969). Mortality and survival: comparison of eunuchs with intact men and women in a mentally retarded population. Journal of Gerontology, 24(4), 395-411.

Hamilton, W. D., & Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites?. Science, 218(4570), 384-387.

Hanna, J. M., & Fitzgerald, M. H. (1993). Acculturation and symptoms: a comparative study of reported health symptoms in three Samoan communities. Social Science & Medicine, 36(9), 1169-1180.

Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., & Blackman, M. R. (2001). Longitudinal effects of aging on serum total and free testosterone levels in healthy men. The Journal of Clinical Endocrinology & Metabolism, 86(2), 724-731.

Harner, M. J. (1972). The Jivaro: People of the sacred waterfalls (Vol. 648). Univ of California Press.

Hau, M. (2007). Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays, 29(2), 133-144.

Hausfater, G., & Watson, D. F. (1976). Social and reproductive correlates of parasite ova emissions by baboons. Nature, 262(5570), 688.

Hendricks, J. W. (1988). Power and knowledge: discourse and ideological transformation among the Shuar. American Ethnologist, 15(2), 216-238.

Herricks, J. R., Hotez, P. J., Wanga, V., Coffeng, L. E., Haagsma, J. A., Basáñez, M. G., ... & Fürst, T. (2017). The global burden of disease study 2013: What does it mean for the NTDs?. PLoS neglected tropical diseases, 11(8), e0005424.

Hey, J. A. K., & Klak, T. (1999). From protectionism towards neoliberalism: Ecuador across four adminis- trations (1981–1996). Studies in Comparative International Development, 31(3), 66–97.

Hill, K. (1993). Life history theory and evolutionary anthropology. Evolutionary Anthropology: Issues, News, and Reviews, 2(3), 78-88.

Hill, K., & Kaplan, H. (1999). Life history traits in humans: Theory and empirical studies. Annual Review of Anthropology, 28(1), 397-430.

Hodges-Simeon, C. R., Gurven, M., Puts, D. A., & Gaulin, S. J. (2014). Vocal fundamental and formant frequencies are honest signals of threat potential in peripubertal males. Behavioral Ecology, 25(4), 984-988.

Holmbeck, G. N. (2002). Post-hoc probing of significant moderational and mediational effects in studies of pediatric populations. Journal of pediatric psychology, 27(1), 87-96.

Hooper, P. L. (2011). The structure of energy production and redistribution among Tsimane' forager-horticulturalists. PhD Dissertation, Evolutionary Anthropology, University of New Mexico.

Horton, R., Wilming, L., Rand, V., Lovering, R. C., Bruford, E. A., Khodiyar, V. K., ... & Wain, H. M. (2004). Gene map of the extended human MHC. Nature Reviews Genetics, 5(12), 889.

Hotez, P. J., Molyneux, D. H., Fenwick, A., Ottesen, E., Sachs, S. E., & Sachs, J. D. (2006). Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. PLoS Med, 3(5), e102.

Hotez, P. J., Bottazzi, M. E., Franco-Paredes, C., Ault, S. K., & Periago, M. R. (2008). The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. PLoS Negl Trop Dis, 2(9), e300.

Hotez, P. J., & Kamath, A. (2009). Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden. PLoS Negl Trop Dis, 3(8), e412.

Hotez, P. J., Alvarado, M., Basáñez, M. G., Bolliger, I., Bourne, R., Boussinesq, M., ... & Carabin, H. (2014). The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. PLoS neglected tropical diseases, 8(7), e2865.

Hotez, P. J. (2016). Blue marble health: an innovative plan to fight diseases of the poor amid wealth. JHU Press.

Hotez, P. J. (2017). The poverty-related neglected diseases: Why basic research matters. PLoS biology, 15(11), e2004186.

Houck, K., Sorensen, M. V., Lu, F., Alban, D., Alvarez, K., Hidobro, D., ... & Ona, A. I. (2013). The effects of market integration on childhood growth and nutritional status: The dual burden of under-and over-nutrition in the Northern Ecuadorian Amazon. American Journal of Human Biology, 25(4), 524-533.

Hurtado, A.M., Frey, M., Hill, K., Hurtado, I., Baker, J. (2008). The role of helminths in human evolution: implications for global health in the 21st century. In: Elton S, O'Higgins P (Eds.). Medicine and Evolution: Current Applications, Future Prospects. New York: Taylor and Francis. p 83-97.

Huss-Ashmore, R., Schall, J., & Hediger, M. (Eds.). (1992). Health and lifestyle change (Vol. 9). UPenn Museum of Archaeology.

IMF. (2000). Ecuador and the IMF. Retrieved from https://www.imf.org/external/np/speeches/2000/051900.htm

Index Mundi. (2015). Ecuador Inflation Rate (consumer Prices). Retrieved from http://www.indexmundi.com/ecuador/inflation_rate_(consumer_prices).html

INEC. (2001). Situacion de la salud en el Ecuador: Indicadores basicos por region y provincia. Quito: Instituto Nacional de Estadistica y Censos (INEC). Poblacion y principales causas de muerte por provincia de residencia habitiual de la person fallecida y sexo. Tasa de mortalidad por 10000 habitantes.

INEC. (2010). Población y Demografía. Retrieved from http://www.ecuadorencifras.gob.ec/censo-de-poblacion-y-vivienda/

Jackson, J. A., Friberg, I. M., Little, S., & Bradley, J. E. (2009). Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies?. Immunology, 126(1), 18-27.

James, G. D., Baker, P. T., Jenner, D. A., & Harrison, G. A. (1987). Variation in lifestyle characteristics and catecholamine excretion rates among young Western Samoan men. Social Science & Medicine, 25(9), 981-986.

Janssen, I., Katzmarzyk, P. T., & Ross, R. (2002). Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines. Archives of internal medicine, 162(18), 2074-2079.

Jarrett, E., & Bazin, H. (1974). Elevation of total serum IgE in rats following helminth parasite infection. Nature, 251(5476), 613.

Jasienska, G., Thune, I., & Ellison, P. T. (2000). Energetic factors, ovarian steroids and the risk of breast cancer. European Journal of Cancer Prevention,9(4), 231-240.

Jasienska, G. (2001). Why energy expenditure causes reproductive suppression in women. An evolutionary and bioenergetic perspective. In: Ellison, P. T. (Ed.). Reproductive ecology and human evolution. Transaction Publishers.

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

Jasieńska, G., Ziomkiewicz, A., Ellison, P. T., Lipson, S. F., & Thune, I. (2004). Large breasts and narrow waists indicate high reproductive potential in women. Proceedings of the Royal Society B: Biological Sciences, 271(1545), 1213.

Jasienska, G. (2009). Reproduction and lifespan: Trade-offs, overall energy budgets, intergenerational costs, and costs neglected by research. American Journal of Human Biology, 21(4), 524-532.

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

Jenny, N. S., Yanez, N. D., Psaty, B. M., Kuller, L. H., Hirsch, C. H., & Tracy, R. P. (2007). Inflammation biomarkers and near-term death in older men. American Journal of Epidemiology, 165(6), 684-695.

Jiménez de La Espada, M. (1965). Relaciones geográficas de Indias. Madrid: Ediciones Atlas 1.

Johnstone, R. A. (1995). Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. Biological Reviews, 70(1), 1-65.

Jokisch, B.D., McSweeney, K. (2011). Assessing the potential of indigenous run demographic/health surveys: The 2005 Shuar survey, Ecuador. Hum Ecol 39:683–698.

Jones, B. C., Feinberg, D. R., Watkins, C. D., Fincher, C. L., Little, A. C., & DeBruine, L. M. (2012). Pathogen disgust predicts women’s preferences for masculinity in men’s voices, faces, and bodies. Behavioral Ecology, 24(2), 373-379.

Kalick, S. M., Zebrowitz, L. A., Langlois, J. H., & Johnson, R. M. (1998). Does human facial attractiveness honestly advertise health? Longitudinal data on an evolutionary question. Psychological science, 9(1), 8-13.

Kalinchenko, S. Y., Tishova, Y. A., Mskhalaya, G. J., Gooren, L. J., Giltay, E. J., & Saad, F. (2010). Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: The double‐blinded placebo‐controlled Moscow study. Clinical endocrinology, 73(5), 602-612.

Kaplan, H., Hill, K., Lancaster, J., & Hurtado, A. M. (2000). A theory of human life history evolution: diet, intelligence, and longevity. Evolutionary Anthropology: Issues, News, and Reviews, 9(4), 156-185.

Kaplan, H. S., & Gangestad, S. W. (2005). Life history theory and evolutionary psychology. The handbook of evolutionary psychology, 68-95.

Kapoor, D., Goodwin, E., Channer, K. S., & Jones, T. H. (2006). Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. European Journal of Endocrinology, 154(6), 899-906.

Kapoor, D., Clarke, S., Stanworth, R., Channer, K. S., & Jones, T. H. (2007). The effect of testosterone replacement therapy on adipocytokines and C-reactive protein in hypogonadal men with type 2 diabetes. European Journal of Endocrinology, 156(5), 595-602.

Karsten, R. (1935). Headhunters of western Amazonas: the life and culture of the Jı´baro Indians of eastern Ecuador and Peru. Commentationes Humanarum Littararum VII(I). Helsinki: Societas Scientiarum Fennica.

Katz, N., Chaves, A., & Pellegrino, J. (1972). A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo, 14(6), 397-400.

Kavaliers, M., Colwell, D. D., Ossenkopp, K. P., & Perrot-Sinal, T. S. (1997). Altered responses to female odors in parasitized male mice: neuromodulatory mechanisms and relations to female choice. Behavioral Ecology and Sociobiology, 40(6), 373-384.

Kealey, A. (2010). Neglected tropical diseases: infection, modeling, and control. Journal of health care for the poor and underserved, 21(1), 53-69.

Khaw, K. T., & Barrett-Connor, E. (1988). The association between blood pressure, age, and dietary sodium and potassium: a population study. Circulation, 77(1), 53-61.

Kidd, S. A., Eskenazi, B., & Wyrobek, A. J. (2001). Effects of male age on semen quality and fertility: a review of the literature. Fertility and sterility, 75(2), 237-248.

Kidon, M. I., Stein, M., Geller-Bernstein, C., Steinberg, S., Handzel, Z. T., & Bentwich, Z. (2005). Serum immunoglobulin E levels in Israeli-Ethiopian children: environment and genetics. group, 4(10), 15.

Kiesecker, J. M., Skelly, D. K., Beard, K. H., & Preisser, E. (1999). Behavioral reduction of infection risk. Proceedings of the National Academy of Sciences, 96(16), 9165-9168.

Kim, H. K., Tiberio, S. S., Capaldi, D. M., Shortt, J. W., Squires, E. C., & Snodgrass, J. J. (2015). Intimate partner violence and diurnal cortisol patterns in couples. Psychoneuroendocrinology, 51, 35-46.

King, C. L., & Nutman, T. B. (1993). IgE and IgG subclass regulation by IL-4 and IFN-gamma in human helminth infections. Assessment by B cell precursor frequencies. The Journal of Immunology, 151(1), 458-465.

King, M. (2009). An overall approach to health care for indigenous peoples. Pediatric Clinical North America, 56, 1239–42.

Kirchengast, S., & Christiansen, K. (2017). Androgen Levels and Body Size Are Associated with Directional as Well as Fluctuating Asymmetry Patterns in Adult! Kung San and Kavango Males from Northern Namibia. Symmetry, 9(5), 72.

Kirkwood, T. B. (1979). The evolution of ageing and longevity. Proc. R. Soc. Lond. B, 205(1161), 531-546.

Klein, S. L., & Nelson, R. J. (1999). Influence of social factors on immune function and reproduction. Reviews of Reproduction, 4(3), 168-178.

Klein, S. L. (2000). The effects of hormones on sex differences in infection: from genes to behavior. Neuroscience & Biobehavioral Reviews, 24(6), 627-638.

Klein, S. L. (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite immunology, 26(6-7), 247-264.

Klein, S. L. (2008). Sex Differences in Infectious and Autoimmune Diseases. In: Becker, J. B. et al. (Eds.). Sex Differences in the Brain from Genes to Behavior. New York: Oxford University Press p 329-353.

Kley, H. K., Deselaers, T., Peerenboom, H., & Kruskemper, H. L. (1980). Enhanced conversion of androstenedione to estrogens in obese males. The Journal of Clinical Endocrinology & Metabolism, 51(5), 1128-1132.

Kley, H. K., Deselaers, T., & Peerenboom, H. (1981). Evidence for hypogonadism in massively obese males due to decreased free testosterone. Hormone and Metabolic Research, 13(11), 639-641.

Kokko, H., & Brooks, R. (2003, January). Sexy to die for? Sexual selection and the risk of extinction. In Annales Zoologici Fennici (pp. 207-219). Finnish Zoological and Botanical Publishing Board.

Kroeger, A. (1980). Housing and health in the process of cultural adaptation: a case study among jungle and highland natives of Ecuador. The Journal of tropical medicine and hygiene, 83(2), 53-69.

Kruger, D. J. (2008). Male financial consumption is associated with higher mating intentions and mating success. Evolutionary Psychology, 6(4), 147470490800600407.

Kudielka, B. M., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. Psychoneuroendocrinology, 28(1), 35-47.

Kuzawa, C. W., Gettler, L. T., Muller, M. N., McDade, T. W., & Feranil, A. B. (2009). Fatherhood, pairbonding and testosterone in the Philippines. Hormones and Behavior, 56(4), 429-435.

Kuzawa, C. W., Tallman, P. S., Adair, L. S., Lee, N., & McDade, T. W. (2012). Inflammatory profiles in the non-pregnant state predict offspring birth weight at Cebu: evidence for inter-generational effects of low grade inflammation. Annals of human biology, 39(4), 267-274.

Lagranja, E. S., Phojanakong, P., Navarro, A., Valeggia, C. R. (2015). Indigenous populations in transition: an evaluation of metabolic syndrome and its associated factors among the Toba of northern Argentina. Annuals of Human Biology, 42, 84–90.

Lancaster, J. B., & Kaplan, H. S. (2009). The endocrinology of the human adaptive complex. In: Ellison PT, Gray PG, editors. Endocrinology of Social Relationships. Cambridge, MA: Harvard University Press. p 95–119.

Laraque, A., Ronchail, J., Cochonneau, G., Pombosa, R., & Guyot, J. L. (2007). Heterogeneous distribution of rainfall and discharge regimes in the Ecuadorian Amazon basin. Journal of hydrometeorology, 8(6), 1364-1381.

Larrea, C., & Kawachi, I. (2005). Does economic inequality affect child malnutrition? The case of Ecuador. Social Science & Medicine, 60(1), 165-178.

Lasry, J. E., & Sheridan, B. W. (1965). CHAGAS ‘MYOCARDITIS AND HEART FAILURE IN THE RED UAKARI Cacajao rubicundus. International Zoo Yearbook, 5(1), 182-184.

Lassek, W. D., & Gaulin, S. J. (2008). Waist-hip ratio and cognitive ability: is gluteofemoral fat a privileged store of neurodevelopmental resources?. Evolution and Human Behavior, 29(1), 26-34.

Lassek, W. D., & Gaulin, S. J. (2009). Costs and benefits of fat-free muscle mass in men: Relationship to mating success, dietary requirements, and native immunity. Evolution and Human Behavior, 30(5), 322-328.

Lee, K. A. (2006). Linking immune defenses and life history at the levels of the individual and the species. Integrative and Comparative Biology, 46(6), 1000-1015.

Lee, S., McCann, D., & Messenger, J. C. (2007). Working Time Around the World: Trends in working hours, laws, and policies in a global comparative perspective (London and Geneva, Routledge and ILO).

Lenk, G., Hansson, J., Beck, O., & Roxhed, N. (2015). The effect of drying on the homogeneity of DBS. Bioanalysis, 7(16), 1977-1985.

Leonard, W. R., Galloway, V. A., Ivakine, E., Osipova, L., & Kazakovtseva, M. (2002). Ecology, health and lifestyle change among the Evenki herders of Siberia. Cambridge Studies in Biological and Evolutionary Anthropology, 206-235.

Leshchinsky, T. V., & Klasing, K. C. (2001). Divergence of the inflammatory response in two types of chickens. Developmental & Comparative Immunology, 25(7), 629-638.

Liebert, M. A., Snodgrass, J. J., Madimenos, F. C., Cepon, T. J., Blackwell, A. D., & Sugiyama, L. S. (2013). Implications of market integration for cardiovascular and metabolic health among an indigenous Amazonian Ecuadorian population. Annals of human biology, 40(3), 228-242.

Liebert, M. A. (2016). Stress and Cultural Change among Indigenous Shuar from Amazonian Ecuador: Integrating Evolutionary Developmental, and Biocultural Perspectives. Retrieved from Scholars' Bank.

Lind, A. (2003). Feminist post-development thought:" women in development" and the gendered paradoxes of survival in Bolivia. Women's Studies Quarterly, 31(3/4), 227-246.

Little, R. J. (1988). A test of missing completely at random for multivariate data with missing values. Journal of the American Statistical Association, 83(404), 1198-1202.

Little, A. C., DeBruine, L. M., & Jones, B. C. (2010). Exposure to visual cues of pathogen contagion changes preferences for masculinity and symmetry in opposite-sex faces. Proceedings of the Royal Society of London B: Biological Sciences, rspb20101925.

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity?. Oikos, 88(1), 87-98.

Lohman, T., Roche, A., Martorell, R. (1988). Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books.

López-Cevallos, D. F., & Chi, C. (2009). Health care utilization in Ecuador: a multilevel analysis of socio-economic determinants and inequality issues. Health policy and planning, czp052

Lopez-Class, M., Castro, F. G., & Ramirez, A. G. (2011). Conceptions of acculturation: A review and statement of critical issues. Social science & medicine, 72(9), 1555-1562.

Lorenzo Garcia, O. C. D. L. (1985). Historia de las Misiones en la Amazonia Ecuatoriano. Edicions Abya Yalla, Quito.

Lourenco, A. E., Santos, R. V., Orellana, J. D., Coimbra, C. E. Jr. (2008). Nutrition transition in Amazonia: obesity and socioeconomic change in the Surui Indians from Brazil. American Journal of Human Biology, 20, 564–71.

Lu, F. (2007). Integration into the market among indigenous peoples: a cross-cultural perspective from the Ecuadorian Amazon. Current anthropology, 48(4), 593-602.

Madimenos, F. C., Snodgrass, J. J., Blackwell, A. D., Liebert, M. A., Cepon, T. J., & Sugiyama, L. S. (2011). Normative calcaneal quantitative ultrasound data for the indigenous Shuar and non-Shuar Colonos of the Ecuadorian Amazon. Archives of osteoporosis, 6(1-2), 39-49.

Madimenos, F. C., Snodgrass, J. J., Liebert, M. A., Cepon, T. J., & Sugiyama, L. S. (2012). Reproductive effects on skeletal health in Shuar women of Amazonian Ecuador: A life history perspective. American Journal of Human Biology, 24(6), 841-852.

Madimenos, F. C., Liebert, M. A., Cepon-Robins, T. J., Snodgrass, J. J., & Sugiyama, L. S. (2015). Determining osteoporosis risk in older Colono adults from rural Amazonian Ecuador using calcaneal ultrasonometry. American Journal of Human Biology, 27(1), 139-142.

Mali, R. G., & Mehta, A. A. (2008). A review on anthelmintic plants. Natural Product Radiance, 7(5): 466-475.

Mårin, P. (1995). Testosterone and regional fat distribution. Obesity, 3(S4).

Marlowe, F., & Wetsman, A. (2001). Preferred waist-to-hip ratio and ecology. Personality and Individual Differences, 30(3), 481-489.

Martin, L. B., Scheuerlein, A., & Wikelski, M. (2003). Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs?. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1511), 153-158.

Mascie-Taylor, C. N., & Peters, J. (Eds.). (2004). The changing face of disease: implications for society. CRC Press.

Matson, K. D., Ricklefs, R. E., & Klasing, K. C. (2005). A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Developmental & Comparative Immunology, 29(3), 275-286.

Mauras, N., Hayes, V., Welch, S., Rini, A., Helgeson, K., Dokler, M., ... & Urban, R. J. (1998). Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. The Journal of Clinical Endocrinology & Metabolism, 83(6), 1886-1892.

Mazur, A., Booth, A., & Dabbs Jr, J. M. (1992). Testosterone and chess competition. Social Psychology Quarterly, 70-77.

Mazur, A., Susman, E. J., & Edelbrock, S. (1997). Sex difference in testosterone response to a video game contest. Evolution and human behavior, 18(5), 317-326.

Mazur, A., & Michalek, J. (1998). Marriage, divorce, and male testosterone. Social Forces, 77(1), 315-330.

McDade, T. W., Stallings, J. F., & Worthman, C. M. (2000). Culture change and stress in Western Samoan youth: methodological issues in the cross-cultural study of stress and immune function. American Journal of Human Biology: The Official Journal of the Human Biology Association, 12(6), 792-802.

McDade, T. W. (2001). Lifestyle incongruity, social integration, and immune function in Samoan adolescents. Social Science & Medicine, 53(10), 1351-1362.

McDade, T. W., & Adair, L. S. (2001). Defining the "urban" in urbanization and health: a factor analysis approach. Social Science & Medicine, 53(1), 55-70.

McDade, T. W. (2002). Status incongruity in Samoan youth: a biocultural analysis of culture change, stress, and immune function. Medical Anthropology Quarterly, 16(2), 123-150.

McDade, T. W. (2003). Life history theory and the immune system: steps toward a human ecological immunology. American journal of physical anthropology, 122(S37), 100-125.

McDade, T. W., Burhop, J., & Dohnal, J. (2004). High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. Clinical chemistry, 50(3), 652-654.

McDade, T. W. (2005). Life history, maintenance, and the early origins of immune function. American Journal of Human Biology, 17(1), 81-94.

McDade, T. W., Williams, S., & Snodgrass, J. J. (2007). What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography, 44(4), 899-925.

McDade, T. W., Rutherford, J. N., Adair, L., & Kuzawa, C. (2008). Adiposity and pathogen exposure predict C-reactive protein in Filipino women. The Journal of nutrition, 138(12), 2442-2447.

McDade, T. W., Nyberg, C. H. (2010). Acculturation and health. In: Muehlenbein, M. P., (ed). Human evolutionary biology. New York: Cambridge University Press. p 581-601.

McDade, T. W. (2012). Early environments and the ecology of inflammation. Proceedings of the National Academy of Sciences, 109(Supplement 2), 17281-17288.

McDade, T. W., Tallman, P. S., Madimenos, F. C., Liebert, M. A., Cepon, T. J., Sugiyama, L. S., & Snodgrass, J. J. (2012). Analysis of variability of high sensitivity C-reactive protein in lowland ecuador reveals no evidence of chronic low-grade inflammation. American Journal of Human Biology, 24(5), 675-681.

McDade, T. W., Georgiev, A. V., & Kuzawa, C. W. (2016). Trade-offs between acquired and innate immune defenses in humans. Evolution, Medicine, and Public Health, 2016(1), 1-16.

McElroy, A. (1990). Biocultural models in studies of human health and adaptation. Medical Anthropology Quarterly, 4(3), 243-265.

Medzhitov, R., Schneider, D. S., & Soares, M. P. (2012). Disease tolerance as a defense strategy. Science, 335(6071), 936-941.

Mehta, P. H., & Josephs, R. A. (2010). Testosterone and cortisol jointly regulate dominance: Evidence for a dual-hormone hypothesis. Hormones and behavior, 58(5), 898-906.

Meikle, A. W., Bishop, D. T., Stringham, J. D., & West, D. W. (1986). Quantitating genetic and nongenetic factors that determine plasma sex steroid variation in normal male twins. Metabolism, 35(12), 1090-1095.

Meikle, A. W., Stringham, J. D., Bishop, D. T., & West, D. W. (1988). Quantitating genetic and nongenetic factors influencing androgen production and clearance rates in men. The Journal of Clinical Endocrinology & Metabolism, 67(1), 104-109.

Migliano, A. B., Vinicius, L., & Lahr, M. M. (2007). Life history trade-offs explain the evolution of human pygmies. Proceedings of the National Academy of Sciences, 104(51), 20216-20219.

Miller, G. F., & Todd, P. M. (1995). The role of mate choice in biocomputation: Sexual selection as a process of search, optimization, and diversification. In Evolution and Biocomputation (pp. 169-204). Springer, Berlin, Heidelberg.

Minchella, D. J. (1985). Host life-history variation in response to parasitism. Parasitology, 90(01), 205-216.

Minority Rights Group International. (2014, August). World Directory of Minorities and Indigenous Peoples – Ecuador. Retrieved from http://www.refworld.org/docid/4954ce3223.html

Mohr, B. A., Bhasin, S., Link, C. L., O’Donnell, A. B., & McKinlay, J. B. (2006). The effect of changes in adiposity on testosterone levels in older men: longitudinal results from the Massachusetts Male Aging Study. European journal of endocrinology, 155(3), 443-452.

Møller, A. P., & Thornhill, R. (1998). Bilateral symmetry and sexual selection: a meta-analysis. The American Naturalist, 151(2), 174-192.

Montanini, V., Simoni, M., Chiossi, G., Baraghini, G. F., Velardo, A., Baraldi, E., & Marrama, P. (1988). Age-related changes in plasma dehydroepiandrosterone sulphate, cortisol, testosterone and free testosterone circadian rhythms in adult men. Hormones, 29(1), 1-6.

Moore, F. R., Cornwell, R. E., Smith, M. L., Al Dujaili, E. A., Sharp, M., & Perrett, D. I. (2010). Evidence for the stress-linked immunocompetence handicap hypothesis in human male faces. Proceedings of the Royal Society of London B: Biological Sciences, rspb20101678.

Morales-Montor, J., Chavarria, A., De Leon, M. A., Del Castillo, L. I., Escobedo, E. G., Sanchez, E. N., ... & Larralde, C. (2004). Host gender in parasitic infections of mammals: an evaluation of the female host supremacy paradigm. Journal of Parasitology, 90(3), 531-546.

Morens, D. M., Folkers, G. K., & Fauci, A. S. (2004). The challenge of emerging and re-emerging infectious diseases. Nature, 430(6996), 242–9.

Morens, D. M., & Fauci, A. S. (2007). The 1918 influenza pandemic: insights for the 21st century. The Journal of infectious diseases, 195(7), 1018-1028.

Moseley, M. E., Nash, D. J., Williams, P. R., Miranda, A., & Ruales, M. (2005). Burning down the brewery: Establishing and evacuating an ancient imperial colony at Cerro Baúl, Peru. Proceedings of the National Academy of Sciences of the United States of America, 102(48), 17264-17271.

Muehlenbein, M. P., & Bribiescas, R. G. (2005). Testosterone-mediated immune functions and male life histories. American Journal of Human Biology, 17(5), 527-558.

Muehlenbein, M. P. (2006). Intestinal parasite infections and fecal steroid levels in wild chimpanzees. American Journal of Physical Anthropology, 130(4), 546-550.

Muehlenbein, M. P., Hirschtick, J. L., Bonner, J. Z., & Swartz, A. M. (2010). Toward quantifying the usage costs of human immunity: altered metabolic rates and hormone levels during acute immune activation in men. American Journal of Human Biology, 22(4), 546-556.

Muehlenbein, M. P., & Watts, D. P. (2010). The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. BioPsychoSocial Medicine, 4(1), 21.

Muller, M. N., & Wrangham, R. W. (2004). Dominance, aggression and testosterone in wild chimpanzees: a test of the 'challenge hypothesis'. Animal Behaviour, 67(1), 113-123.

Muller, M. N., Marlowe, F. W., Bugumba, R., & Ellison, P. T. (2009). Testosterone and paternal care in East African foragers and pastoralists. Proceedings of the Royal Society of London B: Biological Sciences, 276(1655), 347-354.

Müller-Graf, C. D. M., Collins, D. A., & Woolhouse, M. E. J. (1996). Intestinal parasite burden in five troops of olive baboons (Papio cynocephalus anubis) in Gombe Stream National Park, Tanzania. Parasitology, 112(5), 489-497.

Murdock, G. P., & Provost, C. (1973). Factors in the division of labor by sex: A cross-cultural analysis. Ethnology, 12(2), 203-225.

Murray, C. J., Ezzati, M., Flaxman, A. D., Lim, S., Lozano, R., Michaud, C., ... & Wikler, D. (2012). GBD 2010: design, definitions, and metrics. The Lancet, 380(9859), 2063-2066.

Nagata, J. M., Barg, F. K., Valeggia, C. R., Bream, K. D. (2011). Coca-colonization and hybridization of diets among the Tz'utujil Maya. Ecology of Food Nutrition, 50, 297–318.

Nevill, A. M., Stewart, A. D., Olds, T., & Holder, R. (2006). Relationship between adiposity and body size reveals limitations of BMI. American journal of physical anthropology, 129(1), 151-156.

Newton, K., & Dixit, V. M. (2012). Signaling in innate immunity and inflammation. Cold Spring Harbor perspectives in biology, 4(3), a006049.

Ngo, S. T., Steyn, F. J., & McCombe, P. A. (2014). Gender differences in autoimmune disease. Frontiers in neuroendocrinology, 35(3), 347-369.

Nicastri, E., Girardi, E., & Ippolito, G. (2001). Determinants of emerging and re-emerging infectious diseases. Journal of biological regulators and homeostatic agents, 15(3), 212-217.

North, L. (1999). Austerity and Disorder in the Andes. NACLA Report on the Americas, 33(1), 6-9.

Nunn, C. L., Gittleman, J. L., & Antonovics, J. (2000). Promiscuity and the primate immune system. Science, 290(5494), 1168-1170.

Nunn, C. L., & Altizer, S. (2004). Sexual selection, behaviour and sexually transmitted diseases. Sexual selection in primates: new and comparative perspectives. Cambridge University Press, Cambridge, 117-130.

Nyberg, C. H. (2012). Diurnal cortisol rhythms in Tsimane'Amazonian foragers: new insights into ecological HPA axis research. Psychoneuroendocrinology, 37(2), 178-190.

Nylin, S., & Gotthard, K. (1998). Plasticity in life-history traits. Annual review of entomology, 43(1), 63-83.

Oh, J. Y., Barrett-Connor, E., Wedick, N. M., & Wingard, D. L. (2002). Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. Diabetes care, 25(1), 55-60.

Ohenjo, N., Willis, R., Jackson, D., Nettleton, C., Good, K., Mugarura, B. (2006). Health of Indigenous people in Africa. The Lancet, 367, 1937–46

Ong, A. D., Fuller-Rowell, T. E., Bonanno, G. A., & Almeida, D. M. (2011). Spousal loss predicts alterations in diurnal cortisol activity through prospective changes in positive emotion. Health Psychology, 30(2), 220.

Organización Panamericana de la Salud. (2008). Perfil de Sistema de Salud: Ecuador, monitoreo y análisis de los procesos de cambio y reforma. Retrieved from http://www1.paho.org/hq/dmdocuments/2010/Health_System_Profile-Ecuador_2008.pdf

Orr, C. M., Dufour, D. L., & Patton, J. Q. (2001). A comparison of anthropometric indices of nutritional status in Tukanoan and Achuar Amerindians. American Journal of Human Biology, 13, 301-309.

Osei-Atweneboana, M. Y., Lustigman, S., Prichard, R. K., Boatin, B. A., & Basáñez, M. G. (2012). A research agenda for helminth diseases of humans: health research and capacity building in disease-endemic countries for helminthiases control. PLoS neglected tropical diseases, 6(4), e1602.

Palmer, A. R., & Strobeck, C. (1986). Fluctuating asymmetry: measurement, analysis, patterns. Annual review of Ecology and Systematics, 17(1), 391-421.

Pan, W. K. Y., Erlien, C., & Bilsborrow, R. E. (2010). Morbidity and mortality disparities among colonist and indigenous populations in the Ecuadorian Amazon. Social science & medicine, 70(3), 401-411.

Pan-American Health Organization. (2001). Profile of the Health Services System of Ecuador. Washington, DC: Pan-American Health Organization.

Panter-Brick, C., Lotstein, D. S., & Ellison, P. T. (1993). Seasonality of reproductive function and weight loss in rural Nepali women. Human Reproduction, 8(5), 684-690.

Pawłwski, B., & Dunbar, R. I. (1999). Impact of market value on human mate choice decisions. Proceedings of the Royal Society of London B: Biological Sciences, 266(1416), 281-285.

Patz, J. A., Graczyk, T. K., Geller, N., & Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. International journal for parasitology, 30(12-13), 1395-1405.

Pearson, J. D., James, G. D., & Brown, D. E. (1993). Stress and changing lifestyles in the Pacific: Physiological stress responses of Samoans in rural and urban settings. American Journal of Human Biology, 5(1), 49-60.

Penn, D., & Potts, W. K. (1998). Chemical signals and parasite-mediated sexual selection. Trends in Ecology & Evolution, 13(10), 391-396.

Penton-Voak, I. S., Perrett, D. I., Castles, D. L., Kobayashi, T., Burt, D. M., Murray, L. K., & Minamisawa, R. (1999). Menstrual cycle alters face preference. Nature, 399(6738), 741.

Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update. The Journal of clinical investigation, 111(12), 1805-1812.

Perlman, R. (2013). Evolution and medicine. OUP Oxford.

Perreault, T. (2003). Changing places: transnational networks, ethnic politics, and community development in the Ecuadorian Amazon. Political Geography, 22(1), 61-88.

Peters, M., Simmons, L. W., & Rhodes, G. (2008). Testosterone is associated with mating success but not attractiveness or masculinity in human males. Animal Behaviour, 76(2), 297-303.

Petney, T. N. (2001). Environmental, cultural and social changes and their influence on parasite infections. International Journal for Parasitology, 31(9), 919-932.

Piperata, B. A., Spence, J. E., Da-Gloria, P., & Hubbe, M. (2011). The nutrition transition in Amazonia: rapid economic change and its impact on growth and development in Ribeirinhos. American journal of physical anthropology, 146(1), 1-13.

Plotkin, S. A. (Ed.). (2011). History of vaccine development. Springer Science & Business Media.

Plymate, S. R., Tenover, J. S., & Bremner, W. J. (1989). Circadian variation in testosterone, sex hormone-binding globulin, and calculated non-sex hormone-binding globulin bound testosterone in healthy young and elderly men. Journal of andrology, 10(5), 366-371.

Pohle, P., & Reinhardt, S. (2004). Indigenous knowledge of plants and their utilization among the Shuar of the lower tropical mountain forest in southern Ecuador. Lyonia, 7(2), 133-149.

Poulin, R. (1996). Sexual inequalities in helminth infections: a cost of being a male? American Naturalist, 287-295.

Pound, N., Lawson, D. W., Toma, A. M., Richmond, S., Zhurov, A. I., & Penton-Voak, I. S. (2014). Facial fluctuating asymmetry is not associated with childhood ill-health in a large British cohort study. Proceedings of the Royal Society of London B: Biological Sciences, 281(1792), 20141639.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Jama, 286(3), 327-334.

Prall, S. P., & Muehlenbein, M. P. (2014). Testosterone and immune function in primates: a brief summary with methodological considerations. International Journal of Primatology, 35(3-4), 805-824.

Pritchard, J., Després, J. P., Gagnon, J., Tchernof, A., Nadeau, A., Tremblay, A., & Bouchard, C. (1998). Plasma adrenal, gonadal, and conjugated steroids before and after long term overfeeding in identical twins. The Journal of Clinical Endocrinology & Metabolism, 83(9), 3277-3284.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology, 28(7), 916-931.

Puts, D. A. (2010). Beauty and the beast: Mechanisms of sexual selection in humans. Evolution and Human Behavior, 31(3), 157-175.

Puts, D. (2016). Human sexual selection. Current opinion in psychology, 7, 28-32.

Quizhpe, E., San Sebastián, M., Hurtig, A.K., Llamas, A. (2003). Prevalence of anaemia in schoolchildren in the Amazon area of Ecuador. Revista Panamericana de Salud Pública, 13(6), 355–361.

Radcliffe, S. A. (1996). Imaginative geographies, postcolonialism, and national identities: Contemporary discourses of the nation in Ecuador. Ecumene, 3(1), 23–42.

Radcliffe, S. A., & Westwood, S. (1996). Remaking the nation: Place, identity and politics in Latin Americ. London: Routledge.

Rantala, M. J., Coetzee, V., Moore, F. R., Skrinda, I., Kecko, S., Krama, T., ... & Krams, I. (2013). Adiposity, compared with masculinity, serves as a more valid cue to immunocompetence in human mate choice. Proc. R. Soc. B, 280(1751), 20122495.

Resko, J. A., & Eik-Nes, K. B. (1966). Diurnal testosterone levels in peripheral plasma of human male subjects. The Journal of Clinical Endocrinology & Metabolism, 26(5), 573-576.

Rhodes, G., Chan, J., Zebrowitz, L. A., & Simmons, L. W. (2003). Does sexual dimorphism in human faces signal health?. Proceedings of the Royal Society of London B: Biological Sciences, 270(Suppl 1), S93-S95.

Ridker, P. M., Buring, J. E., Cook, N. R., & Rifai, N. (2003). C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation, 107(3), 391-397.

Roberts, C. W., Satoskar, A., & Alexander, J. (1996). Sex steroids, pregnancy-associated hormones and immunity to parasitic infection. Parasitology Today, 12(10), 382-388.

Roberts, M. L., Buchanan, K. L., & Evans, M. R. (2004). Testing the immunocompetence handicap hypothesis: a review of the evidence. Animal Behaviour, 68(2), 227-239.

Roberts, S. B., & Rosenberg, I. (2006). Nutrition and aging: changes in the regulation of energy metabolism with aging. Physiological reviews, 86(2), 651-667.

Robinson, J.G., & Bennett, E.L. (2004). Having your wildlife and eating it too: an analysis of hunting sustainability across tropical ecosystems. Animal Conservation, 7, 397–408.

Roche, M. L., Creed-Kanashiro, H. M., Tuesta, I., & Kuhnlein, H. V. (2008). Traditional food diversity predicts dietary quality for the Awajun in the Peruvian Amazon. Public health nutrition, 11(5), 457-465.

Roe, C. F., & Kinney, J. M. (1965). The caloric equivalent of fever II. Influence of major trauma. Annals of surgery, 161(1), 140.

Roney, J.R. (2009). Sex hormones in the initiation of human mating relationships. In: Ellison, P. T., & Gray, P. B. (Eds.). (2009). Endocrinology of social relationships. Harvard University Press.

Rose, R. M., Kreuz, L. E., Holaday, J. W., Sulak, K. J., & Johnson, C. E. (1972). Diurnal variation of plasma testosterone and cortisol. Journal of endocrinology, 54(1), 177-178.

Ross, K. M., Murphy, M. L., Adam, E. K., Chen, E., & Miller, G. E. (2014). How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. Psychoneuroendocrinology, 39, 184-193.

Rubenstein, S. (2001). Colonialism, the Shuar Federation, and the Ecuadorian state. Environment and planning D: Society and Space, 19(3), 263-293.

Rubenstein, S. L., Calavia, O., Course, M., Crook, T., Mentore, G., & Rubenstein, S. L. (2012). On the importance of visions among the Amazonian Shuar. Current Anthropology, 53(1).

Rubin, D. B. (1978). Multiple imputations in sample surveys-a phenomenological Bayesian approach to nonresponse. In Proceedings of the survey research methods section of the American Statistical Association (Vol. 1, pp. 20-34). American Statistical Association.

Rubin, D. B., & Schenker, N. (1986). Multiple imputation for interval estimation from simple random samples with ignorable nonresponse. Journal of the American statistical Association, 81(394), 366-374.

Rudel, T. K., Bates, D., & Machinguiashi, R. (2002). Ecologically noble Amerindians? Cattle ranching and cash cropping among Shuar and colonists in Ecuador. Latin American Research Review, 144-159.

Salazar, E. (1981). The Federacion Shuar and the Colonization Frontier. In: Cultural Transformations and Ethnicity in Modern Ecuador. Whitten Jr., N. E. (ed.). University of Illinois Press, Urbana, IL. pp 589-613.

San Sebastián, M., Santi, S. (1999). The health status of rural school children in the Amazon basin of Ecuador. Journal of Tropical Pediatrics, 45(6), 379–382. [PubMed: 10667014]

San Sebastián M, Hurtig, A. K. (2007). Review of health research on indigenous populations in Latin America, 1995–2004. Salud Publica Mexicana, 49, 316–20.

Sapolsky, R. M. (1997). The trouble with testosterone. New York: Scribner, p 147-159.

Sattenspiel, L. (2000). Tropical environments, human activities, and the transmission of infectious diseases. American journal of physical anthropology, 113(S31), 3-31.

Schmid-Hempel, P., & Ebert, D. (2003). On the evolutionary ecology of specific immune defense. Trends in Ecology & Evolution, 18(1), 27-32.

Schmid-Hempel, P. (2011). Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. USA: Oxford University Press.

Schmitt, D. P. (2005). Fundamentals of human mating strategies. The handbook of evolutionary psychology.

Scott, I. M., Clark, A. P., Josephson, S. C., Boyette, A. H., Cuthill, I. C., Fried, R. L., ... & Honey, P. L. (2014). Human preferences for sexually dimorphic faces may be evolutionarily novel. Proceedings of the National Academy of Sciences, 111(40), 14388-14393.

Sell, A., Cosmides, L., Tooby, J., Sznycer, D., von Rueden, C., & Gurven, M. (2009). Human adaptations for the visual assessment of strength and fighting ability from the body and face. Proceedings of the Royal Society of London B: Biological Sciences, 276(1656), 575-584.

Sell, A., Hone, L. S., & Pound, N. (2012). The importance of physical strength to human males. Human Nature, 23(1), 30-44.

Seymour-Smith, C. (1988). Shiwiar: Identidad étnica y cambio en el Río Corrientes. Ediciones ABYA-YALA.

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution, 11(8), 317-321.

Shephard, R. J., & Rode, A. (1996). The Health Consequences of 'Modernisation': Evidence from Circumpolar Peoples (Vol. 17). Cambridge University Press.

Shirtcliff, E. A., & Essex, M. J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. Developmental psychobiology, 50(7), 690-703.

Shoenfeld, Y., Tincani, A., & Gershwin, M. E. (2012). Sex gender and autoimmunity. Journal of Autoimmunity, 38(2-3), J71-J73.

Shores, M. M., Smith, N. L., Forsberg, C. W., Anawalt, B. D., & Matsumoto, A. M. (2012). Testosterone treatment and mortality in men with low testosterone levels. The Journal of Clinical Endocrinology & Metabolism, 97(6), 2050-2058.

Singh, D. (1993). Adaptive significance of female physical attractiveness: role of waist-to-hip ratio. Journal of personality and social psychology, 65(2), 293.

Singh, D. (1995). Female judgment of male attractiveness and desirability for relationships: Role of waist-to-hip ratio and financial status. Journal of personality and social psychology, 69(6), 1089.

Smith, J. M., & Harper, D. (2003). Animal signals. Oxford University Press.

Snodgrass, J. J., Leonard, W. R., Sorensen, M. V., Tarskaia, L. A., Alekseev, V. P., Krivoshapkin, V. (2006). The emergence of obesity among indigenous Siberians. Journal of Physiological Anthropology, 25, 75–84.

Snodgrass, J. J., Sorensen, M. V., Tarskaia, L. A., & Leonard, W. R. (2007). Adaptive dimensions of health research among indigenous Siberians. American Journal of Human Biology, 19(2), 165-180.

Snodgrass, J. J. (2012). Human energetics. Human Biology: An Evolutionary and Biocultural Perspective, Second Edition, 325-384.

Snodgrass, J. J. (2013). Health of indigenous circumpolar population. Annual Reviews of Anthropology, 42, 69–87.

Snyder, P. J., Peachey, H., Hannoush, P., Berlin, J. A., Loh, L., Lenrow, D. A., ... & Strom, B. L. (1999). Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. The Journal of Clinical Endocrinology & Metabolism, 84(8), 2647-2653.

Spratt, D. I., O'Dea, L. S., Schoenfeld, D, Butler, J., Rao, P. N., & Crowley Jr, W. F. (1988). Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH, and testosterone. American Journal of Physiology-Endocrinology And Metabolism, 254(5), E658-E666.

Spratt, D. I., Cox, P., Orav, J., Moloney, J., & Bigos, T. (1993). Reproductive axis suppression in acute illness is related to disease severity. The Journal of Clinical Endocrinology & Metabolism, 76(6), 1548-1554.

Spratt, D. I. (2001). Altered gonadal steroidogenesis in critical illness: is treatment with anabolic steroids indicated?. Best Practice & Research Clinical Endocrinology & Metabolism, 15(4), 479-494.

Stearns, S. C. (1977). The evolution of life history traits: a critique of the theory and a review of the data. Annual Review of Ecology and Systematics, 8(1), 145-171.

Stearns, S. C. (1989). Trade-offs in life-history evolution. Functional ecology, 3(3), 259-268.

Stearns, S. C. (2000). Life history evolution: successes, limitations, prospects. Naturwissenschaften, 87(11), 476-486.

Steckel, R. H. (1995). Stature and the Standard of Living. Journal of economic literature, 33(4), 1903-1940.

Steffen, P. R., Smith, T. B., Larson, M., & Butler, L. (2006). Acculturation to Western society as a risk factor for high blood pressure: a meta-analytic review. Psychosomatic medicine, 68(3), 386-397.

Stein, A. D., Thompson, A. M., & Waters, A. (2005). Childhood growth and chronic disease: evidence from countries undergoing the nutrition transition. Maternal & child nutrition, 1(3), 177-184.

Stellato, R. K., Feldman, H. A., Hamdy, O., Horton, E. S., & Mckinlay, J. B. (2000). Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. Diabetes care, 23(4), 490-494.

Stephens, C., Porter, J., Nettleton, C., Willis, R. (2006). Disappearing, displaced, and undervalued: a call to action for Indigenous health worldwide. The Lancet, 367, 2019–28.

Stirling M. 1938. Historical and ethnographical material on the Jivaro Indians. Government Printing Office. Washington, D.C.

Stoehr, A. M., & Kokko, H. (2006). Sexual dimorphism in immunocompetence: what does life-history theory predict?. Behavioral Ecology, 17(5), 751-756.

Strachen, D. P. (1989). Hay fever, hygiene, and household size. British Medical Journal, 299, 1259-1260.

Streeter, S. A., & McBurney, D. H. (2003). Waist–hip ratio and attractiveness: New evidence and a critique of “a critical test”. Evolution and Human Behavior, 24(2), 88-98.

Stutzman, R. (1981). El mestizaje: an all-inclusive ideology of exclusion. In N. E. Whitten Jr. (Ed.), Cultural transformations and ethnicity in modern Ecuador (pp. 45–94). University of Illinois Press: Urbana, IL.

Sugiyama, L. S. (1996). In search of the adapted mind: a study of human psychological adaptations among the Shiwiar of Ecuador and the Yora of Peru. Unpublished PhD dissertation, University of California, Santa Barbara.

Sugiyama, L. S., & Chacon, R. (2000). Effects of illness and injury on foraging among the Yora and Shiwiar: pathology risk as adaptive problem. Human behavior and adaptation: an anthropological perspective, 371-395.

Sugiyama, L. S. (2004). Illness, injury, and disability among Shiwiar forager-horticulturalists: Implications of health-risk buffering for the evolution of human life history. American Journal of Physical Anthropology, 123, 371-389.

Sugiyama, L. S. (2005). Physical attractiveness: An adaptationist perspective. The handbook of evolutionary psychology.

Sugiyama, L. S. (2015). Physical Attractiveness: An Adaptionist Perspective. In: Buss, D. M. (Ed.). The handbook of evolutionary psychology. John Wiley & Sons, p 317.

Sullivan, D. A., & Hann, L. E. (1989). Hormonal influence on the secretory immune system of the eye: endocrine impact on the lacrimal gland accumulation and secretion of IgA and IgG. Journal of steroid biochemistry, 34(1-6), 253-262.

Symons, D. (1979). The evolution of human sexuality. Oxford: Oxford University Press.

Symons, D. (1995). Beauty is in the adaptations of the beholder: The evolutionary psychology of human female sexual attractiveness. Sexual nature, sexual culture, 80-118.

Tang, Y. J., Lee, W. J., Chen, Y. T., Liu, P. H., Lee, M. C., & Sheu, W. H. H. (2007). Serum testosterone level and related metabolic factors in men over 70 years old. Journal of endocrinological investigation, 30(6), 451-458.

Tanner, S., & Mcdade, T. W. (2007). Enzyme immunoassay for total immunoglobulin E in dried blood spots. American Journal of Human Biology, 19(3), 440-442.

Tene, V., Malagón, O., Finzi, P. V., Vidari, G., Armijos, C., & Zaragoza, T. (2007). An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. Journal of Ethnopharmacology, 111(1), 63-81.

Thomson, M. D., & Hoffman-Goetz, L. (2009). Defining and measuring acculturation: a systematic review of public health studies with Hispanic populations in the United States. Social science & medicine, 69(7), 983-991.

Thornhill, R., & Gangestad, S. W. (2003). Do women have evolved adaptation for extra-pair copulation?. In Evolutionary aesthetics (pp. 341-368). Springer, Berlin, Heidelberg.

Thornhill, R., & Gangestad, S. W. (2006). Facial sexual dimorphism, developmental stability, and susceptibility to disease in men and women. Evolution and Human Behavior, 27(2), 131-144.

Thornhill, R., & Fincher, C. L. (2015). The parasite-stress theory of sociality and the behavioral immune system. In Evolutionary perspectives on social psychology (pp. 419-437). Springer, Cham.

Tooby, J., & Cosmides, L. (1992). The psychological foundations of culture. The adapted mind: Evolutionary psychology and the generation of culture, 19.

Travison, T. G., Araujo, A. B., Kupelian, V., O'donnell, A. B., & McKinlay, J. B. (2006). The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. The Journal of Clinical Endocrinology & Metabolism, 92(2), 549-555.

Trinchieri, G. (2007). Interleukin-10 production by effector T cells: Th1 cells show self control. Journal of Experimental Medicine, 204(2), 239-243.

Trumble, B. C., Cummings, D. K., O'Connor, K. A., Holman, D. J., Smith, E. A., Kaplan, H. S., & Gurven, M. D. (2013). Age-independent increases in male salivary testosterone during horticultural activity among Tsimane forager-farmers. Evolution and Human Behavior, 34(5), 350-357.

Trumble, B. C., Blackwell, A. D., Stieglitz, J., Thompson, M. E., Suarez, I. M., Kaplan, H., & Gurven, M. (2016). Associations between male testosterone and immune function in a pathogenically stressed forager-horticultural population. American journal of physical anthropology, 161(3), 494-505.

Tsai, L. W., & Sapolsky, R. M. (1996). Rapid stimulatory effects of testosterone upon myotubule metabolism and sugar transport, as assessed by silicon microphysiometry. Aggressive Behavior, 22(5), 357-364.

Tybur JM, Gangestad SW. 2011. Mate preferences and infectious disease: theoretical considerations and evidence in humans. Philos Trans R Soc [Biol] 366(1583): 3375-3388.

Uchida, A., Bribiescas, R. G., Ellison, P. T., Kanamori, M., Ando, J., Hirose, N., & Ono, Y. (2006). Age related variation of salivary testosterone values in healthy Japanese males. The aging male, 9(4), 207-213.

Udry, J. R., & Talbert, L. M. (1988). Sex hormone effects on personality at puberty. Journal of Personality and Social Psychology, 54(2), 291.

Udry, J. R. (1990). Biosocial models of adolescent problem behaviors. Social Biology, 37(1-2), 1-10.

United Nations Department of Economic and Social Affairs. (1999). The World at Six Billion. Retrieved from the United Nations: http://www.un.org/esa/population/publications/sixbillion/sixbilpart1.pdf

United Nations Department of Economic and Social Affairs. (2017). World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100. Retrieved from the United Nations: https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html

United Nations Statistics Division (UNSD). (2008). United Nations Common Database (UNCDB). New York: UN.

Urlacher, S. S. (2016). Growing Up Shuar: Life History Tradeoffs and Energy Allocation in the Context of Physical Growth Among an Indigenous Amazonian Population (Doctoral dissertation).

Urlacher, S. S., Liebert, M. A., Josh Snodgrass, J., Blackwell, A. D., Cepon-Robins, T. J., Gildner, T. E., ... & Sugiyama, L. S. (2016). Heterogeneous effects of market integration on sub-adult body size and nutritional status among the Shuar of Amazonian Ecuador. Annals of human biology, 43(4), 316-329.

Urlacher, S. S., Ellison, P. T., Sugiyama, L. S., Pontzer, H., Eick, G., Liebert, M. A., ... & Snodgrass, J. J. (2018). Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists. Proceedings of the National Academy of Sciences, 201717522.

Valeggia, C., & Ellison, P. T. (2009). Interactions between metabolic and reproductive functions in the resumption of postpartum fecundity. American Journal of Human Biology, 21(4), 559-566.

Valeggia, C. R., Burke, K. M., & Fernandez-Duque, E. (2010). Nutritional status and socioeconomic change among Toba and Wichí populations of the Argentinean Chaco. Economics & Human Biology, 8(1), 100-110.

Valeggia, C. R., & Snodgrass JJ. (2015). Health of indigenous peoples. Annual Review of Anthropology, 44, 117-135.

Valen, L. V. (1962). A study of fluctuating asymmetry. Evolution, 16(2), 125-142.

Van Anders, S. M., & Watson, N. V. (2006). Relationship status and testosterone in North American heterosexual and non-heterosexual men and women: Cross-sectional and longitudinal data. Psychoneuroendocrinology, 31(6), 715-723.

Van der Heijden, G. J., Donders, A. R. T., Stijnen, T., & Moons, K. G. (2006). Imputation of missing values is superior to complete case analysis and the missing-indicator method in multivariable diagnostic research: a clinical example. Journal of clinical epidemiology, 59(10), 1102-1109.

Viney, M. E., Riley, E. M., & Buchanan, K. L. (2005). Optimal immune responses: immunocompetence revisited. Trends in Ecology & Evolution, 20(12), 665-669.

Waldhauser, F., Weissenbacher, G., Frisch, H., & Pollak, A. (1981). Pulsatile secretion of gonadotropins in early infancy. European journal of pediatrics, 137(1), 71-74.

Walker, R. S., Sattenspiel, L., & Hill, K. R. (2015). Mortality from contact-related epidemics among indigenous populations in Greater Amazonia. Scientific reports, 5, 14032.

Wallen, K., & Hassett, J. (2009). Neuroendocrine mechanisms underlying social relationships. Endocrinology of social relationships, 32-53.

Walsh, J. F., Molyneux, D. H., & Birley, M. H. (1993). Deforestation: effects on vector-borne disease. Parasitology, 106(S1), S55-S75.

Wang, C., Plymate, S., Nieschlag, E., & Paulsen, C. A. (1981). Salivary testosterone in men: further evidence of a direct correlation with free serum testosterone. The Journal of Clinical Endocrinology & Metabolism, 53(5), 1021-1024.

Wasserstrom, R. (2010, October). Roads, oil and native people: A controlled comparison on the Ecuadorian frontier. In Latin American Studies Association (LASA) annual meeting, Toronto, October (Vol. 6).

Wells, J. C. (2007). Sexual dimorphism of body composition. Best practice & research Clinical endocrinology & metabolism, 21(3), 415-430.

Wells, J. C. (2010). The evolutionary biology of human body fatness: thrift and control (Vol. 58). Cambridge University Press.

Wells, J. C. (2012). Sexual dimorphism in body composition across human populations: associations with climate and proxies for short-and long-term energy supply. American Journal of human biology, 24(4), 411-419.

Wetsman, A., & Marlowe, F. (1999). How universal are preferences for female waist-to-hip ratios? Evidence from the Hadza of Tanzania. Evolution and Human behavior, 20(4), 219-228.

Whitehead, R. D., Re, D., Xiao, D., Ozakinci, G., & Perrett, D. I. (2012). You are what you eat: Within-subject increases in fruit and vegetable consumption confer beneficial skin-color changes. PLoS One, 7(3), e32988.

Wilder, R. L. (1998). Hormones, pregnancy, and autoimmune diseases. Annals of the New York Academy of Sciences, 840(1), 45-50.

Wiley, A. S., & Allen, J. S. (2009). Medical anthropology: A biocultural approach. New York: Oxford University Press.

Wingfield, J. C., Hegner, R. E., Dufty Jr, A. M., & Ball, G. F. (1990). The" challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. The American Naturalist, 136(6), 829-846.

Wirsing, R. L., Logan, M. H., Micozzi, M. S., Nyamwaya, D. O., Pearce, T. O., Renshaw, D. C., & Schaefer, O. (1985). The health of traditional societies and the effects of acculturation [and comments and replies]. Current Anthropology, 26(3), 303-322.

Wolowczuk, I., Verwaerde, C., Viltart, O., Delanoye, A., Delacre, M., Pot, B., & Grangette, C. (2008). Feeding our immune system: impact on metabolism. Clinical and Developmental Immunology, 2008.

Wood, W., & Eagly, A. H. (2002). A cross-cultural analysis of the behavior of women and men: Implications for the origins of sex differences. Psychological bulletin, 128(5), 699.

Wood, B. M., & Marlowe, F. W. (2013). Household and kin provisioning by Hadza men. Human Nature, 24(3), 280-317.

World Health Organization (WHO). (2006). Neglected tropical diseases: hidden successes, emerging opportunities. Retrieved from http://apps.who.int/iris/bitstream/10665/69367/1/WHO_CDS_NTD_2006.2_eng.pdf

World Health Organization. (2004). Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet, 363, 157-63.

World Health Organization (WHO) & UNICEF. (2004). Review of National Immunization Coverage, 1980–2003: Ecuador. Geneva, Switzerland: World Health Organization, Department of Immunization Vaccines and Biologicals.

World Health Organization (WHO). (2015). Investing to overcome the global impact of neglected tropical diseases: third WHO report on neglected diseases 2015. Retrieved from WHO: http://apps.who.int/iris/bitstream/10665/152781/1/9789241564861_eng.pdf

World Health Organization (WHO). (2016). Neglected tropical diseases. Retrieved from http://www.who.int/neglected_diseases/diseases/en/

Worm, M., & Henz, B. M. (1997). Molecular regulation of human IgE synthesis. Journal of molecular medicine, 75(6), 440-447.

Yashar, D. (1998). Contesting citizenship: indigenous movements and democracy in Latin America. Com- parative Politics, 31(1), 23–42.

Yu, D. W., & Shepard Jr, G. H. (1998). Is beauty in the eye of the beholder?. Nature, 396(6709), 321.Zuk, M., & McKean, K. A. (1996). Sex differences in parasite infections: patterns and processes. International journal for parasitology, 26(10), 1009-1024.

Zahavi, A., & Zahavi, A. (1997). The Handicap Principle. New York: Oxford University Press.

Zapata-Ríos, G., Urgilés, C., & Suárez, E. (2009). Mammal hunting by the Shuar of the Ecuadorian Amazon: is it sustainable?. Oryx, 43(03), 375-385.

Zhao, Y., Li, M., Sun, Y., Wu, W., Kou, G., Guo, L., ... & Zhao, B. (2017). Life-history dependent relationships between body condition and immunity, between immunity indices in male Eurasian tree sparrows. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 210, 7-13.

Zilioli, S., Caldbick, E., & Watson, N. V. (2014). Testosterone reactivity to facial display of emotions in men and women. Hormones and Behavior, 65(5), 461-468.

Zilioli, S., Sell, A. N., Stirrat, M., Jagore, J., Vickerman, W., & Watson, N. V. (2015). Face of a fighter: Bizygomatic width as a cue of formidability. Aggressive Behavior, 41(4), 322-330.

Zuk, M. (1990). Reproductive strategies and disease susceptibility: an evolutionary viewpoint. Parasitology today, 6(7), 231-233.

Zuk, M., & McKean, K. A. (1996). Sex differences in parasite infections: patterns and processes. International journal for parasitology, 26(10), 1009-1024.

Zuk, M., & Stoehr, A. M. (2002). Immune defense and host life history. The American Naturalist, 160(S4), S9-S22.

Zuk, M. (2009). The sicker sex. PLoS pathogens, 5(1), e1000267.