Testosterone, Energetics, and Male Life-History

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Abstract

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Testosterone-mediated behavior and muscle mass convey advantages to male fitness. However, high-testosterone phenotypes entail energetic and immunologic costs: the physiological and somatic effects of testosterone require significant caloric maintenance, and may disrupt immune function. Human studies of acute testosterone change have focused on male-male competition among healthy young men in industrialized populations with higher, potentially evolutionarily novel, levels of testosterone compared to environmentally stressed subsistence populations. These studies provide little information about other fitness enhancing hormone-behavior interactions in evolutionarily relevant environments. The overarching objective of this dissertation is to examine the conditions and selective pressures that resulted in flexible testosterone response to environmental and social stimuli. Field and laboratory studies were conducted among US and Tsimane men to examine the relative dynamics of acute testosterone change for men engaged in: 1) fasting; 2) physical competition (soccer); 3) non-competitive physically-intensive food production (tree-chopping); 4) non-physical competition (soccer penalty kicks); and 5) a semi-competitive physical food production activity (hunting). Despite lower basal testosterone levels, Tsimane forager-horticulturalists express relative increases in testosterone similar to those seen in industrialized populations. Physical challenges (soccer, tree-chopping, hunting) increased salivary testosterone more than a non-physical challenge (penalty kicks). A competitive challenge (soccer) resulted in increased testosterone, but did not augment testosterone as much as physically intensive food production (tree-chopping). These studies underscore the importance of moving beyond a unidimensional focus on competitive interactions in order to understand the importance of acute testosterone-behavior interactions during other reproductively important activities, providing a broader understanding of human male life-histories and adaptive variation.

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DEDICATION

Dedicated to my family: to my father who taught me about science and my mother who taught me about people; and to Megan Costa, who reminds me why it all matters.

Chapter 1: Introduction

Overview

All organisms face life-history trade-offs in the allocation of scarce energetic resources between survival and reproduction, and hormones are often a mediating force in the distribution of energetic resources. Industrial society relaxes many evolutionary pressures, as its members live in relatively parasite- and pathogen-free environments with excess caloric availability. Thus, in order to understand the range of trade-offs that humans faced in their evolutionary history, field research in populations from varied environmental settings is important. Only then can we hope to understand how natural selection shaped human physiology and behavior.

Human behavior and physiology are inextricably linked: we cannot fully comprehend behavior without appreciating the underlying physiology, nor can we pretend to comprehend human physiology without understanding the behavioral strategies that favored selection for this physiological capacity (Tinbergen 1963). Understanding how environmental conditions impact hormones is central for understanding how hormones interact with behavior. This dissertation combines data from laboratory and field studies with the goal of improving our understanding of acute changes in testosterone across different behavioral domains. It contains five interrelated data papers examining acute changes in testosterone for men engaged in: 1) fasting; 2) physical competition (soccer); 3) non-competitive physical food production (tree-chopping); 4) non-physical competition (penalty kick shootout); and 5) semi-competitive physical food production activity (hunting).

Examining testosterone dynamics in each of these five domains will help parse the conditions under which acute changes in testosterone occur, providing greater understanding of the role of testosterone-behavior interactions in male life-history. The first chapter elucidates the responsiveness of the hypothalamic-pituitary-gonadal axis to a brief fast, providing evidence of rapid decreases in testosterone production due to the reduced energetic availability in US males. The following chapters focus on work conducted among the Tsimane and offer a detailed examination of hormone-behavior interactions in a population with significantly lower levels of testosterone compared to men in industrialized populations. Because most of the research into the role of hormones in human behavior has been conducted in industrialized populations with high, potentially novel levels of testosterone, it is important to examine whether these same hormone-behavior interaction patterns occur in a subsistence population with relatively low testosterone.

The second chapter investigates testosterone changes during male-male competition (soccer match), a domain of behavior important in most mammalian reproductive strategies (Geary 2000). Competition among human males tends to focus less on direct competition, with greater emphasis on resource acquisition and provisioning compared to other mammals (Geary 2000), thus the third chapter measures acute changes in testosterone during a physically intensive horticultural activity (tree-chopping). The fourth chapter examines testosterone changes during non-physical competition (soccer penalty kicks), to further disentangle competition from physical activity. The fifth and final data chapter examines hormonal changes in males engaged in hunting. Hunting is an important male provisioning activity that is associated with reproductive success, and considered by many to be a form of indirect competition (Smith 2004; Gurven and Hill 2009).

Examining acute changes in male testosterone across these five domains contributes to a broader understanding of the conditions in which testosterone change occurs in response to various stressors, ranging from energetic deficits to the relative importance of competitive and non-competitive physical and non-physical tasks.

Theoretical Background

Costs of high testosterone

Testosterone promotes muscle growth, physical performance, and somatic maintenance (Bribiescas 2001), which are beneficial for male reproductive fitness. However, skeletomuscular tissue is energetically costly, with adult human males spending approximately 20% of the basal metabolic rate on muscle tissue development and maintenance (Bribiescas 2010). While potentially beneficial for reproductive fitness, the somatic and physiological effects of high testosterone come with an energetic and immunologic trade-off (Muehlenbein and Bribiescas 2005). Life history theory predicts (Charnov 1993; Ricklefs and Wikelski 2002), and studies support that during energetic stress from reduced caloric intake (Cameron 1996), increased caloric expenditure (Nindl et al. 2007), or parasitic and pathogenic stress (Muehlenbein and Bribiescas 2005; Boonekamp et al. 2008; Muehlenbein and Watts 2010), the male hypothalamic-pituitary-gonadal (HPG) axis will down regulate, reducing testosterone production. There is evidence in avian models that exogenous testosterone can negatively impact immune function, reducing both humeral and cell mediated immune function (Duffy et al. 2000).

Given these life-history trade-offs, we can generate a series of predictions regarding expected levels of testosterone under different environmental conditions. For example, many subsistence populations live in environments with high parasite and pathogen burdens and relatively low energetic availability. In contrast, many industrialized populations exist in relatively parasite and pathogen free settings with excess caloric availability. In these differing environments, we can expect to find variation in male testosterone; life-history theory predicts that individuals in energetically plentiful environments should be able to maintain higher levels of testosterone than those in more challenging environments. What are the physiological and behavioral effects of high testosterone in industrial populations? Do the same hormone-behavior interactions occur in populations with relatively lower baseline testosterone?

Hypothalamic-Pituitary-Gonadal (HPG) Axis

Under normal baseline conditions in primates, male testosterone production is controlled by the HPG axis (Figure 1.1). The hypothalamus releases gonadotropin releasing hormone (GnRH), which stimulates the release of the gonadotropins luteinizing hormone (LH), and follicle stimulating hormone (FSH) from the anterior pituitary. Gonadotropins travel to the testes, where LH stimulates testosterone synthesis in the leydig cells (Nelson 2000). Testosterone is released into the bloodstream, where it travels to target tissues throughout the body. Once homeostatic levels of testosterone are detected by the hypothalamus and pituitary, gonadotropin release is down-regulated, decreasing testosterone production. Most circulating testosterone is bound to the carrier proteins sex hormone binding globulin (SHBG) and albumin, with only a small portion remaining free for use in tissues (Vermeulen et al. 1999b). The bond between testosterone and albumin is relatively weak, and testosterone can be dissociated for use in target tissues (Vermeulen et al. 1999b).

Studies in primates and humans suggest that decreases in testosterone during energetic stress are due to hypothalamic and pituitary down-regulation, resulting in lower levels of LH, and decreased testosterone production (Cameron 1996; Trumble et al. 2010). In humans, an injection of synthetic LH causes an increase in testosterone within 45-75 minutes (Veldhuis and Iranmanesh 2004). However, during competitive situations in laboratory and industrialized settings, increases in salivary testosterone occur within 5-15 minutes (Archer 2006; Pound et al. 2009).

This suggests that acute increases in testosterone during challenge situations may occur via another hormonal mechanism independent of the HPG axis. The endocrine pathway by which acute increases in human testosterone occur has not been established (Flinn et al. 2012), nor is it limited to males; female athletes also express increases in testosterone during competition (Edwards et al. 2006; Edwards and Kurlander 2010). Studies of human competition have focused largely on salivary testosterone (Archer 2006), which only represents the free portion of circulating testosterone (Granger et al. 2004). Most proteins are too large to cross the cellular membrane into saliva, and thus LH, as well as testosterone bound to SHBG or albumin cannot be measured from saliva specimens (Granger et al. 2004). While the studies in this dissertation contribute to our understanding of the conditions under which acute increases in testosterone can occur, they remain agnostic as to the hormonal mechanism responsible. Directions for future research regarding potential endocrine mechanism and their importance in the evolution of hormone-behavior interactions will be discussed in the concluding chapter.

Testosterone and signaling behavior

In evolutionary biology, signaling theory is the broad framework used to discuss how organisms pass information to observers. Examples of signaling vary in the animal kingdom, from butterflies exhibiting bright colors denoting toxicity, to stotting behavior in African ungulates, to human courtship displays (Zehavi and Zahavi 1997). Because of the conflicting fitness interests that arise whenever signalers would benefit from deceiving observers, there has been considerable discussion over how signals can achieve evolutionarily stability (Zehavi and Zahavi 1997; Maynard Smith and Harper 2003; Bliege Bird and Smith 2005). One proposed solution involves costly signaling, where the quality of the signaler is tied to the intensity of the signal produced (Johnstone 1994). Handicap signaling, as described by Zahavi (1975), argued that signals must entail fitness costs, so that cheaters (lower-quality individuals) cannot afford to produce the signal (Zahavi 1975). Zahavi proposed the peacock's tail as an example of a handicap signal: high-quality males that could afford a large tail also faced a higher risk of predation, and thus their continued survival signaled to females their high level of phenotypic quality. Later mathematical models of signaling suggest that costly signaling need not necessarily impose a handicap (Grafen 1990; Johnstone 1994; Bliege Bird and Smith 2005).

Current signaling models focus on the argument that lower-quality individuals pay higher marginal signal costs than individuals of higher phenotypic quality (e.g., (Johnstone 1994; Bliege Bird and Smith 2005)). Thus even if low-quality individuals can produce the display, the higher marginal fitness cost they pay means their optimal level of signaling is lower than it is for higher-quality signalers.

In order for costly signaling to be an evolutionarily stable strategy several conditions must be met: 1) there must be some underlying variation in phenotypic quality that is difficult to directly observe; 2) observers must stand to benefit from gaining accurate knowledge of this variation, while signalers could in principle benefit from deceiving observers; 3) signals must be quality-dependent (individuals in better condition pay reduced marginal costs to express the signal); and 4) the signal must be broadcast in such a way that the display can effectively reach an intended audience (Bliege Bird et al. 2001; Bliege Bird and Smith 2005).

In humans, male hunting behavior can be described as a form of costly signaling. The conditions are met in the following ways: men vary in quality, which could include energy reserves, health, ability, and other less tangible phenotypic qualities; hunters in better condition pay reduced marginal costs (time and energy) to successfully acquire meat; and returning with, or without, a kill is visible to all those present (Bliege Bird et al. 2001). Thus even if hunting is primarily an efficient way of obtaining subsistence, it can serve as an honest signal of underlying qualities to competitors, allies, and potential mates (Bliege Bird et al. 2001; Gurven and Hill 2009).

Various researchers have hypothesized that testosterone-dependent phenotypes are costly signals of male quality (Folstad and Karter 1992), in the sense that only high-quality individuals (in this case, those with robust immune systems and good energy reserves) can afford to express testosterone-dependent ornaments (i.e., they do so at a reduced marginal cost compared to lower quality males) (Zahavi 1975; Bliege Bird and Smith 2005). While these testosterone influenced ornaments can include coloration (Blas et al. 2006), antler (Malo et al. 2009) and comb size (Ligon et al. 1990), or other ornaments used in sexual selection, they also may apply to less sexually dimorphic human traits.

Studies conducted as a part of this dissertation will examine potential effects of audience size and sex on acute changes in testosterone, providing a novel method for investigating associations between testosterone and signaling behavior.

Testosterone and Fitness

In humans, testosterone influences facial (Verdonck et al. 1999; Penton-Voak and Chen 2004), voice (Dabbs and Mallinger 1999; Puts et al. 2012), and muscle (Bhasin et al. 1996) characteristics that are hypothesized to be advantageous in attracting mates (Roney and Simmons 2008), or in competition over mates (Archer 2006). Beyond physical features, testosterone is often associated with a suite of competitive and aggressive behaviors (Archer et al. 1998; Archer 2006), thought to be exemplified by dominant, high status individuals (Mazur and Booth 1998; Johnson et al. 2007). In chimpanzees (Muller and Wrangham 2004) and some, though not all primates (Sapolsky 1982; Wobber et al. 2010), higher levels of testosterone are associated with high social status. Few studies have been able to document links between testosterone and high status in humans, which is unsurprising given that human male social status takes many forms, (e.g., (von Rueden et al. 2008; von Rueden et al. 2011)), and is not entirely dependent on direct physical ability. There are numerous studies linking circulating levels of male testosterone to economic game behaviors thought to be associated with status seeking (Burnham 2007; Apicella et al. 2008; Sapienza et al. 2009; Zak et al. 2009), though these studies have largely been conducted among young men in university settings. Cross culturally, high status men have better reproductive success (Kaplan and Hill 1985; Mulder 1987; Smith 2004; Hopcroft 2006; von Rueden et al. 2011); thus, testosterone-mediated status-seeking behavior could have direct reproductive benefits (Johnson et al. 2007).

While studies in bird models suggest that experimentally increased testosterone can result in higher reproductive success through increased mate-guarding and extra-pair fertilizations (Raouf et al. 1997), only one human study to date has provided evidence that men with higher basal testosterone are more likely to get married and reproduce (Gettler et al. 2011). This study did not examine behavioral characteristics of these men, thus it was not possible to establish whether testosterone increased their mate seeking behavior, mate value, or interactions between these non-mutually exclusive pathways to enhance reproductive success. Human studies have not yet been able to evaluate whether higher status men have both higher testosterone and higher reproductive success; this type of association is inherently difficult to examine in human populations where contraception makes it possible to delink sex and reproduction.

There are many routes to attaining high status (Henrich and Gil-White 2001; von Rueden et al. 2008), with varying effects on reproductive success (von Rueden et al. 2011). One route to high status is through increased resource production, which can be viewed in terms of monetary income in industrialized populations (Hopcroft 2006), or activities like hunting or herding in subsistence societies (Smith and Bird 2000; Smith 2004; Gurven and von Rueden 2006; Gurven and Hill 2009; Mulder et al. 2009). Muscle mass is mediated by testosterone, which has extensive benefits in hunter and forager-farmer production strategies, potentially allowing men to achieve greater status and perhaps, greater reproductive success. In non-industrialized populations, testosterone may play a vital role in several pathways to higher reproductive success, including potential behavioral effects on status-seeking behaviors, as well as enhanced muscular performance which can be beneficial for male-male competition and also many subsistence provisioning strategies.

Given the energetic and potentially immunosuppressive costs associated with high testosterone phenotypes (Duffy et al. 2000; Bribiescas 2010), how do men allocate energetic resources between survival and reproductive effort in different immunologic and energetic environments? The challenge hypothesis was originally designed to describe species level variation in avian male testosterone in different reproductive and parenting contexts (Wingfield et al. 1990), suggesting that that high testosterone levels in the mating season facilitate male-male competitive and territorial behaviors, but then decrease dramatically during the non mating season as males focus on parenting. While other researchers have pointed out flaws in the original analyses for failing to correct for non-independence of phylogenetic relatedness among species (Hirschenhauser et al. 2003; Adkins-Regan 2005), the framework has been adapted for numerous other species across various taxa (Hirschenhauser and Oliveira 2006), including humans and other primates (Muller and Wrangham 2004; Archer 2006). The challenge hypothesis has been modified from the original form to a three-level model describing the patterns of testosterone and testosterone change observed in some avian species (Goymann et al. 2007). This framework suggests that baseline levels of testosterone remain low throughout most of the year when males are focused on parenting and survival, to limit the behavioral, energetic, and immunocompromising costs of testosterone. During the mating season, testosterone rises as males engage in various forms of mating effort. A third level of testosterone is seen during direct male-male competition, when testosterone spikes (Wingfield et al. 1990; Goymann et al. 2007; Hau 2007).

The challenge hypothesis and costly signaling are theoretical models that fall under the life-history framework; investing in reproductively advantageous testosterone based signals reduces what can be allocated toward maintenance and survival (Charnov 1993; Andersson 1994; Kokko et al. 2003; McGlothlin et al. 2008). Evidence in bird models suggests that males with higher quality plumage ornamentation are able to produce larger acute increases in testosterone than males with lower quality plumage (McGlothlin et al. 2008). In humans and primates there is some evidence that the development of facial morphology can be influenced by testosterone levels (Verdonck et al. 1999). One study examining masculine face shape found that males with the most masculine face produced the largest increases in testosterone during a competitive interaction (Pound et al. 2009). Thus there is some evidence of interactions between costly signals and the challenge hypothesis, suggesting that males that can produce the largest ornaments are able to sustain the largest acute increases in testosterone.

Research in industrialized populations suggests male-male competition increases testosterone. The typical subjects are young, unmarried, energy-replete, pathogen-free undergraduate students (e.g., (Archer 2006)). Industrialized populations with constant energy availability have higher, potentially evolutionarily novel, testosterone levels compared to pathogenic and parasitically stressed non-industrialized populations with higher energy expenditures and constrained food intake (Ellison et al. 2002). Behavioral ecologists have investigated associations between testosterone-linked behaviors and fitness (Raouf et al. 1997; Gettler et al. 2011), and reproductive ecologists have examined environmental correlates of testosterone (Cameron 1996; Bribiescas 2001; Ellison et al. 2002; Campbell et al. 2006), but these groups have largely worked in isolation. This dissertation is designed to bridge the gap between these disciplines, examining how the pathogenic and energetic environment affects short-term changes in testosterone during various fitness enhancing activities.

Long-Term Changes in Testosterone

Levels of circulating testosterone are responsive to biological and social factors, both across the lifespan, and in the short term. Research to date indicates that human male testosterone peaks in the early twenties and then declines with age, although the slope and speed of the decline appear to be different across populations (Harman et al. 2001; Ellison et al. 2002; Uchida et al. 2006; Travison et al. 2007a). While there is no consensus on the mechanism responsible for declining testosterone with age, obesity, inflammation, heart disease, and other chronic health conditions are associated with lower levels of testosterone (Travison et al. 2007b), and high mortality rates (Shores et al. 2006; Laughlin et al. 2008). In comparison, non-industrialized populations facing energetic constraints and pathogen stress show relatively low levels of testosterone across all adult ages as well a slower and shallower rate of change with age (Worthman and Konner 1987; Bribiescas 1996; Ellison et al. 2002; Muehlenbein and Bribiescas 2005; Bribiescas and Hill 2009; Vitzthum et al. 2009). Though humans are not seasonal breeders, studies have examined seasonal variation in male testosterone, as well as variation in relation to light and cold (Svartberg et al. 2003), and energetic intake and expenditure (Vitzthum et al. 2009). In non-industrialized populations there is some evidence that periods of high energetic expenditure are associated with seasonal decreases in testosterone (Vitzthum et al. 2009). Research in industrialized populations is mixed; studies suggest seasonal variation in some countries (Svartberg et al. 2003), while others report no evidence for seasonal variability in testosterone (Brambilla et al. 2007). Marriage and fatherhood appear to have long term down regulatory effects on testosterone (Gray et al. 2002; Gray et al. 2006; Kuzawa et al. 2009; Muller et al. 2009; Gettler et al. 2011). Theoretic models of post-marriage and fatherhood down-regulation of testosterone hypothesize that the decrease in testosterone reflects a switch from focus on mating behavior to parenting behavior, though the endocrine mechanism responsible in humans is unknown (Wingfield et al. 1990; Gettler et al. 2011). While these changes generally occur in a gradual fashion over the lifespan, testosterone down regulation can occur rapidly via the HPG axis during energetic duress.

Short-Term Changes in Testosterone

When human and non-human male primates face energetic stresses such as caloric deficiency (Cameron 1996), increased energy expenditure (Nindl et al. 2007), short-term fasting (Trumble et al. 2010), pathogens (Muehlenbein et al. 2010), or parasites (Boonekamp et al. 2008), the hypothalamus and pituitary down regulate testosterone production within hours. These responses reduce the energetic expenditure on costly muscle tissue or behaviors that could be disadvantageous during poor environmental conditions (Bribiescas 2001). Rapid increases in testosterone can occur within a matter of minutes during male-male competition, across many taxa (Hirschenhauser and Oliveira 2006), including industrialized human populations (e.g., (Archer 2006)). Though short in duration, acute changes in testosterone play an important role in enhancing muscle physiology and performance (Crewther et al. 2011). Brief increases in testosterone promote muscle metabolism and increase sugar uptake within one minute (Tsai and Sapolsky 1996), allowing rapid performance enhancement. Men who express larger increases in testosterone during resistance training tend to have larger increases in strength over time (Rønnestad et al. 2011).

Studies of acute testosterone change in humans have thus far focused on male-male competition in industrialized populations with excess caloric availability and low parasite and pathogen loads. However, rapid testosterone-mediated performance enhancement during male-male competition or provisioning activities is advantageous even in the face of energetic or pathogenic stresses that normally down-regulate baseline testosterone. For example, many species, including primates, exhibit acute increases in testosterone while engaging in mate guarding and territory defense behaviors (Hirschenhauser and Oliveira 2006), which often entail extensive energetic expenditure, and prolonged periods without foraging, leading to decreases in body weight (Bercovitch 1997; Yoccoz et al. 2002). Do these same increases in testosterone occur in human populations facing energetic constraints and high parasite and pathogen loads? Can the scope of the challenge hypothesis be expanded beyond physical competition to include a repertoire of behaviors and situations where acute increases in testosterone may be beneficial? A central goal of this dissertation is to address these questions systematically with research among the Tsimane.

The Tsimane

There are few forager-horticultural populations whose health and life course have been as extensively studied as the Tsimane (Gurven et al. 2007; Gurven et al. 2008; Leonard and Godoy 2008; Gurven et al. 2009a). In particular, there is a wealth of data examining male social status (von Rueden et al. 2008; Kaplan et al. 2009) and hunting activities (Gurven et al. 2006; Gurven and von Rueden 2006), with detailed characterization of the associations between male status, hunting and reproductive fitness (von Rueden et al. 2011), making the Tsimane an ideal population to examine acute changes in testosterone during these fitness related activities. Additionally, because the Tsimane are forager-horticulturalists living in the Bolivian Amazon, they face energetic constraints resulting from high energy expenditure and a relatively high parasite and pathogen load (Gurven et al. 2008; Tanner et al. 2009; Gurven et al. 2009a). The combination of environmental stimuli associated with testosterone down regulation (Ellison et al. 2002), and extensive research on behaviors associated with male fitness make the Tsimane an excellent test case for extending the challenge hypothesis beyond physical competition.

More than 15,000 Tsimane live in eighty villages of 50-150 people across the La Paz and Beni districts of Bolivia, east of the Andes (Gurven 2004; Godoy et al. 2005; Winking 2005; Gurven et al. 2007; Leonard and Godoy 2008; Schniter 2009), see Figure 1.2. Medical exams suggest that at least two-thirds of adults have intestinal parasites, and one third of all adults examined showed signs of respiratory illness (Gurven et al. 2009a). The Tsimane face relatively high levels of pathogen exposure (McDade et al. 2005; Blackwell et al. 2011), with immune activation, as measured by C-Reactive Protein, suggesting that more than 50% of adults aged 40 and older have elevated CRP by US standards (CRP ≥3.0 mg/dL) (Gurven et al. 2009a). Energetic expenditure for Tsimane men and women aged 40-49 is estimated to be 450-850 kcals per day greater than an aged matched US sample, with the prevalence of obesity eight times less common among the Tsimane (Gurven et al. 2009a). The average BMI for 1266 men and women over the age of 20 was 23.7 kg/m^2 among the Tsimane, while a sample 13,035 US men and women had an average BMI of 28.0 kg/m^2 (Gurven et al. 2009a).

Over the last decade, ongoing research by the Tsimane Health and Life History Project (THLHP) team, led by Professors Hillard Kaplan and Michael Gurven, has led to an excellent relationship with the Tsimane, a well-developed research infrastructure, and extensive demographic data collection. The THLHP only collects blood specimens when immediate medical testing, feedback, and treatment are available. Because specimens collected as part of this dissertation were returned to the US for analysis, and no immediate medical feedback could be provided by the principal investigator, only saliva was collected from Tsimane participants.

Tsimane diet largely consists of hunted meat, fish, plantains, manioc root, corn, and rice (Reyes-García et al. 2004). Small-scale horticulture is the mainstay food production method; men clear-cut and burn jungle plots in order to plant staple crops. There is a high level of variability in reliance on fish versus hunted meat between communities, depending on the location of the community and access to large river ecosystems (Gurven and von Rueden 2006). In the communities studied here, men live in a riverine environment with easy access to fishing; these men nevertheless hunt an average of 1.3 days per week (see Chapter 5). Some men living in close proximity to the market town of San Borja engage in part-time unskilled wage labor, and some individuals sell some food products grown during small scale horticulture; the level of market engagement varies from community to community, and with distance from San Borja (Reyes-García et al. 2004). Hunted meat is primarily acquired for familial consumption, and rarely sold at market (Gurven and von Rueden 2006).

Specific Aims

The overarching goal of this research is to examine whether individuals facing energetic and pathogenic stress are able to mount acute increases in testosterone during fitness related challenges. I will examine whether the Tsimane, despite having significantly lower baseline levels of testosterone than men in industrialized populations (Trumble et al.

2012), have short term increases in testosterone during physical activity, competition, and while hunting. Specifically:

Chapter 1 examines the responsiveness of the HPG axis to an energetic deficit among young US males. This experiment serves as proof-of-concept that brief changes in food intake result in acute short-term changes in testosterone, and helps validate the laboratory methods used throughout this dissertation.

Chapter 2 examines whether Tsimane salivary testosterone is significantly lower than US salivary testosterone in an age-matched sample, and tests whether physical competition (soccer) among Tsimane men results in acute changes in salivary testosterone, despite significantly lower levels of testosterone.

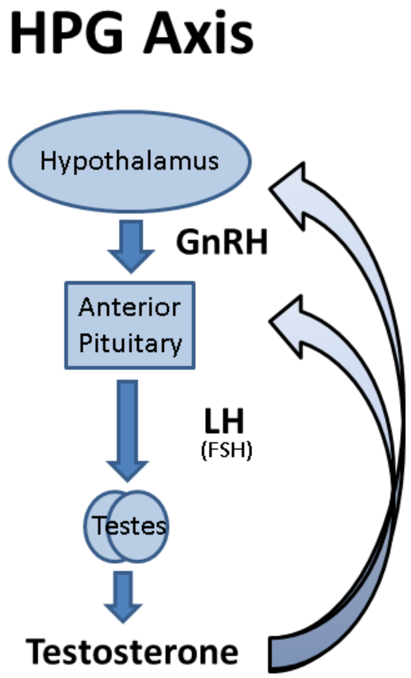
Chapter 3 examines acute changes in Tsimane male salivary testosterone during physical, non-competitive subsistence activity; tree chopping.

Chapter 4 measures acute changes in Tsimane male salivary testosterone during non-physical, competitive activity, a penalty kick shoot out. This study also includes a component examining the effect of the audience on testosterone response.

Chapter 5 examines hormone-behavior interactions while hunting; testing whether testosterone increases occur at the time of the kill, and upon returning home

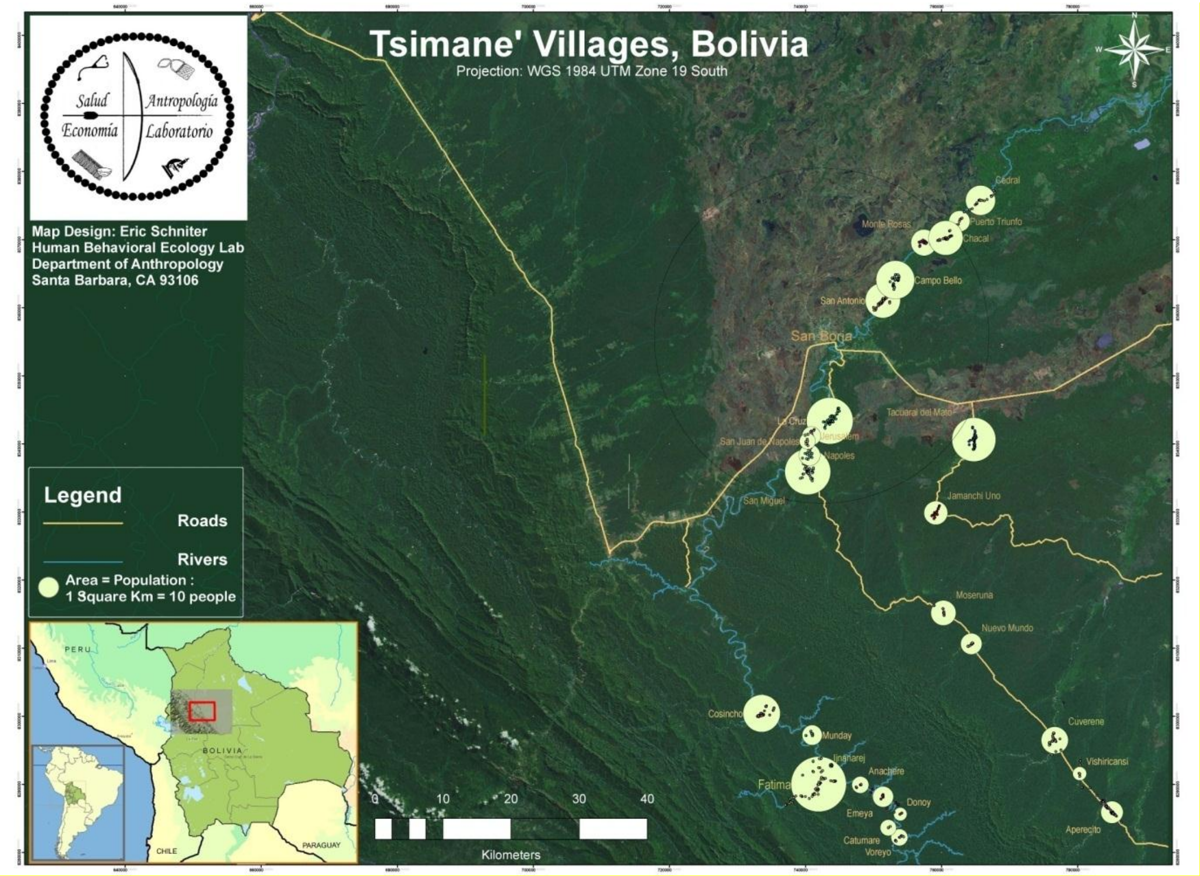
A concluding chapter summarizes and synthesizes these data, examining which domains result in the largest relative increases in testosterone, describing how these studies advance current knowledge of human male life-history, and the implications they have for understanding human evolution. This concluding chapter makes suggestions for amending the anthropocentric version of the challenge hypothesis, noting limitations in studying human male-male competition due to the coalitional nature of human male competitive strategies. These findings also highlight the importance of moving beyond a unidimensional focus on changes in testosterone and male-male aggression, to better understand the reproductive importance of acute testosterone-behavior interactions outside of direct mating effort.

Figure 1.1: HPG Axis



This diagram represents a simplified overview of the hypothalamic-pituitary gonadal (HPG) axis.

Figure 1.2: Map of Tsimane Communities



A detailed map of Tsimane villages in lowland Bolivia (map credit: Eric Schniter).

Chapter 2: Responsiveness of the reproductive axis to a single missed evening meal in young adult males

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Abstract

The male reproductive axis is responsive to energetic deficits, including multi-day fasts, but little is known about brief periods of fasting (<24 hours). Reduced testosterone in low-energy balance situations is hypothesized to reflect redirection of resources from reproduction to survival. This study tests the hypothesis that testosterone levels decrease during a minor caloric deficiency by assessing the effects of a single missed (evening) meal on morning testosterone in 23 healthy male participants, age 19-36. Participants provided daily saliva and urine samples for two baseline days and the morning following an evening fast (water only after 4PM). Testosterone, cortisol, and luteinizing hormone were measured with enzyme immunoassays. Fasting specimens had significantly lower overnight urinary luteinizing hormone (p=0.045) and morning salivary testosterone than baseline (p=0.037). In contrast to morning salivary testosterone, there was a significant increase in overnight urinary testosterone (p=0.000) following the evening fast, suggesting an increase in urinary clearance rates. There was a marginal increase in overnight urinary cortisol (p=0.100), but not morning salivary cortisol (p=0.589). These results suggest the male reproductive axis may react more quickly to energetic imbalances than has been previously appreciated.

Introduction

There is a growing body of evidence suggesting that the phenotypic effects of testosterone are energetically expensive, and may play an energy-allocating role in the life history trade off between reproduction and survival (Folstad and Karter 1992; Charnov 1993; Muehlenbein and Bribiescas 2005; Muehlenbein 2008). High testosterone levels are hypothesized to lead to increased investment in reproduction, including behavioral strategies and secondary sexual characteristics (Muehlenbein and Bribiescas 2005). Testosterone plays an important role in increasing and maintaining male muscle mass, which can enhance competitive ability and mate attraction (Bhasin et al. 1996; Bribiescas 2001). This comes at a cost: not only is muscle energetically expensive (Lassek and Gaulin 2009), but high levels of testosterone also increase the catabolism of fat tissues, depleting reserves that could be essential during periods of food shortage (Bhasin et al. 2005).

Studies of male human and non-human primates suggest that short-term fasts can have significant down-regulatory effects on the hypothalamic-pituitary-gonadal (HPG) axis, prior to any change in body weight or composition (Cameron et al. 1991; Bergendahl and Veldhuis 1995; Aloi et al. 1997). In human males, fasting appears to significantly reduce the number, and amplitude of gonadotropin releasing hormone (GnRH) pulses, thus decreasing luteinizing hormone (LH) pulsatility (Rojdmark 1987; Aloi et al. 1997). It is difficult to directly measure GnRH in humans, as it exists solely within the brain, but administration of intravenous GnRH during caloric restriction restores LH pulses, suggesting that fasting down regulates GnRH production (Aloi et al. 1997). Problems with direct testing for GnRH make it difficult to ascertain whether it is the frequency of GnRH pulses, or their amplitude that is affected by short term fasting (Aloi et al. 1997). Administration of intravenous GnRH has also been shown to restore testosterone levels in fasting subjects (Rojdmark 1987).

Once a fast is broken, LH pulsatility rebounds in male subjects; both in human and non-human primates (Parfitt et al. 1991; Schreihofer et al. 1993a; Schreihofer et al. 1993b; Friedl et al. 2000). LH secretion begins within twenty-to-forty minutes of caloric re-feeding, and is monotonically related to the size of the re-feeding meal (Parfitt et al. 1991). Resumption of LH secretion is unrelated to the actual act of eating; macaques re-fed via gastric cannulae showed an LH rebound statistically identical to macaques that were re-fed naturally (Schreihofer et al. 1993a). When normal weight primates were overfed just prior to the onset of fasting, there was no effect on LH secretion, suggesting that acute caloric deficit plays an important role in hormonal down regulation (Schreihofer et al. 1993b; Cameron 1996).

Previous studies find that multi-day fasts cause a reduction in LH and testosterone. This project tests the responsiveness of the male HPG and HPA to a brief caloric deficit. We hypothesize that the male reproductive axis is sensitive to even minor energetic disruptions, with reductions in LH and testosterone the morning following a missed evening meal. We also hypothesize that testosterone will return to normal levels rapidly after re-feeding. Cortisol is hypothesized to rise due to HPA activation from the psychological and physiological stress of fasting. Understanding the effects of short term caloric reduction on the endocrine system will provide insight into the role of testosterone in the trade-off between survival and reproduction in human males.

Methods

Participants

Twenty four male volunteers were recruited from the University of Washington campus. Individuals who regularly chewed tobacco, reported taking prescription or non-prescription steroids, or other medications known to affect testosterone production were not eligible for the study (Bhasin et al. 1996; Granger et al. 2004). One participant did not complete the study protocol, leaving a total of 23 participants who ranged in age from 19 to 36 years (mean age 26.7), see Table 2.1. Self reported weight and height were used to calculate BMI (mean 23.7 kg/m^2, range 19.5-29.0 kg/m^2), see Table 2.1. All procedures were approved by the Institutional Review Board of the University of Washington.

Specimen Collection

Participants collected first void morning urine specimens as well as waking and late morning (11AM) passive drool saliva specimens each day for three days. The first two days of specimen collection were designed to establish baseline levels of testosterone, cortisol, LH. On the second day of specimen collection, participants were asked not to eat or drink anything aside from water after 4PM. The protocol stressed the importance of not eating extra throughout the day, and not simply eating dinner early was stressed. On the third morning, after providing waking saliva and urine specimens, participants resumed their normal diet and ate breakfast before taking a final late morning (11AM) saliva specimen.

Passive drool saliva was collected in polypropylene centrifuge tubes after participants arose from bed each morning, after participants rinsed their mouths with water, but before eating, drinking, or brushing their teeth. Participants also collected a mid-stream first void morning urine specimen (2-5mL). After going about their normal daily routine until 11 AM, participants provided a second specimen, regardless of their time of wake up. Asking all participants to take the sample at 11AM as opposed to 4 hours after wake-up simplified an already complex study protocol. Waking urine and saliva specimens were frozen immediately, and 11AM specimens were frozen as soon as possible. Specimens were stored in home freezers until the end of the study, at which point they were stored at -80° C in the laboratory for up to 26 days before assay.

To control for differences in morning testosterone caused by behavioral differences rather than fasting, participants were asked to refrain from major exercise, competitive sports, and sexual activity for the duration of the study (Burnham et al. 2003; Gray 2003; Gray et al. 2006). Weight lifting or jogging were considered major exercise, while walking the dog, or across campus were considered acceptable. Participants recorded the times they went to bed and woke up, and the time that each saliva specimen was taken. These data were later used to correct salivary hormone concentrations for time of wake up (Flinn and England 1997). The self-reported age, weight, and height of the participants was also collected to control for inter-individual differences in testosterone production (Burnham et al. 2003; Gray 2003; Gray et al. 2006).

Laboratory Methods

In the lab, saliva specimens was centrifuged at 2800 RPM for 20 minutes, and the aqueous layer was removed and stored at -80° C until assay. Urine specific gravity was measured with a hand refractometer (Model No. 2721 URC-PN, Atago Inc., Farmingdale, NY), and urine specimens were stored at -80° C until assay. All specimens had gone through two freeze thaw cycles when initially assayed. Enzyme immunoassays (EIA) were used to measure testosterone, and cortisol in saliva, and LH, testosterone and cortisol in urine; all specimens were run in duplicate. Urine hormone concentrations were corrected for hydration status using specimen specific gravity and a reference population mean specific gravity of 1.015 (Miller et al. 2004).

The EIA for testosterone used a polyclonal antibody that cross reacts 100% with testosterone, 57.4% with 5α-dihydrotestosterone, 0.27% with androstenodione, and less than 0.05% with other androgens (Muir et al. 2001). All saliva specimens were run neat, and all urine specimens were run at a 1:100 dilution in 0.1% bovine serum albumin (BSA) blocking buffer. The within and between assay coefficients of variation (CV) for this project (n=10 plates) were 3.76% and 8.37% respectively for the high (513.0pg/mL), 4.87% and 8.54% for the medium (412.7pg/mL), and 3.68% and 8.84% for the low (350.3pg/mL) in-house controls.

The EIA for cortisol used a polyclonal antibody that cross-reacted 100% with cortisol and less than 10% with other steroids tested (Munro and Stabenfeldt 1985). All saliva specimens were run at a 1:2 dilution in 0.1% BSA blocking buffer, and urine specimens were diluted to 1:60 in the same buffer. The within and between assay CVs for cortisol for this project (n=7 plates) were 4.27% and 2.44% for the high (1047.7pg/mL), 4.51% and 5.51% for the medium (553.8pg/mL), and 6.79% and 7.30% for the low (337.0pg/mL) in-house controls, respectively.

An immunoenzymometric assay (IEMA) was used to measure the beta (β) sub-unit of LH in urine (Brindle et al. 2006). The cross reactivity was 100% for βLH, 7.60% for intact LH, and 0% for all other cross reactants tested. Specimens were heated to 100° C for 2 minutes before assay to disassociate the β sub-unit of LH, and run neat. The within and between assay CVs for this project (*n*=3 plates) were 16.56% and 27.03% for the high (116.8pg/mL) and 14.37% and 16.47% for the low (87.7pg/mL) in house controls, respectively.

Color reactions were quantified at 405nm (test) and 570nm (reference) for testosterone, cortisol, and LH assays using a Synergy HT microtiter plate reader (Bio Tek Instruments, Inc., Winooski, VT). Concentrations were estimated with a four parameter logistic calibration curve fit (Gen5, Bio Tek Instruments Inc.).

Statistical Methods

Linear mixed effects regression models with maximum likelihood estimation were run to determine the effects of a single skipped meal on individual 1) waking salivary testosterone and cortisol; 2) waking urinary LH, testosterone, and cortisol; and 3) 11 AM salivary testosterone and cortisol. All hormone data were log transformed and normality was graphically assessed. To control for the non-independence of repeated specimens from each participant, individuals were modeled as a random effects (West et al. 2007). Analysis of waking saliva specimens controlled for the time of waking, hours of sleep, an interaction between the amount of sleep and time of waking, BMI, age, and an interaction term for age and BMI. The 11AM salivary analysis controlled for waking hormone concentration, time since waking, BMI, age, and an interaction term for age and BMI. The urinary analysis controlled for the number of hours of sleep, BMI, age, and an interaction term for age and BMI. The threshold for statistical significance was set at α=0.05, and all analyses were completed in STATA 10.1 (College Station, TX).

Results

Twenty four participants began the study; one subject did not complete study protocol, leaving a total of 23 participants. Two specimens were not analyzed due to improper collection time, and one participant missed collection of a single sample. All morning saliva and urine specimens were taken between 5:45AM and 10:40AM, and all 11AM saliva specimens were taken within one hour of 11 AM. Three LH specimens were below the limits of detection, and four cortisol specimens were above the limits of detection for the assay; these specimens were assigned the concentration determined to be the upper or lower limits of detection of the assay. Results are reported in Table 2.1 and Table 2.2.

There was a significant decrease in waking salivary testosterone on the morning following the fast, when compared to two baseline days combined (β=-0.107, p=0.037), and a significant increase in 11 AM salivary testosterone after participants fasted on the previous evening (β=0.149, p=0.012) (Figures 2.1A and 2.2A). In contrast to salivary testosterone, morning urinary testosterone increased significantly (β=0.237, p<0.000) the morning following a single skipped meal (Figures 2.1B and 2.4B).

Salivary cortisol showed no significant change between baseline and fasting levels, either at waking (p=0.589) or 11 AM (p=0.222) (Figures 2.2B and 2.4A). Similar to urinary testosterone, overnight urinary cortisol increased (β=0.180, p=0.100) on the morning following a single missed meal, though the results did not reach statistical significance (Figures 2.3C and 2.4B). Urinary LH decreased significantly (β=-0.152, p=0.045) after a single skipped meal (Figures 2.5 and 2.3A). Due to the high LH CVs, an additional bootstrapped statistical analysis (1000 replications) was run building within plate variability into the model as a second random effect. This analysis also concluded that urinary LH decreased significantly (p=0.010) following an evening fast.

Discussion

This study examined the effects of a single missed evening meal on the male reproductive axis. As hypothesized, urinary LH and waking salivary testosterone decreased significantly following the fast. These results are consistent with previous research in humans and animals which suggest that even minor energetic disturbances from fasting down-regulate the male reproductive axis (Klibanski et al. 1981; Cameron et al. 1991; Bergendahl and Veldhuis 1995; Aloi et al. 1997; Bergendahl et al. 1998). Previous studies of energetic imbalances have reported decreases in serum LH (Cameron et al. 1991; Veldhuis et al. 1993; Bergendahl and Veldhuis 1995; Aloi et al. 1997), this report extends these findings to urinary LH. These results are also consistent with other research demonstrating that mild energetic imbalances, either from increased exercise (Nindl et al. 2001), or immune system activation (Muehlenbein and Bribiescas 2005; Simmons and Roney 2009) cause decreases in circulating testosterone in human males.

Research in animal models suggests that decreases in hypothalamic GnRH production are responsible for decreased LH and testosterone (Cameron et al. 1985). Participants in this study showed significantly higher 11 AM testosterone (post-breakfast) levels the morning after fasting compared to baseline days. This is consistent with previous work showing that re-feeding is associated with immediate increases in GnRH, LH, and testosterone, regardless of the macronutrient content of the meal (Parfitt et al. 1991; Schreihofer et al. 1993a; Schreihofer et al. 1993b; Friedl et al. 2000). To assess if larger than normal breakfast sizes could have been responsible for the increase in 11AM salivary testosterone, an analysis of relative meal size was conducted. As a measure of fasting compliance, participants recorded the size of their meals compared to their average meal size (coding each meal as smaller then usual, normal, or larger then usual). This provided a crude measure of relative food intake, making it possible to examine whether the increases in 11AM salivary testosterone were caused by significantly larger breakfasts the morning following the fast. A mixed effects regression was conducted on self reported relative breakfast size. This revealed that breakfasts the morning following the fast did not differ significantly in size from breakfasts on other days (p=0.307). While it is not possible to rule out the possibility that individuals ate breakfasts of different caloric or macronutrient content the morning following the fast, participants reported no differences in relative breakfast sizes in this crude measure of dietary intake.

In contrast to the waking salivary testosterone decrease following the fast, we observed a significant increase in first morning urinary testosterone following the fast. Studies testing paired urine and blood specimens in fasting individuals found that while blood hormone concentrations of LH and FSH decreased (Klibanski et al. 1981), or stayed stable (Beitins et al. 1980), over the course of a multi-day fast, there were significant increases in urinary hormone concentrations during fasting. This suggested to that clearance rates increased significantly during a fast. Similar increases in peripheral testosterone clearance have been shown in patients in the days following surgery or trauma, while serum testosterone decreased (Spratt et al. 2006). Increased urinary clearance of testosterone may be an adaptive response to rapidly eliminate circulating testosterone during energetic stress.

Future studies should examine energetic stress and changes in testosterone clearance rates in greater detail.

Morning and 11 AM salivary cortisol showed no increase the morning following a missed meal compared to baseline; however, there was an increase in urinary cortisol the morning of the fast. Previous studies have reported increased serum cortisol following brief (1-2 day) fasts in primates (Schreihofer et al. 1993b). Increasing cortisol following fasting is hypothesized to result from both the psychological stress of missing a meal, as well as the metabolic actions of cortisol (Cameron 1996; Lukas et al. 2005). In our study, increases in cortisol from both psychological and metabolic stress may have occurred close to the time of the fast, and were thus only partially observable in the pooled overnight urinary measure of cortisol. Because the increase in urinary cortisol was not accompanied by a decrease in salivary cortisol the following morning, we believe that there was a significant increase in circulating cortisol the previous evening and into the night, either from the psychological or metabolic stress of going to sleep without eating (Cameron 1996; Lukas et al. 2005). It is not possible to rule out increased urinary clearance of cortisol, thought the authors are unaware of any previous literature showing increased cortisol excretion during caloric deficit. In addition, high morning levels due to the cortisol awakening response may have swamped any expected morning increase in salivary cortisol (Clow et al. 2004)

Circulating glucose and insulin rise immediately after eating, and then gradually decline as the time from the meal increases (Van Cauter et al. 1992). Insulin receptors are expressed at high levels in the hypothalamus, pituitary, and testis, and it has been suggested that changes in insulin concentration could play a role in reproductive down-regulation during fasting (Bruning et al. 2000). Previous research indicates that extreme hypoglycemia induced by glycemic clamps can cause an acute decrease in LH and testosterone (Oltmanns et al. 2001; Oltmanns et al. 2005). However, these studies generated severe hypoglycemia, unlike the very mild changes in glycemic levels seen during natural fasting in young adult males (Merimee and Tyson 1974; Haymond et al. 1982), thus it is unlikely that missing a single meal caused severe enough hypoglycemia to down-regulate LH and testosterone (Cameron 1996). Primate research on post-fast refeeding finds that different iso-caloric mixtures of protein, fats and carbohydrates make no difference in the LH rebound, suggesting that naturally circulating glucose levels are not responsible for changes in LH secretion (Schreihofer et al. 1993a; Schreihofer et al. 1993b; Cameron 1996; Schreihofer et al. 1996). Further studies of natural changes in circulating glucose and insulin are necessary to understand the mechanism by which energetic status down-regulates human male reproductive hormones.

There were several limitations to this study. Participants were not physically monitored during the study, and although no participants reported any food intake during the fast on daily meal logs, this self report was not verified. Nutrient content of the meals was not assessed. When providing saliva samples, participants took their first saliva specimen upon waking and a second sample at 11AM. For some participants, the time of wake up may have been closer to 11AM than others, thus there was less time between waking and specimen collection. That said, there was no association between fasting and hours of sleep (p=0.819), or time of wake (p=0.720).

Another limitation was the use of self reported BMI. Previous studies have shown that men tend to overestimate height, which can skew BMI (Bolton-Smith et al. 2000; Gorber et al. 2007). BMI in the study ranged from 19.5-29.0 kg/m^2, with a mean of 23.7 kg/m^2, and thus our results may not generalize to individuals with lower or higher BMI. The majority of the participants were well educated, white undergraduate and graduate students at the University of Washington, thus the results may not be generalizable to all males. The young age distribution of our sample precludes generalization to older men who have lower levels of testosterone (Harman et al. 2001; Travison et al. 2007a) from reduced hypothalamic-pituitary gonadotropin feedback and secretion (Mulligan et al. 1997; Veldhuis et al. 2001), as well as reduced testicular function (Harman and Tsitouras 1980; Midzak et al. 2009).

In sum, these results support that the male reproductive axis is more responsive to very brief caloric deficiencies than has previously been shown. Not only do overnight LH, and morning salivary testosterone decrease, but our data suggests that clearance rates of testosterone increase, resulting in a rapid drop in circulating testosterone levels. These results are consistent with previous studies of multi-day fasting, as well as studies examining energetic stresses such as immune activation (Cameron 1996; Muehlenbein and Bribiescas 2005; Muehlenbein 2008). This study adds to a growing body of literature suggesting that during energetic deficit testosterone production is down regulated, and may play a key mediating role in the regulation of trade-offs between survival and reproduction.

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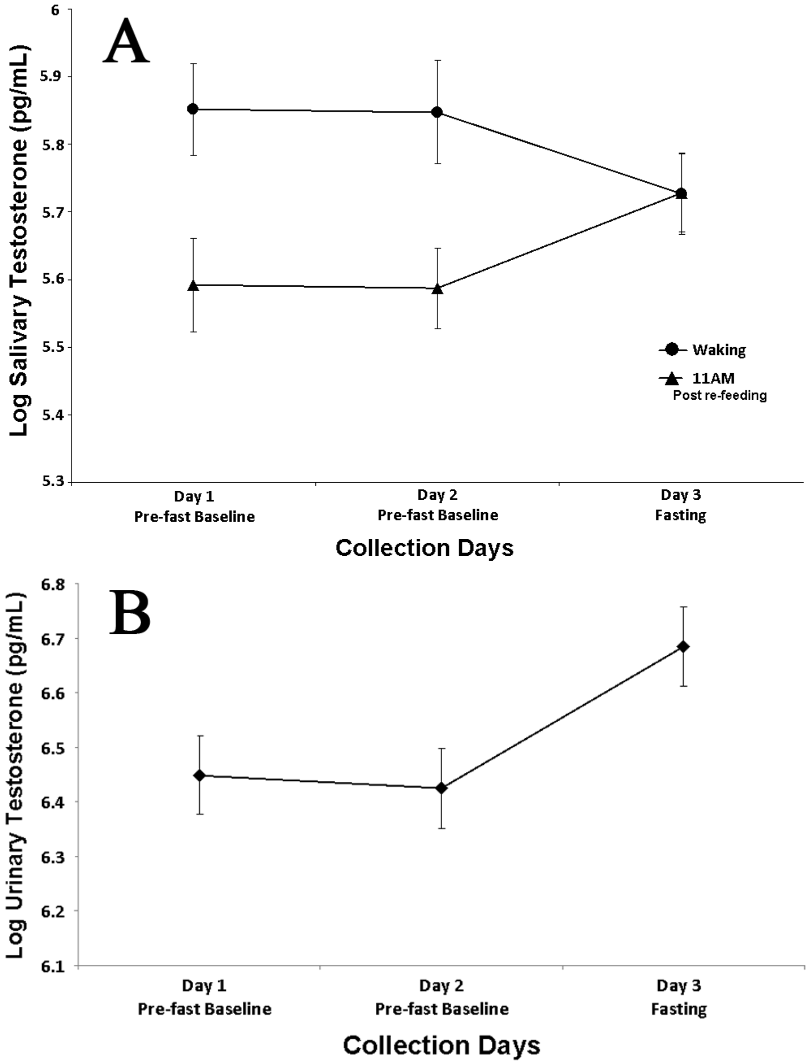
Table 2.1: Summary statistics for hormonal and anthropometric characteristics over two baseline days (averaged), and following a brief fast (*n*= 23 men)

	Time	**Mean ±SE**	**Minimum**	**Maximum**	**95% CI**
Age (yr)	Baseline	26.7 ±5.3	19	36	(25.2, 28.3)
BMI (kg/m^2)	Baseline	23.7 ±2.2	19.5	29.0	(24.5, 29.1)
Waking Salivary Testosterone (pg/mL)	Baseline	385.7 ±14.8	269.7	708.3	(356.0, 415.3)
	Fasting	337.3 ±19.8	223.8	573.6	(297.8, 376.9)
Waking Salivary Cortisol (pg/mL)	Baseline	3340.8 ±129.2	1859.4	6114.9	(3082.5, 3599.1)
	Fasting	3062.4 ±158.7	1781.6	4983.5	(2745.1, 3379.6)
11AM Salivary Testosterone (pg/mL)	Baseline	283.1 ±10.2	190.9	490.2	(262.6, 303.5)
	Post re-feeding	321.4 ±14.5	230.4	534.5	(292.5, 350.3)
11AM Salivary Cortisol (pg/mL)	Baseline	2241.8 ±140.2	791.8	4845.4	(1961.5, 2522.1)
	Post re-feeding	2485.5 ±152.5	1507.3	4742.0	(2180.6, 2790.4)
Urinary Testosterone (pg/mL)	Baseline	670.6 ±18.2	422.3	992.0	(634.2, 706.9)
	Fasting	892.1 ±29.8	662.1	1217.3	(832.5, 951.8)
Urinary Cortisol (pg/mL)	Baseline	2745.3 ±171.3	1034.4	5875.8	(2403, 3087.7)
	Fasting	3581.8 ±240.1	1919.5	6633.2	(3101.8, 4061.8)
Urinary LH (pg/mL)	Baseline	286 ±12.2	128.8	517.4	(261.5, 310.5)
	Fasting	252.8 ±18	92.2	519.4	(216.9, 288.7)

Table 2.2: Results of separate linear mixed effects models examining the response of logged endocrine measures to fasting (each hormone modeled separately) and the control variables for each model.

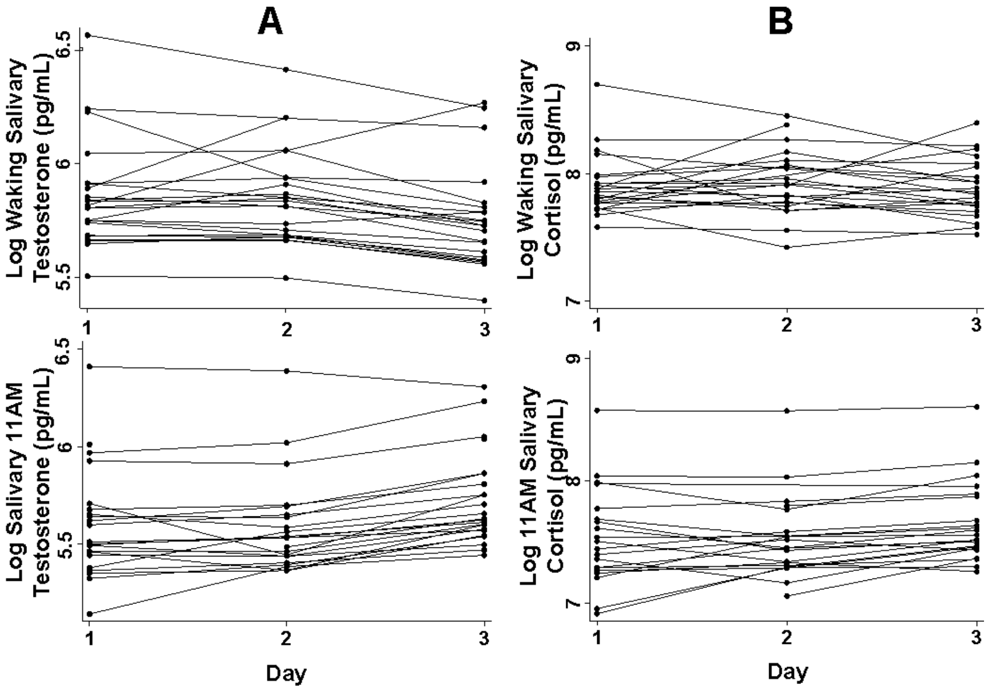
	Coefficient	**Std. Error**	***p*-value**	**95% CI**
Waking Salivary Testosterone**	-0.1072	0.0514	0.037	(-0.2079, -0.0064)
Waking Salivary Cortisol	-0.0561	0.1038	0.589	(-0.2596, 0.1474)
Waking salivary specimen analysis controlled for time of wakening, hours of sleep, age, BMI, and interactions between time of wake and hours of sleep^{T} as well as age and BMI. Individuals were treated as random effects.				
11AM Salivary Testosterone** (Post re-feeding)	0.1487	0.0589	0.012	(0.0333, 0.2641)
11AM Salivary Cortisol (Post re-feeding)	0.1119	0.0916	0.222	(-0.0677, 0.2915)
11AM salivary specimen analysis controlled for waking salivary hormone levels (testosterone controlled for waking salivary testosterone^{T}, cortisol controlled for waking salivary cortisol^{C}), hours since wake, age^{T}, BMI^{T}, and an interaction between age and BMI^{T}. Individuals were treated as random effects.				
Urinary Testosterone***	0.2368	0.0531	0.000	(0.1328, 0.3409)
Urinary Cortisol*	0.1802	0.1095	0.100	(-0.0344, 0.3948)
Urinary LH**	-0.1524	0.0759	0.045	(-0.3012, -0.0037)
First morning void urine specimens were corrected for specific gravity and the analysis controlled for time of wake, age$^{T, C, LH}$, BMI$^{C, LH}$, and an interaction between age and BMI$^{T, C, LH}$. Individuals were treated as random effects.				

Figure 2.1: Daily Fitted Salivary and Urinary Testosterone



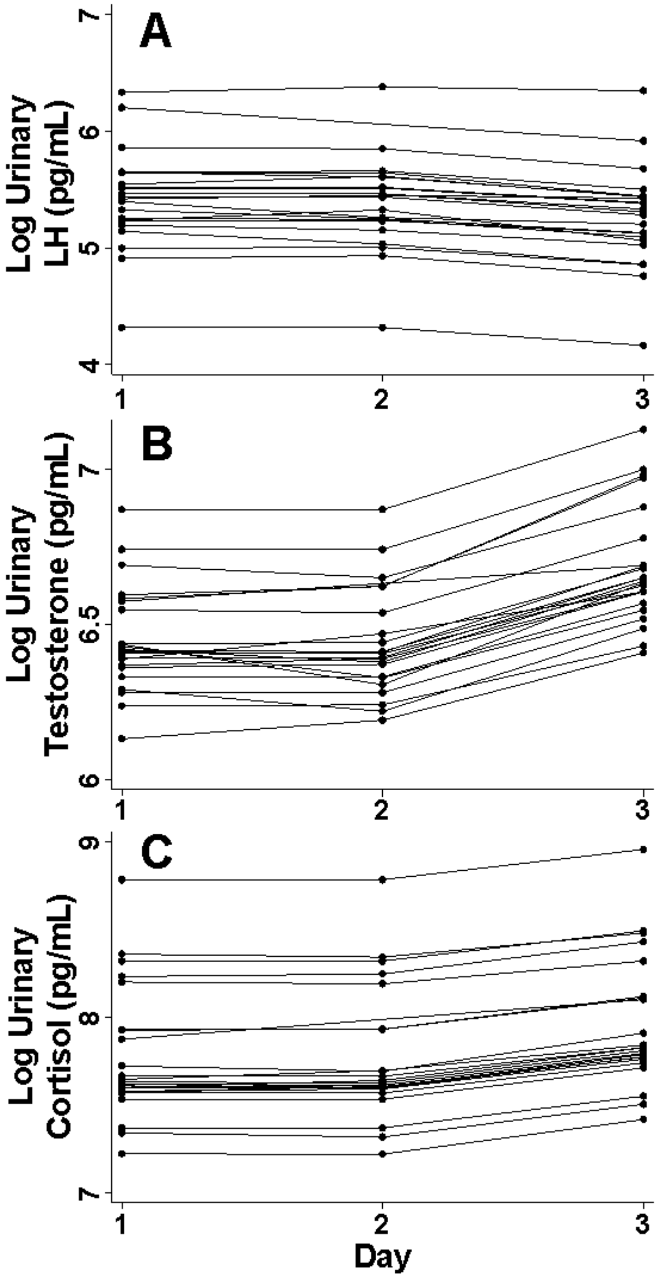
A: Two days of non-fasting baseline waking and 11AM fitted log salivary testosterone levels (mean ± SE), followed by a third day showing the effects of missing a single meal the previous evening. **B**: First morning fitted log urinary testosterone (specific gravity corrected) for two baseline days and the morning following a single missed meal (mean ± SE).

Figure 2.2: Individual Daily Salivary Testosterone and Cortisol



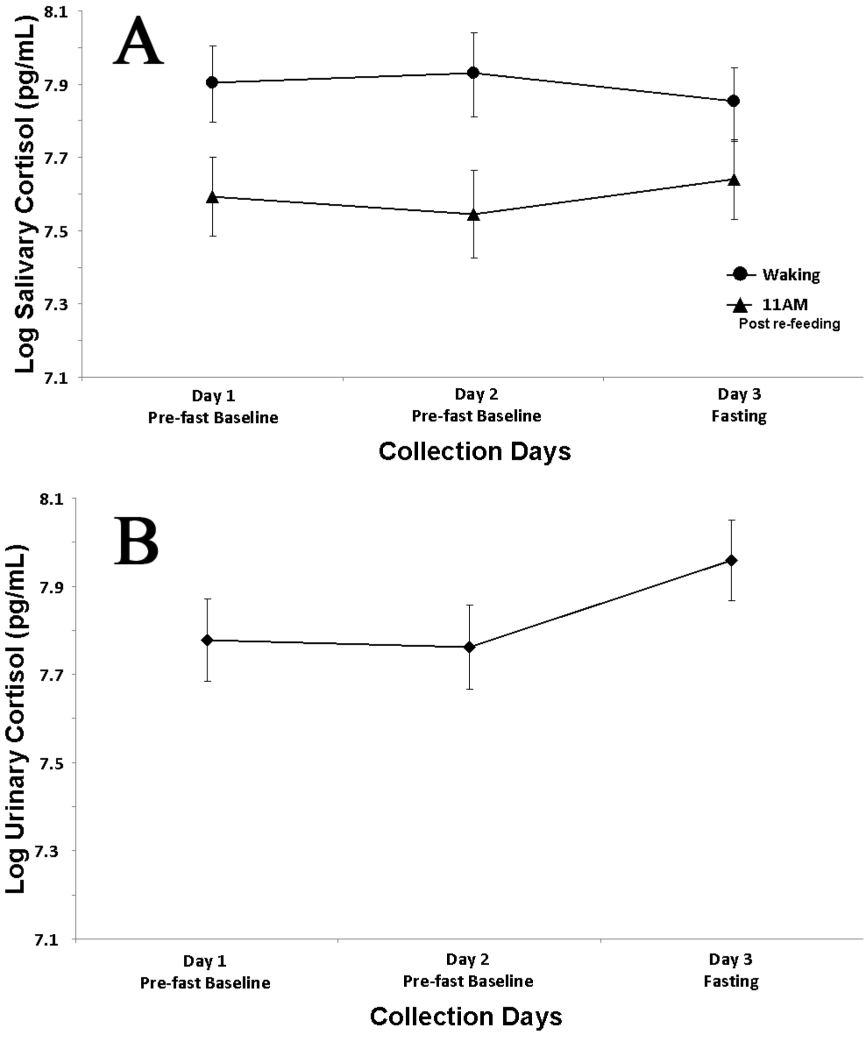
A: Two days of non-fasting baseline waking and 11AM log salivary testosterone levels, followed by a third day showing the individual effects of missing a single meal the previous evening. B: Two days of non-fasting baseline waking and 11AM log salivary cortisol levels, followed by a third day showing the individual effects of missing a single meal the previous evening.

Figure 2.3: Individual Daily Urinary Hormone Measures



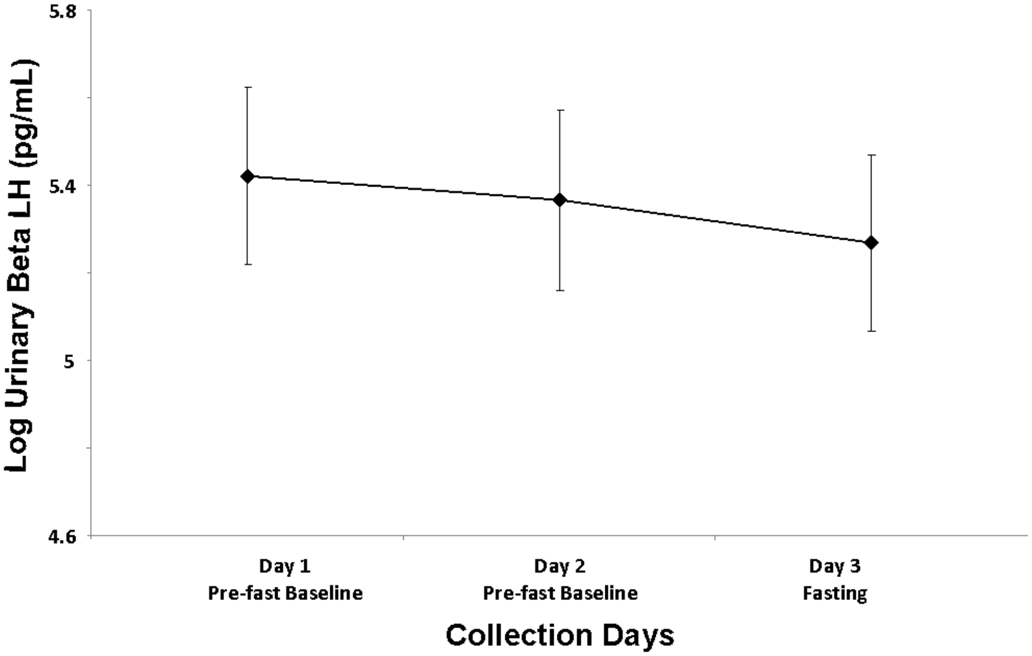
Daily first morning log urinary LH (A) Testosterone (B) and Cortisol (C) for two days of baseline, and the morning following a single missed meal, corrected for specific gravity.

Figure 2.4: Daily Fitted Salivary and Urinary Cortisol



A: Two days of non-fasting baseline waking and 11AM log salivary cortisol levels (mean ± SE), followed by a third day showing the effects of missing a single meal the previous evening. **B**: First morning fitted log urinary cortisol (specific gravity corrected) for two baseline days and the morning following a single missed meal (mean ± SE).

Figure 2.5: Daily Fitted Urinary LH



Day 1 and 2 are non-fasting, baseline log urinary beta LH specimens (specific gravity corrected), while the third sample was taken the morning immediately following an evening fast (mean ± SE).

Chapter 3: Physical competition increases testosterone among Amazonian forager-horticulturalists: a test of the "challenge hypothesis"

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Abstract

The challenge hypothesis posits that acute increases in testosterone during male-male competition enhance performance and survivability while limiting the physiological costs of consistently high testosterone. Human challenge hypothesis research has focused on young men in industrial populations, who have higher baseline testosterone levels than men in subsistence populations. We tested whether the Tsimane, pathogenically stressed forager-horticulturalists of the Bolivian Amazon, would express acute testosterone increases in response to physical competition. Saliva was collected from 88 Tsimane men (aged 16-59 years) before and after a competitive soccer match. Tsimane men had significantly lower baseline levels of testosterone ($\beta=-0.41$, $p<0.001$) compared to age-matched US males. Linear mixed-effects models were used to establish that testosterone increased significantly immediately following competition ($\beta=0.23$, $p<0.001$), remaining high one hour later ($\beta=0.09$, $p=0.007$); equivalent to 30.1% and 15.5% increases in testosterone respectively. We did not find larger increases in testosterone among winners ($p=0.412$), though testosterone increases were positively associated with self-rated performance ($\beta=9.07$, $p=0.004$). These results suggest that despite lower levels of testosterone than US males, Tsimane males exhibit acute increases in testosterone at the same relative magnitude reported by studies in industrialized settings, with larger increases in testosterone for those who report better individual performance.

Introduction

During male-male physical competition, acute spikes in testosterone are observed in a variety of species across numerous taxa (Hirschenhauser and Oliveira 2006), including humans (Archer 2006) and other primates (Muller and Wrangham 2004). Acute increases in testosterone have a multitude of effects on male behavior and physiology (Wingfield et al. 1990), including enhanced muscular performance (Tsai and Sapolsky 1996; Crewther et al. 2011), and immunoredistribution of leukocytes to the periphery to prepare for potential injury (Braude et al. 1999). The challenge hypothesis (Wingfield et al. 1990) postulates that the energetic and immunocompromising costs of testosterone (Folstad and Karter 1992; Muehlenbein and Bribiescas 2005) have led to the evolution of a system where testosterone is low throughout most of the year, when males focus on parenting or survival, spiking only when necessary to mediate the trade-off between reproduction and survival in social situations, including sexual activity, resource defense, status competition, and competition over mates (Wingfield et al. 1990).

In industrialized human societies, acute increases in male testosterone during competition have been reported in a variety of contexts from judo to chess (Archer 2006). The magnitude of testosterone increase in response to a challenge tends to be largest during physically competitive sports, due to energetic mobilization during physical activity (Archer 2006), which limits the interpretation of the results, confounding physical activity and psychological competition. Competitions where social status is at stake tend to have larger increases in testosterone (Mehta and Josephs 2006). Evidence for a victory-induced T-response is mixed, with some studies reporting larger increases in testosterone among winners (Elias 1981; Booth et al. 1989; Gladue et al. 1989; Mazur et al. 1992; Archer 2006), while other studies find no evidence of a T-related winner effect (Gonzalez-Bono et al. 1999; Suay et al. 1999; Wagner et al. 2002; Mehta and Josephs 2006; van der Meij et al. 2008). It has also been reported that players with larger post-game testosterone increases tend to rate their importance in the outcome of the game as higher (Edwards et al. 2006).

Industrialized populations, relatively free of energetic constraints and parasitic or pathogenic stress show elevated levels of testosterone across all adult ages, compared to non-industrialized populations (Bribiescas 1996; Ellison et al. 2002; Bribiescas and Hill 2009). In advantageous energetic environments, males pay reduced marginal costs to put on a display (Zahavi 1975; Bliege Bird and Smith 2005), and thus can invest more energy in exaggerated T-based signals (Folstad and Karter 1992). However, individuals in energetically limited environments face a more restrictive trade-off between investing in survival, or reproductively advantageous but immunologically and metabolically costly testosterone (Folstad and Karter 1992; Muehlenbein and Bribiescas 2005). Muscle tissue is calorically expensive to develop and maintain, particularly given testosterone increases fat catabolism (Bhasin et al. 2001). In healthy industrialized males, reduced energetic availability from increased energy expenditure (Friedl et al. 2000), or fasting (Trumble et al. 2010) appears to reduce testosterone through down-regulation of the hypothalamic-pituitary-gonadal (HPG) axis. Parasitic and pathogenic stress (Muehlenbein and Bribiescas 2005; Boonekamp et al. 2008; Muehlenbein et al. 2010) also down regulate testosterone.

Do the same transient increases in testosterone occur during male-male competition in chronically energetically and pathogenically stressed populations? The Tsimane forager-horticulturalists of the Bolivian Amazon are an ideal population for testing testosterone dynamics in energetically stressful environments. A wealth of demographic, behavioral, and health related data have documented their high energy expenditure and a relatively high parasite and pathogen load (Gurven et al. 2007; Gurven et al. 2009a). More than 15,000 Tsimane live in eighty villages of 30-500 people in the lowlands, East of the Andes (Godoy et al. 2005; Gurven et al. 2007). Medical exams indicate that at least two-thirds of adults present with signs of intestinal parasites, and one third of all adults examined showed signs of respiratory illness (Gurven et al. 2009a). Energetic expenditure for Tsimane men and women aged 40-49 is estimated to be 450-850 kcals per day greater than an aged matched US sample, and the prevalence of obesity is eight times less common among the Tsimane (Gurven et al. 2009a). The Tsimane do not have a recent history of inter-group violent aggression, but intra-village disputes arising from competition over horticultural land, sale of village lumber, sexual jealousy, or accusations of theft are not uncommon. Men's ability to get their way in a group or influence others during community meetings is largely related to coalitional support, while physical size better predicts dyadic fight outcomes (von Rueden et al. 2008).

Predictions

Regardless of basal testosterone or condition, males engaged in physically competitive situations would benefit from acute increases in testosterone, which rapidly enhance muscle performance and redistributes immune resources to prepare for injury. We hypothesize that despite pathogenic and parasitic stress resulting in lower levels of baseline testosterone, Tsimane males will express a relative increase in testosterone during competition similar to males in industrialized populations. We also expect to find larger increases in testosterone among winners, as increased post-game testosterone could augment future competitive ability. Positive associations between self-rated performance and increases in testosterone are also hypothesized, as they may represent a personal victory, independent of team success.

Methods

In August 2010, teams from eight Tsimane communities competed in one of two inter-village soccer tournaments held by the researchers. Soccer is a common form of recreation among the Tsimane; men in these communities reported playing an average of 3.1 (SD=1.5) days per week. The majority of games occur within communities with little-or-no audience present. On weekends, some neighboring communities play against one another, frequently in the presence of audiences. Our tournaments brought distant villages together, with large mixed-sex audiences. Teams from each community were given uniforms, and the winning teams received trophies, creating a competitive atmosphere. Eighty-eight male participants provided saliva specimens via passive drool 15 minutes before, 10 minutes after, and 1 hour after their first soccer match.   All games started at 11AM and 12PM, to control for diurnal variation in testosterone (Bribiescas and Hill 2009). Following the match, players were separated from their teammates and asked to identify the best and worst players on their team, and then how well they played in comparison with a five-point ladder scale. Height, weight, and age were collected from participants for use as control variables, with age ranging from 16-59 years (mean age 26.8), see Table 3.1. Anthropometric measurements were not available for one individual; his body mass index (BMI) was coded as missing. Specimens were frozen in liquid nitrogen until transferred to dry ice and flown to the

University of Washington where they were stored for four months at -80°C. Salivary testosterone is stable for at least 36 months when stored at -80°C (Granger et al. 2004).

Specimens were thawed and centrifuged at 2800 RPM for 20 minutes, and the aqueous layer aliquoted for assay. All specimens had gone through two freeze thaw cycles when initially assayed. Saliva exhibiting blood contamination (n=22 specimens) (Granger et al. 2004), or inadequate volume (n=5 specimens) were excluded, leaving a total of 237 usable specimens from 82 individuals. Salivary testosterone was measured via an in-house enzyme immunoassay (Trumble et al. 2010) that cross reacts 100% with testosterone, 57.4% with 5 α-dihydrotestosterone, 0.27% with androstenodione, and less than 0.05% with other androgens (Muir et al. 2001). Specimens were run in duplicate, with each participant's samples run on the same plate to reduce bias due to inter-plate variation. Individuals were randomized between plates to ensure that teams were not placed together on the same plate. The within and between assay coefficients of variation for this project (n=24 plates) were 4.5% and 9.3% respectively for the low (403.1 pg/mL) and 5.5% and 11.4% for the high (681.4 pg/mL) in-house controls.

US Tsimane Salivary Testosterone Comparison Methods

US male salivary testosterone specimens (n=40 specimens), were collected as a part of a separate, non-competitive experiment (Trumble et al. 2010). Twenty three healthy weight men (mean BMI 23.7 kg/m^2) aged 19-36 years (mean age 26.7) provided saliva specimens at 11 AM on two consecutive control days, while undergoing no experimental manipulations. These US data, analyzed in the same lab using the same enzyme immunoassay (Muir et al. 2001), will be directly compared to Tsimane pre-competition (baseline) salivary testosterone levels to examine differences in basal testosterone.

Statistical Methods

Salivary testosterone concentrations were normalized with a log transformation. To control for the non-independence of repeated specimens from each participant, linear mixed-effects regressions with individuals modeled as a random effect (West et al. 2007) were used to determine the effect of competition on log transformed salivary testosterone 10 minutes and 1 hour after the a soccer match. Adipose tissue can metabolize testosterone into estrogen (e.g., (Bhasin et al. 2001)), so BMI was included as a control variable in all models. Study design controlled the time of day at which specimens were collected; wakeup times were reported to be invariant, thus time of specimen collection was not included as a control variable. In the Tsimane competition data, there was no association between testosterone and age (p=0.805), or testosterone change from before to immediately following the game and age (p=0.371), thus age was not included as a control variable. The effects of age may be non-linear; additional analyses modeling age as quadratic or dummy variables (dividing age into quartiles and decades), found no association between age and testosterone or change in testosterone. Analyses were conducted in STATA 12.1 (College Station, TX).

Results

Tsimane-US Salivary Testosterone Comparison

Tsimane pre-competition saliva specimens (n=82) were compared to age-matched US saliva specimens (n=40) collected as controls during a non-competition study (Trumble et al.

2010) to establish differences in baseline testosterone levels. Tsimane male salivary testosterone (182.9 pg/mL, SD=76.0) was significantly lower (β=-0.411, $p<0.001$) compared to US males (266.8 pg/mL, SD=90.2), controlling for age and BMI. These results were not due to a wider age range of Tsimane participants- a secondary analysis limiting the age range of Tsimane participants to match US participants also found significantly lower salivary testosterone (β=-0.409, $p<0.001$) among Tsimane men.

Tsimane Soccer Competition

T in the Tsimane soccer players was significantly higher after the game compared to baseline levels (β=0.23, $p<0.001$) and remained high one hour after the game (β=0.08, $p=0.009$), see Figure 3.1. The mean percent change in testosterone from 15 minutes before the game to 10 minutes after the game was 30.1%, and from before to one hour after the game was 15.5%, see Table 3.1. Winning was not associated with testosterone ($p=0.558$), nor percent change in testosterone ($p=0.450$). Men with higher baseline testosterone had smaller relative increases in testosterone immediately following the game (β=-32.85, $p< 0.001$) and one hour after the game (β=-46.41, $p< 0.001$).

Additional linear mixed-effects models with individuals and teams coded as random effects established that higher self-rated performance was associated with larger percent increases in testosterone from before the game to immediately following the game (β=8.90, $p=0.005$), but not one hour after the game ($p=0.201$), see Figure 3.2. Baseline testosterone was not associated with self-rated performance ($p=0.493$). Eleven goals were scored by 6 players; those who scored more goals trended towards larger increase in testosterone 10 minutes after the game (β=16.79, $p=0.113$), and one hour after the game (β=32.74, $p=0.006$).

No single position (forward, midfielder, defender, goalkeeper) had a statistically different testosterone response compared to other positions. When stratified into defensive (defenders and goalkeepers) and offensive players (forwards and midfielders), defensive players trended toward smaller increases in testosterone compared to the offense ($\beta=-15.92$, $p=0.054$), controlling for BMI. However, 11 participants reported switching between defensive and offensive positions during the game; excluding these individuals attenuated the trend ($\beta=-14.50$, $p=0.106$). A logistic regression found that defensive players tended to report poorer self-rated performance than offensive players ($\beta=-0.91$, $p<0.001$). Analysis examining the association between relative change in testosterone and position was re-run with self-rated performance as a control variable, finding no association ($p=0.497$).

Discussion

The Tsimane men in this sample had significantly lower baseline (pre-competition) levels of salivary testosterone compared to age matched US males, controlling for age, and BMI. These results replicate the finding that industrialized populations sheltered from many energetic and pathogenic stresses have significantly higher levels of testosterone than men in non-industrialized settings (Ellison et al. 1989; Bentley et al. 1993; Bribiescas 1996; Ellison et al. 2002; Campbell et al. 2006). Previous studies find evidence of a pre-competition rise in testosterone (Booth et al. 1989; Mazur et al. 1992; Suay et al. 1999; Salvador et al. 2003), thus our baseline Tsimane testosterone measure may be artificially inflated, suggesting that basal Tsimane testosterone may be even lower.

Age was not associated with basal Tsimane testosterone levels, or changes in testosterone following competition. These results replicate those of previous studies suggesting that the typical pattern of decreasing testosterone across the lifespan in industrialized populations (e.g., (Travison et al. 2007a)) may not be generalizable to forager-horticulturalist populations (e.g., (Bribiescas 1996; Ellison et al. 2002)). Most (Archer 2006), though not all (Mazur et al. 1992), studies of acute changes in testosterone during competition are conducted in young males, thus little is known about competition related changes in testosterone in older males. A US study reports that age did not affect the magnitude of increase in salivary testosterone from men exposed to sexual stimuli (Escasa et al. 2011), raising the possibility that acute changes in testosterone due to behavioral stimuli may occur independent of age.

Despite lower baseline testosterone, Tsimane males expressed significant increases in testosterone during physical competition. The average increase in testosterone from before the game to immediately following the game was 30.1%. A review of twelve physical competition related studies in industrialized populations found an average testosterone increase of 37%, with a confidence interval ranging from a 13-61% increase in testosterone (Archer 2006). The Tsimane testosterone increase is of a comparable relative change to that observed in industrialized settings, despite lower absolute testosterone levels. Studies of human male-male physical competition have focused on industrialized populations with high, perhaps evolutionarily novel levels of T; this study extends these findings to non-industrialized populations. Caution must be taken when interpreting changes in testosterone as purely due to competition, which omits changes in testosterone due to physical activity; previous studies suggest that physically demanding competitions result in a larger increase in testosterone than non-physical competitions (Archer 2006).

Acute, short-term increases in testosterone are beneficial regardless of current environmental condition, enhancing immediate muscle performance and redistributing leukocytes to peripheral tissues in preparation for injury. Previous studies find that poor environmental conditions or illness can reduce testosterone mediated reproductive or competitive behavior in some avian species (Neave and Wolfson 2003; Munoz et al. 2010). In these studies, lower testosterone was associated with avoiding territorial behavior that could induce a fight; thus facilitating avoidance of dangerous conflicts when an individual is in poor condition. However, these studies examined the role of baseline testosterone on behavior, and did not measure acute changes in testosterone during competition. Once engaged in a physical confrontation, enhanced muscle physiology and immunoredistribution would be advantageous regardless of individual condition, in order to facilitate fighting ability, or escape.

Men with higher baseline testosterone had a decreased relative change in testosterone compared to those with lower baseline testosterone. This is interpreted as a function of the pre-competition rise in testosterone (Booth et al. 1989; Mazur et al. 1992; Suay et al. 1999; Salvador et al. 2003); some men may have had a pre-competition spike in testosterone prior to their first saliva specimen, while others had a spike in testosterone at the onset of the game.

When stratified into defensive and offensive positions, there was a trend toward offensive players presenting a larger relative increases in testosterone. There ceased to be an association between relative change in testosterone and field position type when controlling for self-rated performance, suggesting that change in testosterone is not simply due to increased physical activity among offensive players, and likely is dependent on personal investment, regardless of position.

There was no evidence of a winner effect in this sample. Previous reports find evidence for (Elias 1981; Booth et al. 1989; Gladue et al. 1989; Mazur et al. 1992; Archer 2006), and against (Gonzalez-Bono et al. 1999; Suay et al. 1999; Wagner et al. 2002; Mehta and Josephs 2006; van der Meij et al. 2008) winner effects. Some variation in winner effects may be due to the circumstances in which competitions are held; men playing against in-groups tend to exhibit a reduced testosterone response to winning compared to those competing against out-groups (Wagner et al. 2002; Oxford et al. 2009). In this case, men were competing for their village, against other villages, in a high status event; a situation normally associated with a winner effect (Archer 2006; Oxford et al. 2009). However, Tsimane communities are relatively informal and fluid; migration between communities is not uncommon. The more salient in-group could have been family members or friends who were playing against each other. It is possible that some of the men were competing against in-groups, a situation which reduces a testosterone related winner effect (Wagner et al. 2002; Oxford et al. 2009). We did not collect data beforehand on who was expected to win each match anecdotal evidence suggests that the winning teams of each tournament were reliably predicted before the matches, which could have reduced individual investment in the game (Gonzalez-Bono et al. 1999).

Better individual performance, as measured by either self-rating or goals scored, was associated with a larger relative change in testosterone following the game. These results replicate those of previous projects which suggest that higher self-rated performance (Edwards et al. 2006; Oxford et al. 2009), and individual investment in the game (Gonzalez-

Bono et al. 1999) increases testosterone response to competition. While overall changes in testosterone confound physical activity and psychological competition, individual level performance may indicate personal investment in competition. Individual performance may be tantamount to personal victory, regardless of team success. Better performance, even if a member of a losing coalition, signals individual ability within and between coalitions, and to audience members. It should be noted that the challenge hypothesis was designed to examine changes in testosterone in a bird model, focusing on dyadic conflicts. Human tests involving sports competitions are mediated by coalitional membership, thus in human studies, it may be important to examine individual and coalitional success.

Limitations

These tournaments were high-profile events; it is not clear whether these results could be extended to normal within-community games. A major limitation of this study is that men playing in the soccer tournament were likely self-selected to be the healthiest and most competitive young Tsimane men. A one-sided t-test suggests that tournament participants who had a mean BMI of 23.7 kg/m^2, were not statistically different from the average BMI of 23.5 kg/m^2 for Tsimane men aged 16-59 years (n=728 men measured since 2008). Despite self selection, these men still presented with significantly lower levels of testosterone compared to age matched US males. While this study has a larger age range than many previous studies, it offers limited insight into age related acute testosterone changes among older men.

It should be noted that all studies examining testosterone dynamics during competitive sports confound changes in testosterone resulting from physical activity and psychological factors associated with competition. To disassociate these differences, additional studies were conducted to examine changes in Tsimane testosterone during non-competitive physical activity, and during competitive non-physical activity. Beyond differentiating between physical and non-physical competition, these studies also allowed us to examine changes in testosterone (1) in conjunction with individual measures of health status, (2) in older men, (3) and in the presence of different audiences.

Conclusion

In sum, these results suggest that the Tsimane, who have significantly lower levels of baseline testosterone compared to US males, exhibit an acute increase in testosterone during physical competition. There was no evidence of increased testosterone for winning teams, though individuals who performed better expressed larger increases in testosterone, perhaps underscoring the importance of individual as well as coalitional success. While previous studies suggest that environmentally mediated testosterone may decrease territorial or competitive behavior, we propose that once engaged in physical competition, acute increase in testosterone are physiologically beneficial regardless of individual condition.

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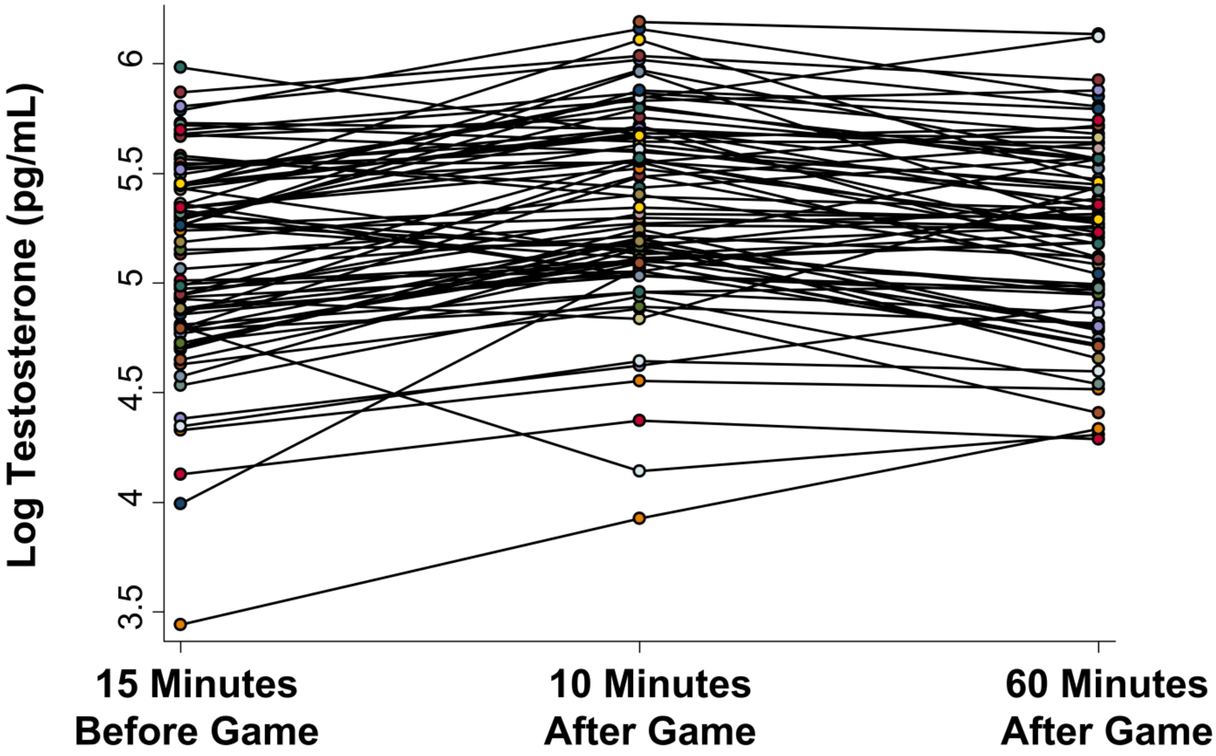
Table 3.1: Descriptive statistics for 82 Tsimane males including (A) age and BMI, (B) testosterone before and after a soccer match, and (C) percent change in testosterone.

		Mean	Std Err	Min	Max	95% CI
A	Age (Years)	26.8	0.99	16	59	(24.8, 28.7)
	BMI (kg/m^2)	23.7	0.29	16.7	29.7	(23.1, 24.3)
B	T 15 Min Pre-Game (pg/mL)	182.9	8.49	31.3	397.0	(166.0, 199.8)
	T 10 Min Post Game (pg/mL)	231.3	10.66	50.7	487.8	(210.1, 252.5)
	T 60 min Post Game (pg/mL)	202.0	9.77	72.9	461.9	(182.6, 221.5)
C	% Change in T 10 Min Post	30.1	4.05	-48.4	193.9	(22.0, 38.2)
	% Change in T 60 Min Post	15.5	6.70	-54.0	212.3	(2.2, 28.9)

Figure 3.1: Testosterone change following a soccer game



Individual log salivary testosterone 15 minutes before, 10 minutes after, and 60 minutes after a soccer match for 82 Tsimane men.

Figure 3.2: Change in testosterone and self-rated performance

Individual change in salivary testosterone 10 minutes post-game and self-rated performance for 82 Tsimane men.

Chapter 4: Age-independent increases in male salivary testosterone during horticultural activity among Tsimane forager-farmers

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Abstract

Testosterone plays an important role in mediating male reproductive trade-offs in many vertebrate species, augmenting muscle and influencing behavior necessary for male-male competition and mating-effort. Among humans, testosterone may also play a key role in facilitating male provisioning of offspring through increases testosterone which can augment muscle performance and enhance neuromuscular facilities. This study examines acute changes in salivary testosterone among 63 Tsimane men ranging in age from 16-80 (mean 38.2) years during one-hour bouts of tree-chopping while clearing trees for horticulture plots. The Tsimane are forager-horticulturalists living in the Bolivian Amazon who experience high energy expenditure associated with food production, as well as high levels of parasites and pathogens, and display significantly lower baseline levels of salivary testosterone than age-matched US males. Mixed-effects models controlling for BMI and time of specimen collection reveal a significant increase in salivary testosterone ($p<0.001$) equivalent to a 48.6% rise in testosterone, after one hour of tree chopping. Age had no effect on testosterone ($p=0.656$), nor did self reported illness ($p=0.488$). A comparison of these results to the relative change in testosterone during a competitive soccer tournament in the same population reveals larger relative changes in testosterone following resource production (tree chopping), compared to competition (soccer). These findings highlight the importance of moving beyond a unidimensional focus on changes in testosterone and male-male aggression, to investigate the importance of testosterone-behavior across additional male fitness-related activities. Increased testosterone during muscularly intensive horticultural food production may facilitate male productivity and provisioning.

Introduction

Testosterone is considered a primary mediating factor in male life history trade-offs, implicated in mate-seeking behaviors, territoriality, and secondary sexual characteristics, at a cost to paternal behavior and maintenance of immune function (Wingfield et al. 1990; Hau 2007). Consistent with this evolutionary model, human studies find evidence for positive associations between testosterone and various aspects of mating effort including male-male competition (Archer 2006), aggression (Archer et al. 1998), and attractiveness (Roney et al. 2006), with negative associations between testosterone and parenting effort (Muller et al. 2009; Gettler et al. 2011) and illness (Muehlenbein and Bribiescas 2005). While male-male physical confrontations dictate reproductive access to females in many mammals (Geary 2000), human males rely on different behavioral strategies to attract and maintain relationships with mates, tending to focus more on resource production and long-term investment in offspring than on agonistic male-male interactions (Kaplan et al. 2000; Pillsworth 2008; von Rueden et al. 2011).

Resource production is an important predictor of male reproductive success in subsistence and industrialized populations (Smith 2004; Gurven and von Rueden 2006; Hopcroft 2006; Fieder and Huber 2007; Gurven and Hill 2009; Mulder and Beheim 2011). Women in subsistence societies often list resource productivity and industriousness as important criteria for marriageable partners (Marlowe 2005; Pillsworth 2008; Gurven et al. 2009b). Much of male food production involves the use of physically active production techniques to acquire nutritionally dense but hard to acquire food resources, hallmarks of the human feeding niche (Kaplan et al. 2000). Testosterone promotes muscle growth and maintenance (Bribiescas 2001), which are not only important in male-male competition, but also vital for physically active food production strategies that would have been the norm throughout most of human evolutionary history.

Although male-male aggression indisputably played a role in human evolution, there are numerous extant and archeological examples of social regulations designed to reduce the damage caused by male-male violence, ranging from axe and club fight protocols to state laws (Prince 1904; Daly and Wilson 1988; Hill and Hurtado 1996; Hill et al. 2009). The coevolution of the human feeding niche, longevity, and brain size may have applied a different set of selective pressures on the role of testosterone in human males compared to other male mammals, placing greater emphasis on production than on direct competition (Geary 2000; Kaplan et al. 2000; Hill et al. 2009). This study looks beyond male-male competition to examine changes in testosterone during horticultural activity, and argues for extending the role of testosterone in reproductive success beyond mating effort.

While much of the research on testosterone and male physiology has focused on the long term up-regulatory effect of testosterone on muscle tissue (Bhasin et al. 1996; Bribiescas 2010), acute increases in testosterone also play an important role in augmenting muscle physiology and metabolism (Tsai and Sapolsky 1996), immediately promoting sugar uptake as well as enhancing neuromuscular performance (Crewther et al. 2011). Individuals who express increases in testosterone during resistance training (e.g., weight lifting) have greater increases in muscle strength compared to those who do not exhibit changes in testosterone (Rønnestad et al. 2011). While human and non-human studies have largely focused on the role of short-term increases in testosterone during competition, the acute benefits of testosterone during muscularly intensive horticultural food production may help also increase male productivity, enhancing reproductive success.

In addition to the physiological effects, acute increases in testosterone produce immediate behavioral changes, increasing aggression and territorial behaviors in many, though not all, vertebrates (Oyegbile and Marler 2005; Wingfield 2005; Hirschenhauser and Oliveira 2006). The pattern of hormone-behavior interactions resulting in increased testosterone during male-male competition is commonly discussed under the framework of the “challenge hypothesis” (Wingfield et al. 1990; Archer 2006; Goymann et al. 2007). The challenge hypothesis, as applied to human males, suggests that testosterone levels are maintained at relatively low levels to avoid the energetic and health costs of high testosterone, but spike when men engage in reproductively important behaviors including male-male competition and sexual activity in order to provide immediate muscular and behavioral advantages (Archer 2006).

Increases in testosterone during physical activity are also seen in women (Edwards et al. 2006; Edwards and Kurlander 2010), and during non-competitive exercise (Vingren et al. 2010). Studies in industrial populations demonstrate that physical activity in the absence of competition can cause short-term increases in testosterone (Kraemer et al. 1998; Pullinen et al. 2002; Vingren et al. 2010). It is unlikely that violent physical competition among females was a significant selective force throughout vertebrate evolution (Clutton-Brock et al. 2006). That human females also exhibit the ability for acute changes in testosterone during physical activity may indicate that this is an ancestral vertebral trait (Aizawa et al. 2008) which allowed for rapid muscular enhancement for productivity that was later co-opted for the purposes of male-male competition. Acute testosterone increases during non-competitive exercise have been reported in both male and female rodents (Aizawa et al. 2008). While male-male competition was certainly important in human evolution, the role of paternal provisioning was likely a much stronger selective pressure in human males compared to other mammals (Geary 2000). This study examines acute changes in testosterone during an ecologically valid horticultural production task, and compares these results to acute changes in testosterone collected during a competitive soccer match, in order to provide a more complete perspective on the conditions under which male testosterone changes.

Testosterone is positively associated with muscle mass in both industrialized (Vermeulen et al. 1999a; Lassek and Gaulin 2009) and non-industrialized populations (Ellison and Panter-Brick 1996; Lukas et al. 2004). Resistance exercises cause a larger increase in testosterone than do aerobic endurance exercises (Jensen et al. 1991; Tremblay et al. 2004), while multi-hour sustained endurance activities (e.g., marathons) are associated with decreases in testosterone (Keizer et al. 1989). However, these studies have been conducted in industrialized populations with constant energy availability and higher, potentially evolutionarily novel testosterone levels compared to pathogenic and parasitically stressed non-industrial populations (Ellison et al. 2002), like the Tsimane (Trumble et al. 2012).

While muscle gain stemming from increased testosterone can be beneficial for physically challenging tasks, the physiological effects of testosterone entail energetic and immunologic trade-offs. Muscle tissue is costly to develop and maintain, with adult human males spending approximately 20% of the basal metabolism on muscle tissue (Bribiescas 2010). Life history theory predicts (Charnov 1993; Ricklefs and Wikelski 2002), and studies report, that energetic stress from reduced caloric intake (Cameron 1996), or parasite and pathogen exposure (Muehlenbein and Bribiescas 2005; Boonekamp et al. 2008; Muehlenbein et al. 2010), down-regulates the hypothalamic-pituitary-gonadal (HPG) axis, decreasing testosterone production. These illness related decreases in testosterone are known to modify downstream behavior in animal models (Ashley et al. 2009), though this has not been explicitly tested in humans.

Males face other trade-offs requiring differential energy allocation across life history stages; reductions in testosterone with marriage (Gray et al. 2004), or fatherhood (Gray et al. 2006; Muller et al. 2009; Gettler et al. 2011) are hypothesized to reflect a switch from mating investment to paternal investment. However, the muscle related advantages of testosterone are not limited to finding a mate, as increased strength is also beneficial for physically intensive food production. Offspring require significant caloric resources, which often necessitate increased food production in subsistence societies (Marlowe 2003; Kaplan et al. 2009; Winking et al. 2009; Madimenos et al. 2011). Thus acute increases in testosterone may be one strategy to gain the performance benefits associated with high testosterone, while avoiding the behavioral and immunocompromising effects of high baseline testosterone.

In industrialized populations, male testosterone peaks in the early twenties and declines with age (Harman et al. 2001; Uchida et al. 2006; Travison et al. 2007a). Declines in testosterone among men in industrialized populations have been linked to inflammation, obesity, metabolic disorders, and other age-linked diseases of affluence (Travison et al. 2007a). Thus the decrease in testosterone with age seen in industrialized populations may not be solely due to senescence of reproductive tissues, but also a host of other physical activity and disease related factors. While baseline testosterone appears to decrease across the lifespan in industrialized societies, most men in these populations do not rely on energy-intensive muscle-based subsistence strategies, and report significantly reduced activity levels compared to men in horticultural populations (Gurven et al. 2009a). Non-industrialized populations facing energetic constraints and pathogenic stress show lower levels of testosterone across all adult ages as compared with men in industrialized nations, as well a slower and shallower rate of change with age (Worthman and Konner 1987; Bribiescas 1996; Ellison et al. 2002; Bribiescas and Hill 2009; Vitzthum et al. 2009; Trumble et al. 2012). Male production needs to remain high throughout life in order to provision offspring and grand-offspring; thus, we predict that men in subsistence-based societies, including the Tsimane, will be able to sustain acute increases in testosterone during horticultural activity across the life.

The Tsimane are forager-horticulturalists living in lowland Bolivia. They face higher levels of parasite and pathogen exposure than individuals in the US (McDade et al. 2005; Gurven et al. 2009a) as evinced by high levels of C-Reactive protein (CRP), a non-specific marker of inflammation (McDade et al. 2005; Vasunilashorn et al. 2010). Two-thirds of Tsimane adults present with signs of intestinal parasites, with one-third reporting symptoms of respiratory illness (Gurven et al. 2009a). The prevalence of obesity is eight times lower among the Tsimane (Gurven et al. 2009a), with energetic expenditure estimated to be 450-850 kcals/day greater for Tsimane men and women aged 40-49 years compared with age-matched US individuals. The average BMI for 1266 Tsimane men and women over the age of 20 was 23.7 kg/m^2, while a sample of 13,035 US men and women had an average BMI of 28.0 kg/m^2 (Gurven et al. 2009a).

An analysis comparing US and Tsimane salivary testosterone found that Tsimane males had significantly lower levels of salivary testosterone compared to age matched US males, controlling for age, BMI, and time of day (Trumble et al. 2012). In addition to hunting, fishing, and gathering, the Tsimane rely on small-scale horticulture for subsistence, clearing small areas of the jungle to grow staple foods including plantains, sweet manioc, rice, and corn (Reyes-García et al. 2004). When clearing fields, smaller vegetation is first removed with machetes, and then the trees are chopped down before burning and planting the field. While women help with harvesting, machete clearing, and planting, the clearing of larger trees is done exclusively by men (Gurven et al. 2009b). Clearing of large trees is arguably the most time-intensive and energy-intensive component of horticulture (Johnson and Johnson 1975; Wilkie and Curran 1993; Kramer 2005; Madimenos et al. 2011). This small-scale slash-and-burn horticulture offers the opportunity to examine changes in testosterone during controlled, intense, non-competitive physical activity; tree chopping. Do Tsimane men express acute changes in testosterone during physical activity similar to the effects of comparable physical activity in industrial populations? Does competitive physical activity produce more of an increase in testosterone than physical activity alone? This study expands the current focus on changes in testosterone during male-male competition and aggression to better understand the importance of acute testosterone-behavior interactions outside of mating-effort, specifically changes in testosterone during tree-chopping for small scale horticulture.

Hypotheses

Despite having salivary testosterone levels one-third lower than age-matched US males (Trumble et al. 2012), Tsimane men engage in production strategies requiring significant muscle mass and cardiovascular performance. The primary hypothesis of this study is that testosterone will increase significantly during physical activity, despite lower overall levels of testosterone. Exhibiting acute increases in testosterone during physical activity may serve to increase and maintain muscle mass, while still allowing Tsimane men to reap the energetic and immune benefits of low baseline testosterone.

A previous study reported acutely increased testosterone during a soccer tournament among Tsimane men (Trumble et al. 2012). If competition is a main driver of acute changes in testosterone, then we would expect tree chopping to result in a smaller change in testosterone in comparison to the soccer tournament. However, if the role of acute changes in testosterone is largely due to physical activity, then we would expect equivalent changes in testosterone during both activities.

If evolutionary pressures asserted by male-male competition resulted in selection for acute increases in testosterone, then one would predict little selective pressure for older men to maintain this ability, as their fertility would already largely be realized by the time their wives reach menopause (Kaplan et al. 2010), and any potential competitive interactions would be occurring in the 'selection shadow' (Kirkwood and Austad 2000), or at least under reduced selection pressure. If instead, resource production was a major driver of male ability to sustain acute increases in testosterone, we would expect older men to be able to sustain acute increases in testosterone, given that older men contribute substantial resources to offspring and grand-offspring (Kaplan et al. 2010) thereby increasing their fitness. Thus a secondary hypothesis for this project is that there will be no age-related differences in testosterone during physical activity.

Previous reports find that illness reduces circulating testosterone (Muehlenbein et al. 2010), and thus we also hypothesize that individuals exhibiting symptoms of illness will have lower levels of testosterone and reduced increases in testosterone during physical activity.

Methods

Participants

From July-September 2010, Tsimane men (n=63) aged 16-80 years participated in a study examining acute changes in salivary testosterone during a heavy physical activity, tree chopping (see Table 4.1). Men were recruited during community meetings, and through word of mouth; this work was approved by the University of Washington Human Subjects Division.

Data Collection

Participants rinsed their mouths with water, and provided 1-2 mL of passive drool saliva 5-15 minutes before beginning to chop trees. All of the men in this sample used metal-headed axes, with some men also employing machetes to cut vines or smaller branches off of trees to facilitate tree clearing. In this sample, the trees being chopped ranged in size and hardness, though all men recruited were chopping trees >100 cm on the day saliva specimens were collected. After one hour of intense tree chopping, participants provided a second saliva specimen. The time of initiation of tree chopping ranged from 7:15AM to 3:26 PM. A subset of participants wore Polar RS 800CX heart rate monitors while chopping (n=31). Saliva specimens were frozen in liquid nitrogen until transferred to dry ice and flown to the Biological Anthropology and Biodemography Laboratory at the University of Washington. There were 10 instances where pre-tree-chopping saliva was missed, as men had begun chopping trees prior to the arrival of the researchers. The time of initial chopping was known, and it was possible to still collect a saliva specimen one hour after beginning physical activity, leading to 10 cases where the initial pre-chopping sample was missing.

Height was measured with a modified SECA 214 stadiometer, and weight was measured with a TANITA scale; these measures were combined to calculate Body Mass Index (BMI) in kg/m^2 (see Table 4.1). At the time of participation, men were asked if they were experiencing any symptoms of pain or illness as a simple assessment of disease state. Nearly three-quarters of participants (*n*=37) reported some type of pain (see Table 4.2), though symptoms were largely mild as these men were able to chop trees. Their reports of symptoms were sub-categorized as illness (*n*=27) if they reported fever, cough, diarrhea, or other symptoms of active infection.

A subset of these individuals (*n*=20) also provided two saliva specimens one hour apart on a day when they were resting. Heart rates were collected with Polar RS 800CX heart rate monitors. The researchers sat with the men while they talked or engaged in non-physically intensive labor (e.g., mending fishing nets), to ensure that changes in testosterone while tree-chopping were not due to the presence of the researchers. In this sample, eighty percent of the resting men (*n*=16) reported some symptom of illness or pain.

Laboratory Methods

Saliva Specimens were later thawed and centrifuged at 2800 RPM for 20 minutes, and the aqueous layer removed and stored at -80°C until assay. All specimens had gone through two freeze thaw cycles when initially assayed. Saliva from men chewing coca (*n*=12 individuals), and specimens exhibiting blood contamination (*n*=4 specimens) (Granger et al. 2004) were excluded from analysis, leaving a total of 90 usable physical activity specimens from 49 individuals, and 38 usable resting specimens from 20 individuals. The ages of two men, and BMI for four men were unknown, and coded as missing for analysis. Salivary testosterone was measured via in-house enzyme immunoassay that cross reacts 100% with testosterone, 57.4% with 5 α-dihydrotestosterone, 0.27% with androstenedione, and less than 0.05% with other androgens (Muir et al. 2001; Trumble et al. 2012). All specimens were run in duplicate, and all of the samples collected from each participant were run on the same plate to eliminate inter-plate variation. The within and between assay coefficients of variation for this project (n=24 plates) were 4.49% and 9.29% respectively for the low (403.13 pg/mL) and 5.47% and 11.35% for the high (681.37 pg/mL) Biorad level one and two commercial controls diluted 1:10 in assay buffer (0.1% bovine serum albumin, sodium phosphate buffer, pH 7.0).

Statistical Analyses

All testosterone data were log transformed for analysis to control for non-normality. Maximum likelihood linear mixed-effects models examined the absolute levels of testosterone change after one hour of intense physical activity. Individuals were coded as a random effect to control for the non-independence of repeated measures from each participant (West et al. 2007). Linear regression models examined relative changes in testosterone. Potential covariates (fixed effects) included age (linear, quadratic, and dummy variables), BMI, time of day, symptoms of pain (binary), and symptoms indicative of illness (binary). Analyses were conducted in STATA 12.1 (College Station, TX).

Results

Tree chopping

Tree chopping is an intense physical activity. To quantify the degree of physical activity, heart rates from individuals resting for one hour were compared to individuals who were chopping trees for one hour (see Table 4.1). Percent of maximum heart rate was calculated (Tanaka et al. 2001), and indicated that tree chopping increased heart rate to an average 70.4% (SD 6.6) of maximum heart rate. In the subset of individuals for whom heart rate was collected during tree chopping (*n*=31), a linear regression demonstrated that age trended toward a negative effect on average heart rate (β=-0.32, *p*=0.081), controlling for time of specimen collection and BMI. Age had no effect on the percent of maximum heart rate, in this study (*p*=0.406). Additional information on age, cardiovascular output, and work productivity will be presented elsewhere (Cummings et al., *in prep*). A logistic regression suggests that there were no age related differences in reporting pain (*p*=0.516) or illness with age (*p*=0.648) for individuals engaged in tree chopping.

Testosterone and tree chopping

Prior to chopping trees, the average salivary testosterone level for these men was 201.9 pg/ml (SD=111.9); following one hour of tree chopping, the mean testosterone increased to 300.0 pg/mL (SD=143.6). Percent change in un-logged testosterone was calculated (Δ= [Post Chopping testosterone – Pre Chopping testosterone]/Pre Chopping testosterone), indicating a 48.6% increase in testosterone following one hour of physical activity. The most parsimonious linear mixed effects model as determined by likelihood ratio tests indicates that log testosterone increased significantly following an hour of tree chopping (β =0.45, *p*<0.001), controlling for BMI, and time of specimen collection (Table 4.3 models 1, Figure 4.1). Covariates for age (*p*=0.656), reporting pain (*p*=0.625), and reporting symptoms of illness (*p*=0.488), were not significant (Table 4.3, models 2-4). Additional analyses modeled age as quadratic, binary (below median age, above median age), and dummy variables (dividing age into terciles and decades), finding no significant impact of age on testosterone levels or change in testosterone. Linear regression models found no association between age and the percent change in testosterone (*p*=0.530), pain and percent change in testosterone (*p*=0.386), or illness and percent change in testosterone (*p*=0.142), controlling for time of specimen collection and BMI. There was no association between testosterone and average heart rate while tree chopping (*p*=0.888, model 5), controlling for BMI, time of specimen collection, and age.

Testosterone and Resting

Separate linear mixed effects models were constructed to examine salivary testosterone collected before and after men rested for one hour (Table 4.3). Unlike tree chopping, resting had no effect on testosterone ($\beta = -0.09$, *p*=0.244), controlling for BMI and time of day, equivalent to a 4.47% decrease in testosterone. In a separate analysis of the resting sample (not reported in Table 3), there was a trend toward older men reporting more symptoms indicative of pain than younger men ($\beta = .01$, *p*=0.052), and thus additional models were constructed including age and pain symptoms as covariates. In model 3, men reporting symptoms of pain had lower levels of testosterone ($\beta = -0.47$, *p*=0.047), Table 4.3, model 3.

Censoring

In order to account for the left censoring caused by 10 missing pre-physical activity saliva specimens, a likelihood model was built in *mle* 2.2.17 (Holman 2003). The model for non-censored data was:

$$\mathbf{L}=f_{\mathbf{pre}}(\mathbf{t}_{\mathbf{pre}})f_{\mathbf{post}}(\mathbf{t}_{\mathbf{post}})f_{\Delta}(\mathbf{t}_{\mathbf{post}}-\mathbf{t}_{\mathbf{pre}})$$

Where $f_{\mathbf{pre}}(t_{\mathbf{pre}})$ represents the distribution of log testosterone values prior to physical activity, $f_{\mathbf{post}}(t_{\mathbf{post}})$ represents the distribution of log testosterone values after exercise, and $f_{\Delta}(t_{\mathbf{post}}-t_{\mathbf{pre}})$ denotes the difference between post and pre exercise testosterone values. In the cases where the pre-physical activity testosterone values were not observed, the censored model was:

$$\mathbf{L}= f_{\mathbf{post}}(\mathbf{t}_{\mathbf{post}})\int f_{\mathbf{pre}}(\mathbf{z})f_{\Delta}(\mathbf{t}_{\mathbf{post}}-\mathbf{z})$$

Where $f_{\mathbf{post}}(t_{\mathbf{post}})$ represents the distribution of log testosterone values after exercise, and $\int f_{\mathbf{pre}}(z)f_{\Delta}(t_{\mathbf{post}}-z)$ integrates over the distribution of possible pre exercise values multiplied by the difference in post and possible pre-exercise values. Covariates were modeled on both the mean and variance, though no covariates were significant, nor did the inclusion of any covariates increase the model fit, thus the most parsimonious model (no covariates) was chosen. The mean results of the pre- and post-exercise testosterone distributions were exponentiated and used to calculate the average percent change in testosterone from before to after physical activity. Using this method, the average percent change in testosterone including all left-censored cases was calculated to be 54.9%.

Discussion

Despite having lower baseline testosterone levels than men in industrialized populations (Trumble et al. 2012), Tsimane men experienced a significant increase (48.6-54.9%) in testosterone following heavy physical activity, at a magnitude similar to or above levels of change reported in studies of competitive sports in industrialized populations (Archer 2006). Age had no effect on testosterone or on change in testosterone during physical activity. There was a non-significant change in testosterone (equivalent to a 4.47% decrease in T) when a sub-group of men rested in the presence of researchers, suggesting that the presence of researchers had no effect on T levels measured in this study. Were there any effect, it would have been constant for all men.

This study was designed to replicate the sampling framework of a previous study which reported a 30.1% increase in Tsimane salivary testosterone during a one hour competitive soccer match. To our knowledge this is the first study to directly compare differences in testosterone change for competitive versus subsistence-based physical activity using the same protocols and following the same analytical methods in the same population. Soccer is cardiovascularly intensive, but requires little resistance-based muscular activity. It has been reported that resistance based physical activity may produce larger increases in testosterone than aerobic activities in some (Tremblay et al. 2004) but not all studies (Hackney et al. 1995). In addition to being a cardiovascular activity, soccer is also highly competitive, which confounds the association between competition, physical activity, and testosterone. Additional studies are necessary to differentiate between the physical activity and psychological competition aspects of soccer in order to understand the role of testosterone in different activities.

While many studies have focused on testosterone and aggressive or competitive activities related to mating effort, our results underscore the importance of testosterone in male physical labor and production strategies. Enhanced muscular performance is beneficial across life history stages; studies of short-term increases in testosterone need not limit themselves to mating-effort contexts.

These results also raise questions about whether acute changes in testosterone in humans were selected for their ability to facilitate muscle physiology in competitive interactions. Current and paleoanthropological evidence of sexual dimorphism suggests that male-male competition has played an important role in human evolution, though human sexual dimorphism is much lower than the levels of dimorphism noted in ancestral populations (Plavcan and van Schaik 1997). Modern human sexual dimorphism could have been selected for during periods when male-male conflict played a more important role in male reproductive success. Or perhaps the coevolution of human brain size and feeding niche led to sexual division of labor that favored physically intensive male production strategies, and therefore an advantage in retaining sexual dimorphism (Kaplan et al. 2000; Gurven et al. 2009b). If the role of acute testosterone increases in human males was primarily to facilitate male-male competition, then one would expect the ability to sustain acute increases in testosterone to decrease with age, as men would have less fitness to gain from, and be less engaged in, mating effort. However, if selection for acute testosterone change is in part because of physical activity, then maintenance of this ability in older men would be important, as provisioning offspring and grand offspring benefits inclusive fitness even late in life (Kaplan et al. 2010). It is a false dichotomy to argue that selective pressures would have favored increases in testosterone due to physical activity as opposed to competitive situations, as it would likely be some combination of the two factors; this paper argues that that relative levels of importance for these two activities may be different among human populations as opposed to other non-human primates, or other mammals with less paternal involvement (Geary 2000).

Our results are similar to those reported by other researchers examining exercise-induced testosterone change in industrialized populations, which find that physical activity (Hackney et al. 1995; Vingren et al. 2010), especially resistance-based activities (Tremblay et al. 2004), result in increased circulating testosterone. Studies in the US find this to be true of younger and middle-aged (Kraemer et al. 1999; Hakkinen et al. 2000; Baker et al. 2006) as well as older men (Hakkinen et al. 1998; Hakkinen et al. 2000; Baker et al. 2006). Contrary to our findings, which suggest no age-related differences in testosterone response to physical activity, several studies in industrialized populations suggest that the magnitude of testosterone change during some resistance activities is diminished in older men compared to younger men (Kraemer et al. 1999; Hakkinen et al. 2000; Baker et al. 2006; Vingren et al. 2010). The differences between our results and these studies may be related to the higher levels of testosterone reported in industrialized populations, and the age-related differences in testosterone reported in industrialized populations, but not among subsistence populations (Ellison et al. 2002; Trumble et al. 2012). Studies of non-physical competitive activities also report no age-related differences in testosterone change following a dominoes competition (Flinn et al. 2012), and after exposure to sexual stimuli (Escasa et al. 2011).

Evidence is growing that non-industrial populations do not show the same scale of decrease in testosterone with age (Worthman and Konner 1987; Bribiescas 1996; Ellison et al. 2002; Bribiescas and Hill 2009; Vitzthum et al. 2009; Trumble et al. 2012). While many have argued that decreases in testosterone with fatherhood would increase investment in current offspring (Gray et al. 2002; Gettler et al. 2011), in subsistence populations dependents necessitate increased food production, and thus a diminished testosterone response during physical activity could have negative effects on physically intensive food production strategies. Studies of testosterone supplementation show that the muscles of older males are as responsive as young men to the anabolic effects of testosterone on muscle tissue (Bhasin et al. 2005). Thus when older Tsimane men express acute increases in testosterone during physical activity, they are likely to receive the same benefit of increased muscle performance as younger men (Tsai and Sapolsky 1996; Crewther et al. 2011; Rønnestad et al. 2011). That said, it seems unlikely that increases in testosterone are able to offset other age-related physical changes, including decreased cardiovascular ability, which result in decreased subsistence productivity for men engaged in hunting (Walker et al. 2002; Gurven et al. 2006), foraging (Walker et al. 2002) and clearing trees for small-scale horticulture (Cummings et al., *in prep*).

The Tsimane are subsistence-level foragers-horticulturalists living in a parasite- and pathogen-rich environment (Gurven et al. 2009a), which is one explanation for why they have lower levels of testosterone than US males (Ellison et al. 2002; Trumble et al. 2012). While low testosterone is advantageous for reducing physiological costs, T-mediated muscle mass is important for strength-related food production activities, like clearing forest for planting subsistence crops. If men are able to lower physiological costs by having low testosterone most of the time, but still exhibit acute increases in testosterone during physical activity, they would maximize muscle performance with minimum cost. This pattern is noted in relation to male-male competition across many taxa (Wingfield et al. 1990; Wingfield 2005). These results support expanding human applications of the challenge hypothesis to include acute changes in testosterone during non-competitive situations that are associated with increased reproductive success (such as subsistence labor).

Study participants were asked if they were experiencing any pain or symptoms of illness, as a simple measure of disease load. Although the majority (72.5%) of men engaged in tree chopping expressed some symptom of pain or illness, there was no difference in testosterone level or in change in testosterone when compared to men with no symptoms. This may indicate that the benefits of increased testosterone while performing physically intensive labor outweigh the potentially immunosuppressive costs of acutely increased testosterone, at least for men suffering from morbidities minor enough that they can still work. Among men who were resting, 80% indicated some type of symptom. Previous reports suggest that injuries, illness, parasites and pathogens (Boonekamp et al. 2008; Spratt et al. 2008; Muehlenbein and Watts 2010), including minor immune insults like flu vaccinations (Simmons and Roney 2009) cause significant reductions in testosterone. Among a self-selected group of men who were healthy enough to be working on their fields, there was no association between testosterone and self-reported symptoms. Among a self-selected group of men who were resting and choosing not to work that day, those indicating they had some sort of symptom or pain had significantly lower levels of testosterone.

Limitations

The disease status assessment was simple, and future work will quantify immune status with greater precision; saliva specimens precluded using serum-based biomarkers of health status. As noted earlier, self-selection is a significant issue in this study; men who were healthy enough to work in their fields are a self-selected group, as are those who chose to rest. The subset of resting men also suffered from a small sample size (n=20).

Conclusions

This study suggests that Tsimane men exhibit significant increases (48.6-54.9%) in testosterone when engaged in intense physical activity typical of horticulturalists. To our knowledge, this is the first study to directly compare differences in testosterone change during resistance based physical activity versus competitive aerobic activity, finding evidence of larger relative changes in testosterone following tree chopping, compared to soccer. While many models of male fatherhood in industrialized populations predict decreased testosterone with age, as males invest more in current over future offspring, this could actually be detrimental during physically active subsistence production. These results can be interpreted using the theoretical framework developed by the challenge hypothesis: men facing high parasite and pathogen loads exhibit low baseline testosterone levels to avoid energetic and immunosuppressive costs associated with basal testosterone. When engaged in heavy physical activity, testosterone increases, allowing the rapid muscular performance enhancement, without the long-term costs of consistently high baseline testosterone. These findings highlight the importance of moving beyond a unidimensional focus on changes in testosterone and male-male aggression, to better understand the reproductive importance of acute testosterone-behavior interactions outside of direct mating-effort.

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Table 4.1: Descriptive statistics for key variables and results for Tsimane men

	Mean	**Std Dev**	**Min**	**Max**
Age	38.2	18.83	16	80
BMI kg/m^2	22.9	2.17	16.2	28.4
Pre-Chop Testosterone pg/mL (*n*=41)	201.9	111.9	31.3	543.9
Post-Chop Testosterone pg/mL (*n*=49)	300.0	143.6	79.1	622.3
Average Resting Heart Rate (*n*=18)	73	7.80	63	90
Average Chopping Heart Rate (*n*=31)	129.9	15.11	102	173

Table 4.2: Reports of pain for individuals who were (A) engaged in tree chopping (*n*=51) or (B) resting (*n*=20). Their responses were then sub-categorized as illness if indicative of infection/parasitism. Note that some men were included in both samples.

A: Tree-Chopping

		Report Pain		
		No	**Yes**	**Total**
Illness	**No**	14	10	24
	Yes	0	27	27
	Total	14	37	51

B: Resting

		Report Pain		
		No	**Yes**	**Total**
Illness	**No**	4	9	13
	Yes	0	7	7
	Total	4	16	20

Table 4.3: Results of separate mixed-effects regression models examining changes in testosterone while (A) tree chopping, and (B) resting

A	Model 1	Model 2	Model 3	Model 4	Model 5
Change in Log T	0.45***	0.45***	0.45***	0.45***	0.46***
Time of Day	0.10	0.10	0.11	0.06	-0.80
BMI	-0.04	-0.05	-0.05	-0.04	-0.08*
Intercept	6.13***	6.12***	6.19***	6.11***	7.44***
Age		0.002			0.005
Symptom: Pain			-0.07		
Symptom: Illness				0.09	
Avg. Heart Rate					-0.003

B	Model 1	Model 2	Model 3	Model 4
Change in Log T	-0.09	-0.09	-0.08	-0.09
Time of Day	0.58	0.42	0.12	0.35
BMI	-0.01	-0.01	-0.008	-0.002
Intercept	5.54***	5.31***	5.58***	5.15***
Age		0.005	0.01*	0.007
Symptom: Pain			-0.47*	
Symptom: Illness				-0.19

*$p<0.05$ **$p<0.01$***$p<0.001$

Figure 4.1: Salivary testosterone before and after one hour of tree-chopping

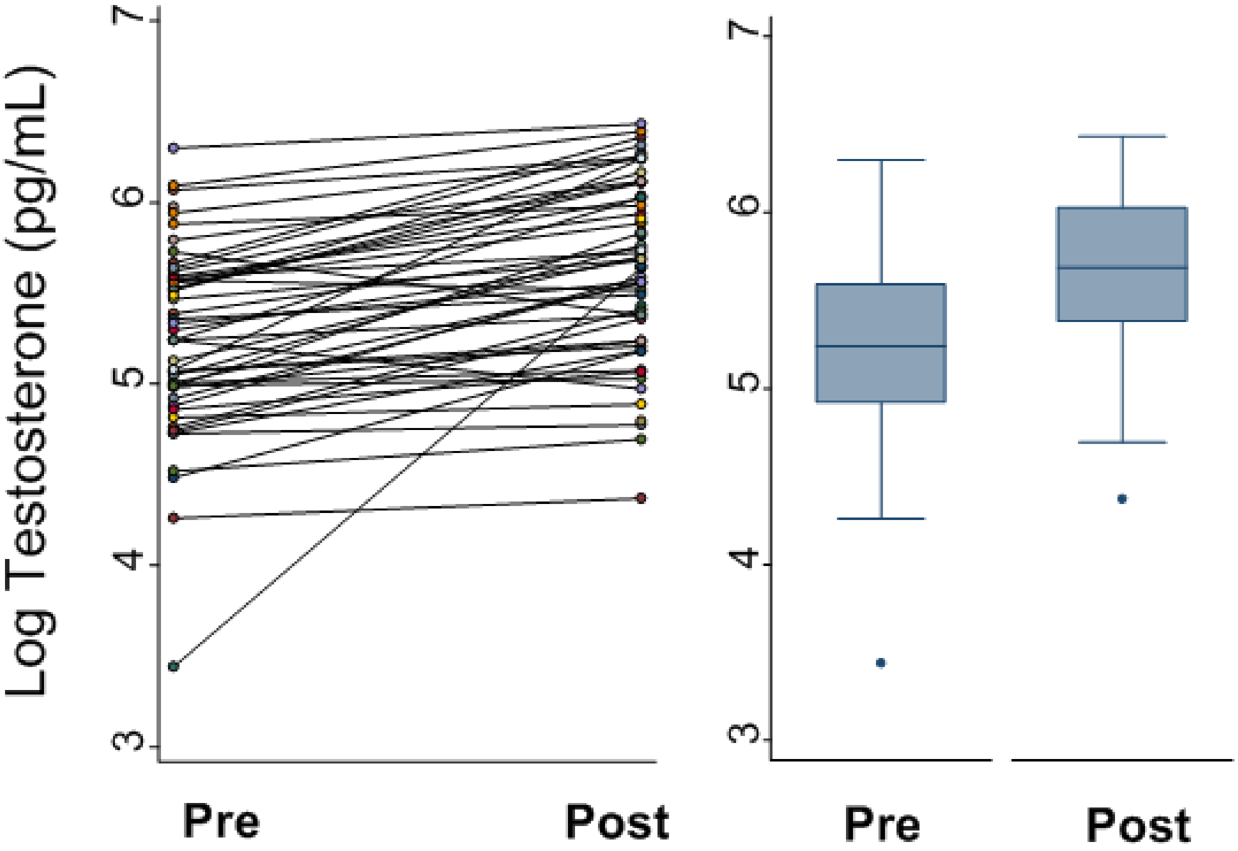

Figure 4.1A represents the individual changes (*n*=51) in log testosterone before and after intense physical activity. Figure 4.1B is the predicted change in testosterone controlling for BMI, and time of day.

Chapter 5: Audience composition does not affect testosterone change among Tsimane men during a penalty kick shootout.

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Abstract

Human behavior changes in various social contexts, particularly in the presence of potential competitors, allies or mates. Studies of hormone-audience interactions find that men express increased testosterone levels in the presence of a female audience. During competitive interactions, men competing in the presence of women tend to have higher levels of testosterone than those competing in the presence of men. This study examines how variation in audience sex and age composition affect acute changes in testosterone before and after men take five soccer penalty kicks. We hypothesized that men would have larger increases in testosterone in the presence of an audience, especially a young reproductive-aged female audience. We also hypothesized moderate increases in the presence of young men who could be potential competitors, and older men who tend to be higher status. We expected to see the smallest increases in testosterone in the presence of older post-reproductive women. Linear mixed effect models for all audience conditions combined show that logged salivary testosterone tended to increase following penalty kicks ($\beta = 0.06$, $p=0.069$), for an average increase of 7.8%. No single audience condition had a statistically different testosterone response from any other audience condition (p values range from $p=0.223$ to $p=0.889$). When audience conditions were pooled there was no difference in testosterone response between male and female audiences ($p=0.418$), or between older and younger audiences ($p=0.803$). While this study suggests that there is no effect of audience composition on testosterone change during penalty kicks, it should be noted that soccer penalty kicks are a relatively trivial form of competition. Future studies will examine the audience effect on testosterone during activities linked to reproductive success, such as hunting ability.

Introduction

Flexible behavioral strategies allow organisms to react to various environmental cues. While the physical, energetic, and immunological environment are all vital cues, so too is the social environment. The behavior of humans and other animal varies in the presence of others, especially when the individuals present are potential competitors, allies, or mates. In humans, the presence of others effects diverse behaviors, from how generous or cooperative participants are in economic games (Milinski et al. 2002; Wedekind and Braithwaite 2002) to how people cross the street (Pawlowski et al. 2008). Even ephemeral audience presence, such as pictures of human eyes (Bateson et al. 2006; Ernest-Jones et al. 2011), robotic eyes (Burnham and Hare 2007), or even more abstract eye-spot designs (Haley and Fessler 2005) modify the way that people behave in both the real world and in economic games.

Not only does behavior change in different social contexts, but so do circulating hormone levels underlying many hormone-behavior interactions. Males of many species exhibit rapid increases in testosterone in the presence of competitors or mates, preparing the body for potential conflicts or mating opportunities (Wingfield et al. 1990; Hirschenhauser and Oliveira 2006; Goymann et al. 2007). From an evolutionary perspective, males who were prepared for various social interactions, whether competitive or mating oriented, would have a behavioral and physiological advantage over unprepared males. In humans, studies report that presence of potential mates increases male testosterone (Roney et al. 2003; Roney et al. 2007; van der Meij et al. 2008), as does engaging in sexual activity (Escasa et al. 2011). While there is a large literature examining changes in testosterone during direct male-male competition (e.g. (Archer 2006)), or during male-male aggression (Cohen et al. 1996), there have been few studies that examine the role of the audience in human male testosterone response to competition.

Male humans and other vertebrates tend to perform better and have larger increases in testosterone when competing in their home territory (Neave and Wolfson 2003; Carre 2009), indicating that the social environment may interact with hormone production to influence competitive ability. Two recent studies of acute changes in testosterone in the presence of differently sexed audiences found that the presence of a female confederate was associated with higher male testosterone while skateboarding (Ronay and Hippel 2010), and that female biased audience sex-ratios were associated with larger changes in testosterone during an Ultimate Frisbee tournament (Miller et al. 2011). Men also experience social modulation of hormone levels even during tasks not associated with competitive or mating cues; a recent study reported increased cortisol levels and perceived stress when undergoing a social-stress challenge in the presence of females (Duchesne et al. 2012).

Varied hormone secretion expressed by individuals during challenges offers insight into their underlying levels of physiological arousal. Researchers report that individuals who are more invested in a competitive activity tend to express larger increases in testosterone during the course of a competitive encounter (Gonzalez-Bono et al. 1999). In some cases, it can be difficult to ascertain the intended audience of certain behaviors: Who are people trying to impress when engaged in competitive athletic behavior?

There is a broad body of literature in evolutionary biology examining how different organisms communicate(Maynard Smith and Harper 2003). Understanding the evolution of various strategies for passing information between two organisms raises complex issues, as it involves coordinated evolution between senders and receivers. In addition, it can often be advantageous to disseminate disingenuous information, and if unchecked such deception would destabilize communications systems (or prevent them from ever evolving). Studies of how organisms with conflicting interests communicate have been conducted in a wide range of fields from plants and insects, to birds and mammals, including primates and humans (Zehavi and Zahavi 1997; Maynard Smith and Harper 2003; Bliege Bird and Smith 2005).

One area of signaling theory has focused on honest, costly, or handicap signaling, where the quality of the signaler is tied to the intensity of the signal produced (Johnstone 1994). Handicap signaling theory suggested that displays that entail fitness costs are a guarantee of honesty: only individuals of high quality can afford to produce a signal in the face of a handicap (Zahavi 1975). The peacock's tail is the quintessential handicap signal: males with large bright tails also face a higher risk of predation: by surviving despite this handicap, they signal to females that they have genes that make them particularly vigorous and therefore high quality mates (Zahavi 1975). Handicap signaling has been since analyzed with mathematical models, and the current literature tends to focus more on costly or honest signaling as opposed to thinking about displays as handicaps (Grafen 1990; Johnstone 1994). Costly signaling need not necessarily impose a handicap; instead, all that is required is that lower quality individuals pay higher marginal signaling costs relative to individuals of higher phenotypic quality (Grafen 1990; Bliege Bird and Smith 2005). In order for a signal to become an evolutionarily stable strategy several conditions must be met- (1) there must be variation in difficult-to-observe underlying quality, (2) signal cost must be quality-dependent (as just outlined), (3) observers must benefit from information on signaler quality and signalers must benefit from being perceived as high quality, and (4) the signal must be broadcast in such a way that it can effectively reach an intended audience (Bliege Bird et al. 2001; Bliege Bird and Smith 2005).

Understanding the intended audience of a signaling behavior may offer insight into why individuals engage in various activities. While soccer is a relatively recent behavior, and penalty kick shootouts likely have had little impact on the evolutionary history of the genus *Homo*, competitive sports interactions are a common aspect of human behavior (Hill et al. 2009). In some cases, competitive male sports activities are thought to take the socially acceptable place of more dangerous male-male aggression, reducing potential violence and damage during male-male conflict (Hill et al. 2009; Van Vugt 2009). They also allow men to display physical and cognitive abilities in front of a wide audience of potential competitors and mates. Public displays of competitive sport activities have a wide broadcast efficiency: numerous individuals will directly see the individual engaged in the display, and others may learn about a player's performance through linguistic communication by a third party.

When men are engaged in a public display of competitive sports prowess, to whom are they directing this behavior? Are they attempting to impress women, or are they aiming to impress other men? It is important to differentiate between these two different groups because they each offer differential paths to potentially increase reproductive success (see Figure 5.1) (e.g., (Gurven and von Rueden 2006; von Rueden et al. 2008)). Men may be signaling prowess to other men as means of intimidation, or in an attempt to gain higher social status or more allies. Higher status men tend to be rated as more attractive (Buss 1991), and among the Tsimane have higher reproductive success (von Rueden et al. 2011). Alternatively, men could be showing-off skill at physical activities to women, who might otherwise spend little time in the presence of unrelated men engaged in physical activity.

This could be signaling their potential ability to provide for women during physically demanding activities, or to provide protection for her and her offspring. In order to examine to whom men are attempting signal when engaged in competitive sports activity, we will examine acute changes in testosterone before and after men take penalty kicks on goal in the presence of various audiences: no audience, young men, young women, old men and old women.

Soccer is a common and important social form of recreation among the Tsimane, with men in these communities reporting playing a game of soccer on an average of 3.1 (SD=1.5) days per week (Trumble et al. 2012). While men are waiting to gather enough teammates to play, they often practice taking shots on goal. Thus taking penalty kicks on goal is a behavior which most men are familiar with and well practiced at, and there is little opportunity for confusion among participants.

Hypotheses

Larger increases in testosterone are expected in the presence of an audience, as opposed to conditions where men are taking shots only against a goalie, as an audience provides a signaling opportunity that would reward better performance (facilitated by elevated testosterone). In addition, previous studies find that audience presence affects both behavior and testosterone (Roney et al. 2003; Pawlowski et al. 2008).

While taking penalty kicks on goal is a skill requiring coordination and practice, it is not likely to be associated with activities that Tsimane women rate as important in their husbands, such as hunting ability or work ethic (Marlowe 2005; Pillsworth 2008; Gurven et al. 2009b). Some weekend games between neighboring communities attract an audience, most games occur in the absence of an audience, so it is unlikely that women have much of an opportunity to witness the majority of male penalty kicks. Thus we hypothesize that it is more likely that penalty kick performance is a signal aimed at other men, with the potential reward of status enhancement.

While soccer is an important social activity for some young men, individual playing ability likely has little impact on older men and women. Thus we hypothesize that the largest increases in testosterone will occur in men who are shooting penalty kicks in the presence of young men and women, as opposed to older men and women. Older men may be of high social status, thus there is the potential that men taking penalty kicks in their presence would feel pressure to impress them. Older women are of low potential mate value (all older women were menopausal), and thus this condition is hypothesized to have the lowest effect on in testosterone.

Methods

In summer 2010, seventy three Tsimane men provided saliva specimens 15 minutes before and 10 minutes after taking five penalty kicks on goal. Research on competitive male interactions indicate that acute increases in testosterone can occur in as little as five minutes (Pound et al. 2009); a ten minute mark was chosen to make the results comparable to previous studies among the Tsimane (Trumble et al. 2012; Trumble et al. *in preparation*). In each of five communities, a goal was built from locally available materials to be approximately 530 x 210 cm in dimension, and a line was demarcated at exactly 11 meters from the goal. Tarps and tent flies were used to cordon off the area from interested onlookers, and a translator actively guarded the entrance to the area to ensure that the participant taking shots on goal was not viewed by any outside community members (see Figure 5.2). In each community 12-16 men took five shots on goal against one of two confederate goalies. The original plan was to use the same goalie for all audience conditions, but fatigue required the use of a second goalie, and statistical methods were used to correct for this difference. Each community was randomly assigned to an audience condition, and men took shots on goal in the presence of either: no audience (n=15), four older men (n=16), four older married post-menopausal women (n=15), four young single women (n=15) or four young age matched males (n=12).

Previous research among the Tsimane found an association between self-rated performance and testosterone response to a soccer came (Trumble et al. 2012), thus in order to make these results comparable, similar questions were asked of each participant. After taking shots on goal, players were kept separate from other community members and asked who in their community they thought would be the best and worst at penalty kicks. Then participants were asked how well their shots on goals went in comparison to the best and worst players in their community, using a five-point ladder scale (Trumble et al. 2012). Height, weight, and age were collected from participants for use as control variables, with age ranging from 16-54 years (mean age 26.1 years). After collection of the second saliva sample, all specimens were stored in liquid nitrogen until transfer to dry ice for shipment to the University of Washington Biodemography and Anthropology Laboratory.

Specimens were stored in a -80°C freezer for up to four months before being thawed and centrifuged at 2800 RPM for 20 minutes, and the aqueous layer aliquoted for assay. All specimens had gone through two freeze thaw cycles when initially assayed. Studies suggest that salivary testosterone is stable for at least 12 months at -80°C (Granger et al. 2004).

Saliva exhibiting blood contamination or inadequate specimen volume (*n*=6 specimens) (Granger et al. 2004), were excluded, leaving a total of 140 usable specimens from 73 individuals. Salivary testosterone was measured using an in-house enzyme immunoassay (Trumble et al. 2010) that cross reacts 100% with testosterone, 57.4% with 5 α-dihydrotestosterone, 0.27% with androstenodione, and less than 0.05% with other androgens (Muir et al. 2001). Specimens were run in duplicate, and to each participant's samples were run on the same plate to avoid inter-plate variation. Individuals were randomly assigned to plates to ensure that audience conditions were not placed together on the same plate. The within and between assay coefficients of variation for this project (*n*=24 plates) were 4.5% and 9.3% respectively for the low (403.1 pg/mL) and 5.5% and 11.4% for the high (681.4 pg/mL) Biorad level 1 and 2 commercial controls diluted to 1:10 in 0.1% PBS buffer.

Statistical Methods

Salivary testosterone concentrations were log transformed. To control for the non-independence of repeated specimens from each participant, multi-level mixed-effects regression models with individuals and goalies modeled as a random effect (West et al. 2007) were used to determine the effect of penalty kicks on log transformed salivary testosterone 10 minutes after taking shots on goal. Adipose tissue can aromatize circulating testosterone into estrogen, and thus BMI was included as a control variable in every model (Bhasin et al. 2001). Testosterone is also subject to circadian variation (Diver et al. 2003), thus time of specimen collection was also included in all models. Age was not associated with testosterone (*p*=0.716) or change in testosterone (*p*=0.831), and thus not included as a control variable.

Results

The average pre-shootout testosterone (236.0 pg/mL) increased to (250.4 pg/mL) following the shootout, for an average 7.8% increase in salivary testosterone. Mixed-effects models controlling for BMI and time of day indicate a trend toward increased testosterone following the soccer shootout ($\beta = 0.06$, $p=0.069$), see Figure 5.3. Individual performance, as measured by number of goals scored ($\beta=-3.69$, $p=0.311$) or self-ratings of performance ($p=0.931$) were not associated with change in testosterone, controlling for time of specimen collection and BMI. When each audience condition was compared to all other conditions, no single audience condition had significantly different change in testosterone from the other conditions; no audience ($p=0.839$), young men ($p=0.677$), young women ($p= 0.548$), old men ($p=0.449$), or old women ($p= 0.223$), controlling for time of specimen collection and BMI, see Figure 5.3. Participants in the male observer condition (old and young men) did not differ from the female audiences (old and young females) in testosterone change ($p=0.418$), controlling for BMI and time of day. There was also no difference in testosterone change ($p=0.803$) for those taking shots on goal in in the presence of an older audience (male and female) compared to those in the presence of a younger audience (male and female), controlling for time of day and BMI. There were no significant interactions between number of goals scored and audience condition or self-rated performance and audience condition.

Discussion

These results suggest that men tended to express a small increase in testosterone during a competitive non-physical task. Contrary to our prediction, the presence and age-sex composition of the audience did not affect the testosterone change experienced by these individuals. Performance did not affect the change in testosterone, as measured by the number of goals scored, or as self-rated performance. There were no differences in testosterone change for any single condition. When grouped by audience composition, there was still no difference in testosterone response among the participants.

Previous examination of audience effects find that the presence an audience, particularly a female audience, affects both behavior (Pawlowski et al. 2008), and underlying endocrine physiology (Roney et al. 2003; Roney et al. 2007; van der Meij et al. 2008). Contrary to our results, studies of competitive male behavior find that the presence of a female audience increases testosterone (Ronay and Hippel 2010; Miller et al. 2011). There are several potential explanations for these differences in results. First, these studies used differing methodologies. While we measured changes in salivary testosterone after exposure to a single sex, or no audience, Ronay and colleagues had participants perform skateboarding tricks in the presence of a male confederate, and then again in the presence of a male or female confederate, taking a single saliva specimen after they finished skateboarding (Ronay and Hippel 2010). Miller and colleagues measured testosterone change, but did so in the presence of a large mixed sex audiences, with varying degrees of female majority (Miller et al. 2011). The size of their audience was not experimentally manipulated, and thus there could be unknown independent effects of size and audience sex. One could hypothesize that a mixed sex audience would have the highest potential to impact on female opinion, as women would be exposed to both the actual male performance, and hear men discussing their evaluations of the performance.

Previous examinations of changes in testosterone during soccer games among the Tsimane found that men with higher self-rated performance, and those who scored more goals tended to have the largest increases in testosterone over the course of a game (Trumble et al. 2012). This study found no association between goals scored, self-rated performance and testosterone. One potential explanation for this discordance in results is that the number of goals scored during the penalty kick task was largely invariant, with a mean of 1.3 goals, (SD=1.1; range 0-4 goals). Because of the low level of variation, it may not have been possible to elucidate difference in performance and testosterone change. Additionally, men playing soccer had significantly more time over the course of the game to express an increase in testosterone; sixty minutes of game time, with specimens collected 10 minutes after the game. Men here only had the several minutes of kicks on goal, followed by a 10 minute wait. Also, the soccer tournament was a much higher status event, and had a large mixed-sex audience (>50 people) including competitors, allies, family, and current or potential mates. Penalty kicks were a low status event, where only the audience would actually know how well an individual performed, as no one else was able to watch. That said, during a soccer game, individuals who are performing poorly can rely on their teammates for victory, while the participants in this study had to rely entirely on their own skill.

The overall methodology and sampling scheme was designed so that it would be possible to compare changes in testosterone during this competitive and non-physical activity with those collected during a competitive physical activity (soccer) (Trumble et al. 2012), and a non-competitive physical activity (tree-chopping) (Trumble et al. *in preparation*). As expected, non-physical competition produced a smaller increase in testosterone (7.8% increase) than physically active competition (30.1% increase), or non-competitive physical activity (48.6% increase). This suggests that while competition alone can lead to increases in testosterone, physical activity has a larger impact on acute testosterone change than non-physical activity. This harmonizes with previous studies in industrialized populations that note that physical competition results in larger increases in testosterone than non-physical competition (Archer 2006).

We believe that this paradigm could be useful for other studies to better understand the intended audience for signaling behavior. The psychological literature suggests that humans have poor self-perception capabilities, and often do not fully understand their internal motivations (Bem 1967). Studies of male recreational risk-taking behavior find that men overestimate how attractive women find behaviors like skydiving and rock-climbing (Farthing 2005). However, men rate participants in such behaviors as high status, and prefer these individuals as friends (Farthing 2005). That said, human poor self-perception applies to women as well as men, thus women may be underestimating their mating choices. This may be a situation where men are attempting to signal behavioral propensities or physical capability to women, but the intended receiver is not necessarily interested. Unintended recipients (other males) may be observing the signal, and conferring higher status on such individuals, which some women may in turn find attractive (Buss 1991; Smith et al. 2003; Farthing 2005). Using a paradigm similar to the penalty kick study could provide hormonal evidence of physiological arousal in order to capture the intended receiver of the signal. Hormonal analyses add a level of objectivity not usually possible in general surveys of human behavior, and should be incorporated into a wider variety of behavioral studies.

Limitations

The audiences in this study were all equal size (*n*=4) to control for potential independent effects of audience size and audience composition. However, different sized audiences may have non-linear effects on testosterone change. Studies of male-female non-competitive interactions tend to be a participant interacting with a single confederate, creating a more intimate setting (Roney et al. 2003; Roney et al. 2007). Other studies have used larger audiences (Miller et al. 2011), which may have differential impact on testosterone change. Future studies will need to vary both audience sex and size to address this issue.

We ensured that all audience members were not a part of the participant's nuclear family, but we had no way of controlling for whether participants had personal history with any member of the audience. There may have been unobserved heterogeneity in individual feelings toward specific audience members that could have impacted performance, or testosterone change. This study did not collect data on whether participants were married or had offspring, and thus it was not possible to examine whether there was an interaction between relationship status and audience condition. It is possible that unmarried men had larger increase in testosterone in the presence of young women, but that we were unable to observe this effect because married men may not have experienced a rise in testosterone in that audience condition. This is of special importance when comparing these results to studies conducted in industrialized populations where the participants are likely to be young unmarried university students.

In humans and potentially some other communicative social animals (Marzluff et al. 2010), it may not be necessary that others directly observe a signaling display in order to gain the information conveyed by the display. Individuals may engage in behaviors in the presence of one audience, but then directly or indirectly inform their intended audience about the experience afterward. While it seems unlikely that penalty kick performance would be discussed outside of the context of this experiment, many other forms behavior could occur in the presence of one audience, and then later be reported to potential competitors, allies, or mates. Thus the evolution of signaling behavior in humans may not be dependent on the actual audience present, but instead on an audience who will eventually be informed about the event (Smith 2010).

Conclusions

This study finds no evidence that audience age or sex impacts testosterone change during penalty kicks. The increases in testosterone observed during these competitive non-physical penalty kicks were smaller than the acute increases observed during competitive physical soccer matches, or physical but non-competitive tree-chopping. Future studies will examine the role of audience effects with greater information on the marital status of participants, and during other activities, especially those linked to reproductive success, such as hunting ability.

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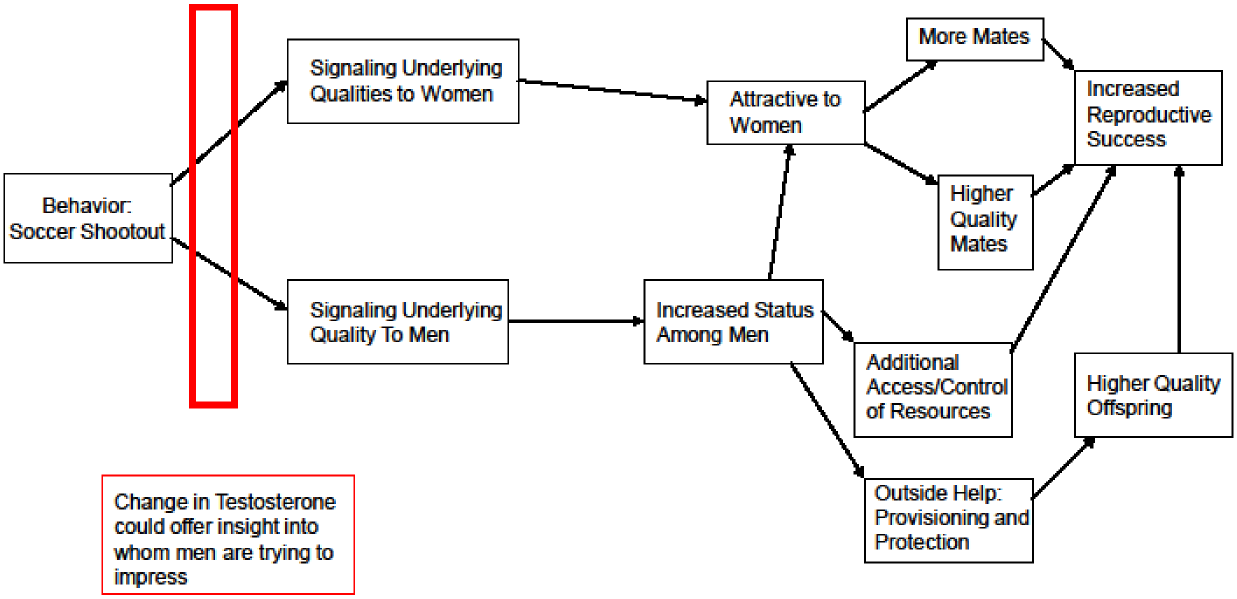
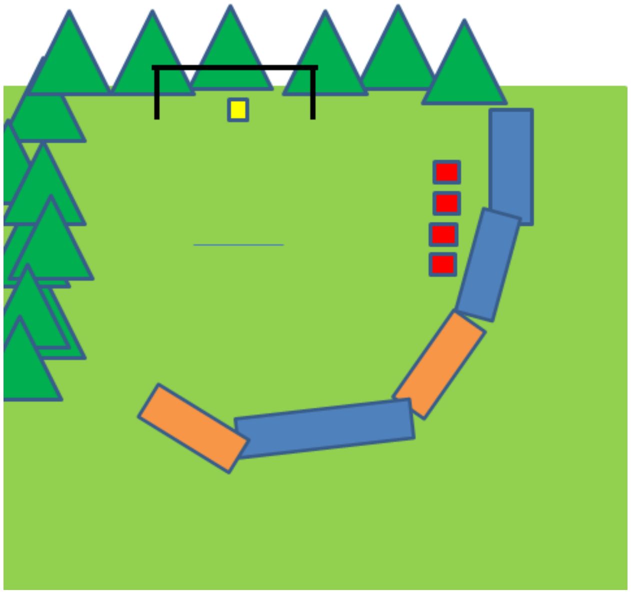
Figure 5.1: Simplified signaling model



This figure represents a simplified model of how a signaling behavior could affect reproductive success via two separate pathways. For similar pathways, see (Gurven and von Rueden 2006; von Rueden et al. 2008). By measuring testosterone change, a measure of physiological arousal, it may be possible to differentiate which audience path men are attempting to signal

Figure 5.2: Penalty kick diagram

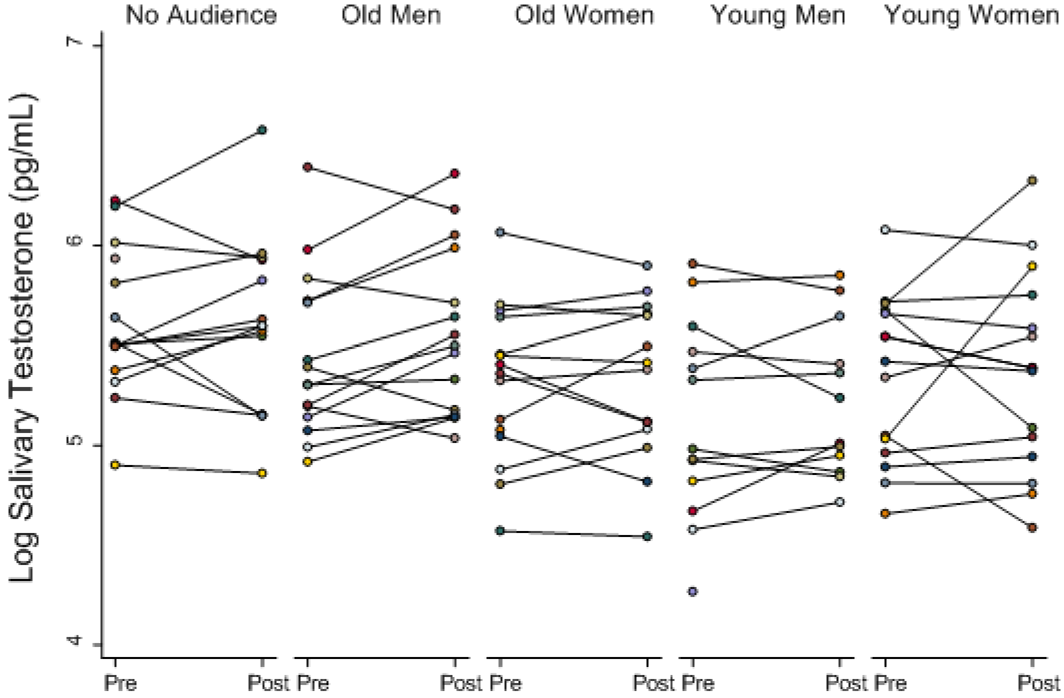
A



B

Figure 5.2: A represents and overhead view of a goal blocked from view, except from four audience members and a goalie. B is an image taken from ground level.

Figure 5.3: Acute changes in log salivary testosterone by audience condition



Acute changes in log salivary testosterone by audience condition for 73 Tsimane men

Chapter 6: Testosterone increases among successful Tsimane hunters: using testosterone to examine provisioning and signaling models of male hunting motivation

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Abstract

Studies of human behavioral endocrinology tend to focus on the role of testosterone in male mating effort, with numerous studies reporting acute increases in testosterone during male-male competition, especially among winners. However, the challenge hypothesis theoretical framework used to interpret these results was designed to examine changes in testosterone during behaviors that affect reproductive success. This study tests whether this pattern of hormone-behavior interactions can be extended to an ecologically valid and reproductively important challenge, examining hunting success and failure among Tsimane forager-horticulturalists. There is considerable disagreement in the literature over the motivation behind male hunting; to what degree to do both family provisioning and the reputational and fitness benefits that good hunters receive motivate hunting behavior? Up to five saliva specimens were collected from 31 Tsimane hunters aged 18-82 years, a) before leaving on a single day hunt, b) after 3 hours of hunting, c) if an encounter ended in a missed shot, d) if an encounter ended in a kill, and e) upon returning home. Salivary testosterone was measured via enzyme immunoassay. Mixed effects models find increased testosterone at the time of kill (p=0.04) compared to other collection times, controlling for age squared, BMI, and time spent hunting, with an average 23.5% increase above baseline testosterone. Hunters returning with meat had 36.1% larger change in testosterone than unsuccessful hunters (p=0.03), controlling for age squared, BMI, and time hunting. The size of the kill, and encountering community members when returning home were not associated with testosterone. Successful hunting is associated with increased testosterone both at the time of the kill and upon returning home, but there is no evidence that presence of an audience is associated with testosterone.

Introduction

Hunting has been an integral part of human provisioning strategies for hundreds of thousands of years (Stiner 2002), with some evidence suggesting that primate hunting behavior may extend prior to genus Homo (Gomes and Boesch 2009). The co-evolution of increasing human brain size, reliance on high quality food items, and learning intensive food acquisition such as hunting helped shape the human adaptive complex (Kaplan et al. 2000; Kaplan and Robson 2002; Kaplan et al. 2010). Even human social organization and the sexual division of labor have been modified by hunting (Washburn and Lancaster 1968). Current bargaining models of the sexual division of labor note that female pregnancy, lactation, and childcare interfere with prolonged periods of skill acquisition necessary for optimal hunting returns, resulting in a male-biased hunting advantage which led to the sexual differentiation of time allocation observed in foraging populations (Gurven and Hill 2009; Gurven et al. 2009b). In sum, hunting has been a strong selective pressure that played a vital role shaping provisioning, learning, behavioral, and social aspects of human evolution.

Hunting has played such a vital role in human evolution because of the associations between hunting and reproductive fitness. Hunting success is positively associated with reproductive success, as measured by age-specific number of surviving offspring, in every subsistence society examined (Kaplan and Hill 1985; Wiessner and Schiefenhövel 1996; Bliege Bird et al. 2001; Wiessner 2002; Marlowe 2004; Smith 2004), including the Tsimane (Gurven and von Rueden 2006; von Rueden et al. 2011). This pattern is repeated across varied environments, and applies to both terrestrial and marine animals (Alvard and Gillespie 2004; Smith 2004). There are several ways in which hunting may impact reproductive success, including direct family provisioning, direct and indirect reciprocity, increases in social status or coalitional memberships, increased access to mates, higher quality mates, or through phenotypic correlation (Smith 2004; Gurven and von Rueden 2006; Gurven and Hill 2009). High levels of correlation between these factors make it difficult to separate out any single factor as being responsible for increased number of surviving offspring; successfully hunting a large game animal may simultaneously provision a family as well as result in various additional forms of benefits to fitness (Smith 2004; Gurven and von Rueden 2006; Gurven and Hill 2009). There is even some evidence that female chimpanzees engage in higher rates of sexual activity with males that share hunted meat (Gomes and Boesch 2009), suggesting that associations between hunting ability and reproductive success have evolutionarily deep roots. The reproductive benefits of hunting are thus not limited to familial provisioning, and can include benefits to social status, reciprocal food-sharing, and coalitional memberships, all of which can enhance reproductive success (Wiessner and Schiefenhövel 1996; Smith 2004; Gurven and von Rueden 2006).

Despite consistent links between reproductive success and hunting, only one study has examined male hunting behavior from an endocrine perspective (Worthman and Konner 1987). Much of the human behavioral endocrinology literature has instead focused on hormone-behavior interactions related to competition and aggression (Archer et al. 1998; Archer 2006), which is only one aspect of mating success. When examining acute changes in testosterone during competition, studies tend to rely on the “challenge hypothesis” as a framework to understand variation in male testosterone in various reproductive contexts (Wingfield et al. 1990; Goymann et al. 2007), suggesting a trade-off between the behavioral and muscular reproductive benefits of higher testosterone and the energetic and immunocompromising costs of elevated testosterone. While this framework has been usefully applied to a number of taxa (Hirschenhauser and Oliveira 2006), human studies have largely focused on long-term down regulation of testosterone in relation to fatherhood (Gray et al. 2002; Muller et al. 2009; Gettler et al. 2011), and acute increases in testosterone during male-male competition (Archer 2006). Studies of short-term testosterone increases have largely focused on organized male-male competition, whilst behavior like hunting offers a more ecologically valid challenge associated with reproductive success than a soccer game or a chess match. Team sports offer the added difficulty that individual performance can be difficult to dissociate from team performance; an individual may play very well though their team still loses (Trumble et al. 2012).

During competitive interactions (Archer 2006), and in some cases of heavy physical activity (Vingren et al. 2010), acute increases in testosterone have been noted, including among the Tsimane (Trumble et al. 2012; Trumble et al. *in preparation*). These acute increases in testosterone benefit muscle performance and capability, immediately increasing sugar uptake (Tsai and Sapolsky 1996; Crewther et al. 2011). While increases in testosterone are generally seen during the course of most physical competitions, winning a competitive interaction is often associated with an additional spike in testosterone (Booth et al. 1989; Oyegbile and Marler 2005). The challenge need not be physical in nature to produce an increase in testosterone following a victory; chess (Mazur et al. 1992), video games (Oxford et al. 2009), watching a favorite sports team win (Bernhardt et al. 1998), and even contrived experimental tasks (Schultheiss et al. 1999; Schultheiss and Rohde 2002) can produce "winner effect" increases in testosterone.

Laboratory studies in animal models report that increased testosterone post-victory prepares the individual for future fights, increasing aggressive behavioral strategies, thereby increasing the probability of winning future encounters (Oyegbile and Marler 2005; Lehner et al. 2011). In humans, the effect of winning on testosterone has not been well characterized among non-industrialized populations, nor has this relationship been measured under conditions that are directly associated with reproductive success. Hunting success offers a socially and calorically important form of success associated with reproductive fitness among the Tsimane (Gurven and von Rueden 2006). Acute increases in testosterone at the time of an encounter would enhance immediate muscle response and hunting ability, and increased testosterone following a successful hunt could influence male behavioral strategies, which may contribute to the greater status or reproductive success seen among better hunters.

While the associations between reproductive benefits and hunting ability appear fairly constant across studies, the roles of provisioning and mating-effort in the motivation to hunt is still debated (Gurven and Hill 2009). There is an inherent level of risk in hunting, both in terms of economic risk due to high variance in hunting returns (e.g., (Hawkes et al. 1991)), and potential physical danger. The animals hunted are often too large to consume in one sitting, and frequently shared with unrelated community members. Thus, questions have been raised as to the motivations and fitness payoffs behind male hunting. The “show-off” hypothesis (Hawkes 1991), which was later integrated with costly signaling models (Bliege Bird et al. 2001; Hawkes and Bliege Bird 2002; Bliege Bird and Smith 2005), proposes that hunting can serve as an honest signal of male quality. Men vary in quality, which could include health status, skill, and other less tangible phenotypic qualities, and hunters in better condition pay reduced marginal costs in terms of time and energy to hunt the same animals. The visibility (broadcast efficiency) of returning with a kill is high, therefore hunting can serve as an honest signal of underlying qualities to competitors, allies, and potential mates

(Bliege Bird et al. 2001). That hunting qualifies as a costly signal is not contested; the area of disagreement lies in whether signaling is a key motivation underlying hunting behavior. It should be noted that provisioning and mating effort models are not necessarily mutually exclusive, and both likely play a role in male hunting motivation.

Although time-allocation, hunting returns, interviews, focal follows and various survey methodologies have been implemented in a number of studies to understand male motivations for hunting (Smith 2004; Gurven and Hill 2009), only one study to date has examined changes in testosterone during hunting (Worthman and Konner 1987). Hormone-behavior interactions offer a different vantage point to examine the role of signaling and provisioning in motivating hunting behavior. Acute changes in testosterone have been used to examine variation in male reproductive and paternal behavior in birds and other species (Hirschenhauser and Oliveira 2006; Goymann et al. 2007; Hau 2007), including primates (Muller and Wrangham 2004) and humans (Archer 2006). Hormones offer an impartial view and can be used to examine differential physiological arousal in the presence, or lack of an audience. For example, men engaged in skateboarding attempt riskier tricks and have larger increases in testosterone in the presence of an audience, particularly a female audience, suggesting that signaling plays a role in motivating risk-taking behavior while skateboarding (Ronay and Hippel 2010).. Acute increases in testosterone and other hormones expressed during competitive tasks offer insight into the underlying levels of physiological arousal. Studies find that individuals who are more invested in a competitive activity tend to express larger increases in testosterone during the course of a competitive encounter (Gonzalez-Bono et al. 1999). If male hunting is partially motivated by mating-effort, and men are attempting to signal underlying quality to competitors, allies, and mates with their hunting ability, then one would expect to see increased testosterone when they have higher broadcast efficiency (e.g., returning to a larger audience). If these men are primarily motivated by familial provisioning, then one would not expect to find a relationship between audience presence and testosterone when returning from a hunting trip. The Tsimane forage-horticulturalists offer an ideal population in which to examine associations between hunting, testosterone, and audience, as Tsimane hunting has been well characterized in the literature, and links between hunting success and reproductive success are well established (Gurven et al. 2006; Gurven and von Rueden 2006; Gurven and Hill 2009).

Hypotheses

We hypothesize that testosterone will increase during hunting encounters, as acute increases in testosterone would benefit muscle physiology. We expect further elevation in testosterone in the event of a kill, as acute increases in testosterone are seen among winners in a variety of trivial competitive situations. Hunting is a non-trivial, ecologically-valid, indirectly competitive task associated with reproductive success. If one is likely to find an effect of winning, it could be expected to be strongest in a reproductively-linked task like hunting. We also hypothesize that men returning home with meat will experience larger increases in T; both from increased workload of carrying meat, and from the psychological impact of success.

A secondary set of hypotheses will be used to examine the signaling explanation of male hunting behavior. The signaling explanation predicts that men who encounter larger audiences when returning with meat will experience larger increases in testosterone than those who encounter no audience other than nuclear family upon return, while a provisioning explanation predicts no audience-mediated testosterone difference. It should be noted that both the signaling and provisioning explanations of hunting behavior overlap in the expectation of increased testosterone following a kill.

Methods

From August to October 2011, thirty-one men provided saliva specimens before, during, and immediately following a single day hunt. All participants provided initial specimens before leaving their house; a second specimen was collected after three hours of hunting for use as a physically active baseline, and a final specimen was collected ten minutes after the participant returned home. Specimens were also collected in the event that the hunter used a firearm, 10-15 minutes after firing a shot (whether resulting in a kill or a miss). Previous studies report diminished hormonal response to continued stimuli (Levine 1978), thus specimens were only collected in the first instance of a miss and first instance of a kill. An Actitrainer accelerometer (Pensacola, FL) and heart rate monitor was attached to each participant before beginning their hunt, and participants were asked questions from a brief behavioral questionnaire including when they last hunted, how many days per week they hunt, and what animal they would like to encounter that day. A handheld GPS was used to measure the total distance traveled throughout the hunt. Hunters were followed until their return to their residence by the lead author and a translator. Prior to re-entering the community following the hunt, all hunters were asked if they were pleased with the results of their hunt, if they would have been happier to encounter the animal they noted as being preferential prior to the hunt, and if their family would be happy with the results of their hunt. All family, community members, or other individuals who witnessed the hunter returning were noted.

All participants left between 5:28-9:11, and returned between 9:03-19:40, for a mean hunting time of 8.4 hours (SD=2.8, range 2.8 to 13.1 hours). The average distance traveled was 17.9 km (SD=5.7, range 6.0-27.5 km). The average age of the hunters was 37.8 (SD= 15.1, range 18-82) years. All of the hunters were married, with two men reporting polygynous marriage (two wives each). All men but one had dependent offspring, which were defined as offspring who still lived in the same household. Of the 31 hunts, 6 men had a second hunter present, either a son (*n*=5), or a cousin (*n*=1). The researcher and a translator were present in all cases (see limitations). There were twenty-two cases where a hunter returned with meat: eighteen men killed an animal, three cases where the second hunter present killed an animal, and one case where a hunter captured a tortoise without a firearm. There were ten cases where men killed an animal large enough to be shared with others (boar, peccary, antelope), though post-hunt food sharing data were not collected. Ten men killed or collected multiple animals, though specimens were only collected from the first shot/miss, and the first encounter/kill for each hunter. Three men did not provide a three-hour control specimen because they made a kill before the three hour mark. Fifteen men hunted with shotguns, and 16 men hunted with .22 caliber rifles. Four men hunted with dogs in addition to their firearm. Eight men also gathered resources along the way, including fish (*n*=3), tortoise (*n*=3), or honey (*n*=2).

Complete heart rate measurements were available from 21 participants. In 9 cases the heart rate monitor shifted during the physical activity of hunting, resulting in incomplete HR data, and in one case the HR monitor did not function. Data were collected on the movements and activities of hunters, and thus it was possible to calculate the average heart rate during an encounter ending in a kill (encounter-kill), and an encounter ending in a missed shot (encounter-miss), see Figure 6.1. These were compared to the average heart rate in the five minutes prior to the encounter, which offer a more useful baseline than the entire trip prior, which included the men resting or eating lunch. All participants ate the same lunch (sardines in tomato sauce and crackers), to control for energetic intake.

Saliva was collected by passive drool, and frozen in liquid nitrogen immediately upon returning to camp. At the end of the study, specimens were shipped on dry ice to the University of Washington. Specimens were thawed and centrifuged at 2800 RPM for 20 minutes, and the aqueous layer aliquoted for assay. All specimens had gone through two freeze thaw cycles when initially assayed. Salivary testosterone was measured using an in-house enzyme immunoassay (Trumble et al. 2010) that cross reacts 100% with testosterone, 57.4% with 5 α-dihydrotestosterone, 0.27% with androstenodione, and less than 0.05% with other androgens (Muir et al. 2001). Specimens were run in duplicate, with each participant's samples run on the same plate to reduce bias due to inter-plate variation. Individuals were randomized between plates to ensure that successful and unsuccessful hunters were evenly mixed across plates. The within- and between-assay coefficients of variation for this project (n=4 plates) were 5.2% and 7.2% respectively for the low (292.5 pg/mL) and 6.7% and 9.3% for the high (696.8 pg/mL) Biorad Level 1 and 2 controls, diluted 1:10 in PBS buffer (0.1% BSA). All procedures were approved by the University of Washington Internal Review Board.

Statistical Methods

Salivary testosterone was log transformed for normality before statistical modeling. Linear mixed effects models were used to examine changes in log salivary testosterone at up to five different time points, a) before leaving on a single day hunt (baseline), b) after 3 hours of hunting, c) if a shot missed, d) if a kill was made, and e) upon returning home. Individuals were modeled as random effects to control for non-independence of multiple specimens collected from the same participant (West et al. 2007). Previous studies report a parabolic association between age and hunting success (Walker et al. 2002; Gurven et al. 2006); our results match those of previous studies (β = -.0005, p= 0.003) and thus age squared was used as a control variable, although age was not directly associated with testosterone. Tsimane men do not have access to clocks, and thus did not know the exact time they awoke. Because all hunts began in the morning, and all hunters reported waking shortly before beginning the hunt, hunting duration was a better proxy for time since wake-up than clock time. Because physical activity can modify testosterone levels, there needs to be a control for total time spent active; because total time hunting and time since wakeup co-vary, only one may be used in the model at any given time. Additional models were run with time of specimen collection as opposed to time hunting; these models did not fit as well as the models using time hunting, and did not change the direction or significance of the associations. All models included BMI, age squared, and hunting bout duration as control variables.

Results

Linear mixed effects models found evidence of increased salivary testosterone at the time of the kill (β=0.13, p=0.04), compared to all other time points, controlling for age squared, BMI, and time spent hunting, see Figure 6.2. As a control measure to differentiate changes in testosterone related to the overall physical activity of hunting from those associated with an encounter, specimens collected after three hours of hunting were compared to specimens collected 10-15 minutes after encounter-kills, revealing a significantly higher testosterone at the time of an encounter-kill ($\beta = 0.17$, $p= 0.025$, n = 18), controlling for age squared, BMI, and time hunting. Of the six men that missed a shot, there was no evidence of increased testosterone following a missed shot ($\beta = 0.002$, $p=0.988$), though the sample size was small.

Hunters that made the kill expressed an average increase in testosterone of 23.5% (SD= 50.5%) over the first sample of the day, and a 21.0% (SD =50.3%) increase over the three-hour mark. Men returning without meat had an average decrease in testosterone of 10.47% (SD= 15.7%) from the beginning to the first sample to the final specimen, with an average decrease of 1.2% (SD=25.0%) from the three-hour mark to the end of the day. Controlling for BMI, age squared, and time spent hunting, hunters returning with meat had a 36.1% higher percent change in testosterone ($p=0.03$) than men who returned without meat (see Figure 6.3). Controlling for age squared, BMI, and time hunting, the 18 men who killed an animal with a firearm tended toward larger increases in testosterone ($p=0.09$), equivalent to a 24.9% larger percent change in testosterone compared to all of those who did not make a kill (see Figure 6.4).

The ten hunters returning with large kills (peccary, boar, or antelope) did not differ in absolute amount nor percent change in testosterone from other successful hunters at the time of the kill ($p=0.801$, $p=0.128$), or upon returning home ($p=0.881$, $p=0.958$), controlling for BMI, age squared, and time hunting. There was no difference in the total testosterone or change in testosterone for successful hunters who encountered individuals other than their nuclear family on the way home (*p*=0.835, *p*=0.344), or at their house (*p*=0.281, *p*=0.308), controlling for age squared, BMI, and time. Interactions between returning with meat and audience, or animal size and audience did not affect total levels of testosterone nor percent change in testosterone.

As a control measure to differentiate changes in testosterone from the overall physical activity of hunting from those associated with an encounter, specimens collected at the beginning of the study were compared to those collected after three hours of hunting. Controlling for age squared, BMI, and time spent hunting, no difference was detected between the initial specimen and the specimen collected after three hours of hunting (β = 0.02, *p*= 0.783).

To test whether increases in testosterone expressed during successful encounters were associated with changes in physical activity, heart-rate data at the time of the encounters was examined in relation to testosterone. A one-sided paired T-test finds that the average heart rate during an encounter that ended in a kill (*p*=0.014) was significantly higher than the average heart rate in the 5 min prior to the encounter. During an encounter that ended in a missed shot, there was no significant difference between the average heart rate and the 5 min prior to the encounter (*p*=0.241). Surprisingly, Testosterone was negatively associated with heart rate during an encounter-kill (β =-0.005, *p*=0.047), and percent change in heart rate during the encounter-kill (β =-0.005, *p*=0.013) controlling for age squared, BMI, and time hunting. This suggests that while testosterone increases on average during an encounter kill, men with the largest increase in heart rate had smaller increases in testosterone than men with attenuated heart rate increases (see discussion). The percent change in testosterone from the start of the study to the kill was not associated with encounter-kill heart rate (β =-1.15, *p*=0.120) or change in heart rate (β =-0.65, *p*=0.262), controlling for age squared, BMI and time spent hunting. There was no association between testosterone and encounter-miss heart rate (β =0.01, *p*=0.257).

Men returning with larger kills tended towards larger increases in heart rate while returning (β =0.88, *p*=0.127); this likely did not reach the threshold for significance partially because the researcher helped carry meat, attenuating the participants' burden (it would have been socially unacceptable to not help). For men who returned with a kill, there was no association between post-kill heart rate (while carrying the meat) and testosterone (β =1.10, *p*=0.660), controlling for age squared, BMI, and time hunting.

Discussion

Tsimane hunters have increased levels of testosterone after making a kill and when returning home with meat. Hunters that missed a shot had no increase in testosterone at the time they took their shot. Unsuccessful hunters tended towards a decrease in testosterone over the course of the day, while those returning with meat expressed an increase in testosterone over the course of a hunt. These results are consistent with those of a previous study examining hunting behavior among the !Kung San (Worthman and Konner 1987). While differences in sampling framework, sample size, and timing of final specimen collection make a direct comparison difficult, both studies find evidence of decreased testosterone on unsuccessful hunting days, and report that successful hunting results in increased testosterone.

The "winner effect" refers to an increase testosterone for men who are victorious during a variety of contexts, including sports (Archer 2006), competitive games (Mazur et al.

1992; Oxford et al. 2009), and even low-investment psychological tasks (Booth et al. 1989; Schultheiss and Rohde 2002). These results suggest that this finding can be extended to hunting behavior, an indirectly competitive task. These findings are also consistent with previous studies among the Tsimane which report positive associations between testosterone and self-rated performance (Trumble et al. 2012), an individual measure of success, though no association between winning a soccer game and success. Increases in testosterone following successful competitive events are hypothesized to augment behavior and muscle tissue, enhancing future competitive success (Booth et al. 1989). Studies in animal models report that the increases in testosterone following a successful competition increase the probability of winning future fights (Oyegbile and Marler 2005; Lehner et al. 2011). Research in humans report that men who want a rematch after losing a game tend to have larger increases in testosterone during competition (Mehta and Josephs 2006), and that men who have larger acute increases in testosterone during resistance training tend to have larger increases in strength (Rønnestad et al. 2011). Studies in humans also indicate that acute increases in testosterone benefit muscle metabolism by increasing the ability for muscle tissue to use sugars (Tsai and Sapolsky 1996), as well as enhancing neuromuscular performance (Crewther et al. 2011). Increases in testosterone during a successful hunt appear to be similar to those seen following successful individual-level competition, and likely modify behavior and perhaps muscle tissue, potentially reinforcing hunting behavior.

Specimens collected at the three hour mark provide an active baseline level of testosterone, allowing differentiation of testosterone change from the physical activity related to traveling through the jungle versus the testosterone change from a successful encounter. These active baseline specimens showed no evidence of increased testosterone over initial specimens, suggesting that any increases in testosterone were not enough to overcome diurnal variation in testosterone production (Granger et al. 2004). Acute increases in testosterone at the time of the kill are hypothesized to reflect the psychological benefits of success, and also of increased activity related to chasing the prey. While there is not yet an understanding of how testosterone increases acutely following competitive activity (Flinn et al. 2012), the rapid nature of these increases at the time of a kill likely rules out the hypothalamic-pituitary-gonadal axis, as luteinizing hormone stimulation does not increase testosterone for 45-75 minutes (Veldhuis and Iranmanesh 2004). Though some have suggested that increases in testosterone are the result of decreased liver clearance of testosterone (Cadoux-Hudson et al. 1985), the increases in testosterone seen here occurred only several minutes following an encounter, and thus are not likely a reflection of this mechanism. Additionally, testosterone and change in testosterone appear to be negatively correlated with heart rate during an encounter-kill, contrary to the supposition that increased aerobic activity alone is responsible for the spikes in testosterone following a successful kill. It is unclear why increases in heart rate during successful encounters were negatively associated with testosterone. Similar results were reported in a previous study among the Tsimane, which found a negative association between heart rate and tree chopping (Trumble et al. *in preparation*). Other studies report that aerobic activities tend to result in smaller increases testosterone than resistance based activities, suggesting that some aspect of aerobic activity may attenuate testosterone response (Tremblay et al. 2004). Future studies will examine the endocrine mechanism responsible for acute increases in testosterone, and may allow us to re-examine and re-interpret associations between heart rate and testosterone.

Unsuccessful hunters experienced a 10.4% decrease in testosterone over the course of the day. This likely reflects diurnal variation in testosterone, and is also consistent with studies of multi-hour endurance exercise (Keizer et al. 1989), which tend to report diminished testosterone during sustained aerobic activity. Similar decreases in testosterone were reported on unsuccessful hunting days in a previous study of hunting (Worthman and Konner 1987). Decreases in testosterone would also likely reduce energetic costs from a day of energetic expenditure, and avoid the potentially immunosuppressive effects of high testosterone levels (Duffy et al. 2000; Muehlenbein and Bribiescas 2005). Successful hunters were able to offset the energetic costs of increased testosterone with the meat they brought back, and thus could afford to maintain higher levels of testosterone. Although a transient effect, increased testosterone following successful competition has been shown to shift behavioral strategies in animal models (Oyegbile and Marler 2005). This raises the possibility that testosterone increase following successful hunting results in behavioral changes, or reinforces hunting behavior. Future studies will examine these possibilities.

Though men returning home with meat had significantly higher levels of testosterone than those returning without meat, the number and size of kills was not associated with testosterone. Additionally, being the hunter to actually make the kill was not necessary to stimulate testosterone, nor did the presence of an audience beyond immediate family have an effect on testosterone. If signaling was a primary driver of men's hunting behavior, one would expect the highest levels of testosterone in cases where men returned with large sharable kills in the presence of community members. While these results do not find any evidence in favor of a signaling model, the Tsimane nuclear families largely consume the meat they hunt, and engage in less widespread meat sharing than many other hunter-gather populations; thus, these results may be context specific (e.g., (Gurven et al. 2006; Gurven and von Rueden 2006; Gurven and Hill 2009)). It is also possible that increased testosterone from the physical labor of carrying back the meat washed out any potential increases that men would have experienced, had they not already had elevated testosterone.

The overall results are consistent with the theoretical framework of the challenge hypothesis, which predicts increases in testosterone during reproductively beneficial challenges (Wingfield et al. 1990). Better hunters have higher reproductive success (Gurven and von Rueden 2006), and thus although hunting is not a direct form of male-male competition, it is still a physically challenging behavior with potential reproductive benefits. Other physically intensive resource production strategies also increase testosterone among the Tsimane. A separate examination of men clearing trees as part of horticultural field preparation also reported increases in salivary testosterone following one hour of tree chopping (Trumble et al. *in preparation*). Together, these examinations of provisioning behaviors provide preliminary evidence that the challenge hypothesis should be expanded to include indirectly competitive, but still reproductively beneficial provisioning activities. While a number of studies of male life-history have reported decreases in testosterone following marriage (Gray 2003; Gray et al. 2004) or fatherhood (Gettler et al. 2011), men in subsistence populations need to be able to maintain muscle mass late into life in order to provision their offspring and grand-offspring (Marlowe 2003; Winking et al. 2009; Kaplan et al. 2010).

Hunting requires extensive skill and training, and hunting returns tend to peak later in life than physical prowess (Walker et al. 2002; Gurven et al. 2006), consistent with models of hunting skill as embodied capital (Kaplan and Robson 2002). Because of the physical nature of hunting, there is a non-linear association between hunting success and age, with hunting returns diminishing cross-culturally by the fifth decade of life (Walker et al. 2002). Previous studies report that among the Tsimane, overall hunting ability peaks at age 39 (Gurven et al. 2006). While studies in industrialized countries show declines in overall testosterone with age (Harman et al. 2001; Travison et al. 2007a), studies in non-industrialized countries tend to find less or no change with age (Ellison et al. 2002), including studies among the Tsimane (Trumble et al. 2012; Trumble et al. *in preparation*). In this study, age was not associated with baseline testosterone, or changes in testosterone. Previous examinations of baseline and change in testosterone during a soccer match (Trumble et al. 2012), and during tree-chopping (Trumble et al. *in preparation*) also report no age related difference in testosterone change during other physical activities. Acute increases in testosterone are associated with strength development (Rønnestad et al. 2011), and could provide one mechanism by which older men can remain productive. This study adds to a growing body of evidence suggesting that men in subsistence populations that rely on physical strength for food production need to be able to maintain testosterone production, and the ability to generate acute changes in testosterone, into later life.

Limitations

Statistically robust hunting data is inherently difficult to collect (Hill and Kintigh 2009). The study design required specimen collection at exact times under constant circumstances, and thus suffers from a relatively small sample size (n=31); that said, the sample in this study (n=31) is five times larger than in the one previous study of hormonal change during hunting (n=6) (Worthman and Konner 1987). While a previous study suggests that there is no researcher effect on testosterone change while resting (Trumble et al. *in preparation*), it is possible that the presence of the researcher and translator modified hunter behavior or testosterone-behavior interactions. The researcher and translator were kept constant throughout the study to minimize variability.

While six out of thirty one hunts had another hunter present, the majority (80%) of hunts were conducted alone. Because hunting among the Tsimane tends to be a solitary task, or one in which only a few closely related individuals are present, we cannot rule out the possibility that the presence of the researcher and translator modified testosterone response during or following the hunt. That said, a previous study among the Tsimane found no evidence that researchers modified testosterone levels among men who were resting (Trumble et al. *in preparation*). The complexity of the sampling framework precluded giving the hunters vials and having them collect specimens on their own.

It is also possible that the broadcast efficiency of hunting relies not on people seeing the hunter returning with meat, but on word-of-mouth following the hunters return. Future work will examine whether direct viewing of a returning hunter is important for community knowledge of hunting success, or whether this information spreads regardless of who views the returning hunter carrying meat.

Conclusions

Testosterone increased at the time of making a kill, and men returning with meat had larger increases in testosterone over the course of the day than men returning without meat. Men returning with larger animals did not differ in testosterone from other successful hunters, nor was actually making a kill associated with a larger increase in testosterone than simply being a member of the party who returned with a kill.

Men who encountered an audience other than their family on their return did not differ in testosterone response compared to those that did return to an audience. Increases in testosterone at the time of the kill and upon return are consistent with both the provisioning and signaling explanations of hunting motivation, which it should be noted are not mutually exclusive; the decision to hunt is surely motivated by a number of factors. The lack of audience effect suggests that familial provisioning may be a primary driver of hunting behavior. In concert with results of previous production behavior among Tsimane males, these data suggest expanding the current mating-effort and competition-centric focus of testosterone-behavior interactions to include subsistence-related activities.

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Figure 6.1: Heart rate while hunting

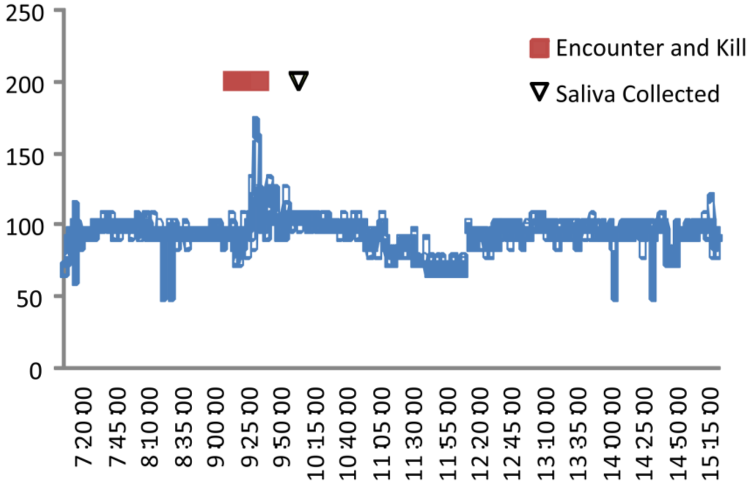
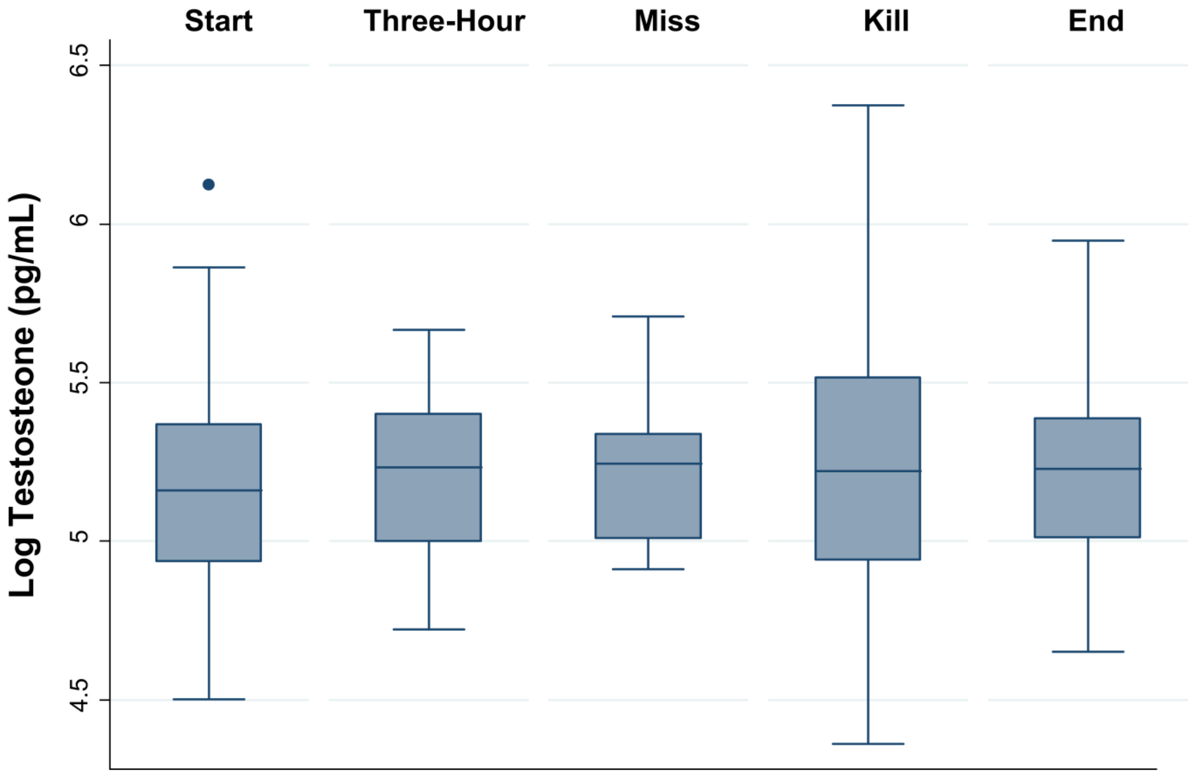


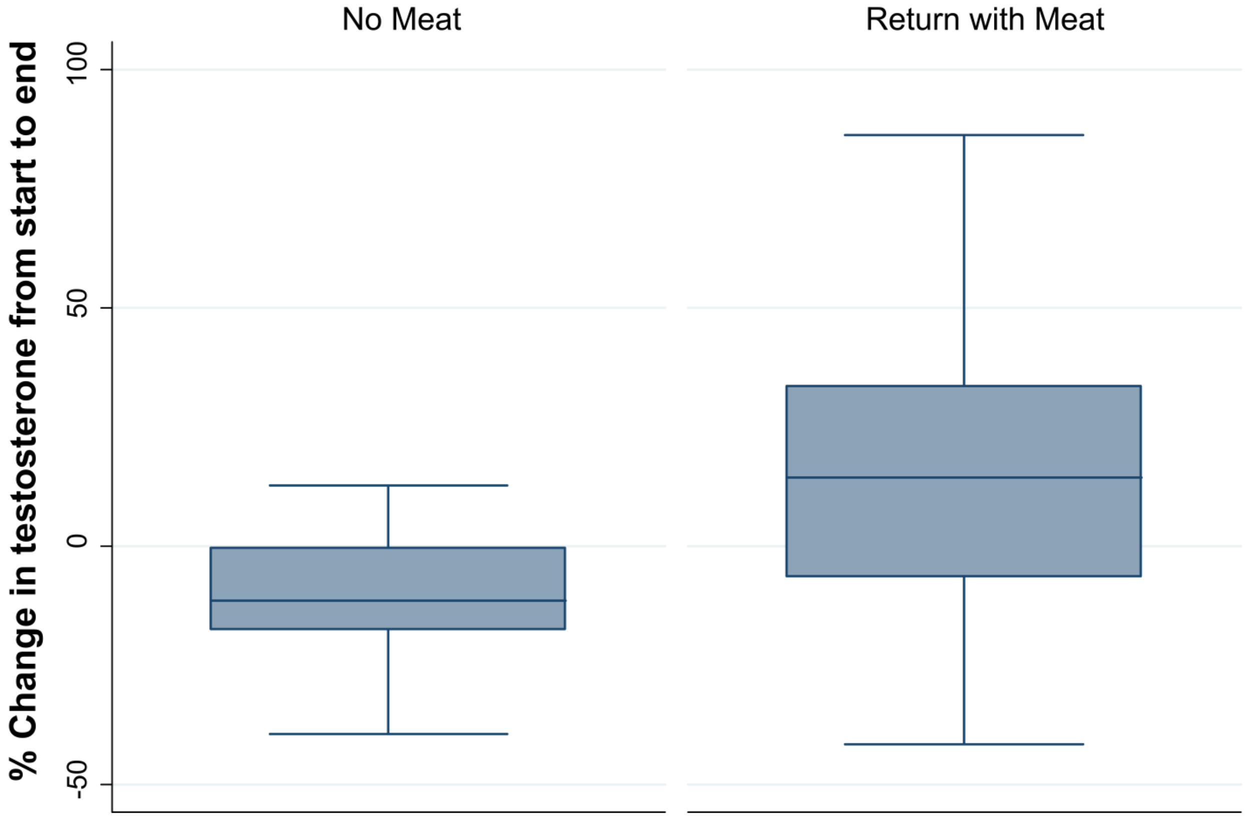
Figure 6.1 depicts an example of changes in heart rate over the course of a hunt in which a small animal (coati) was killed.

Figure 6.2: Testosterone by time point



This figure represents the average log salivary testosterone levels at each time point over the course of the study.

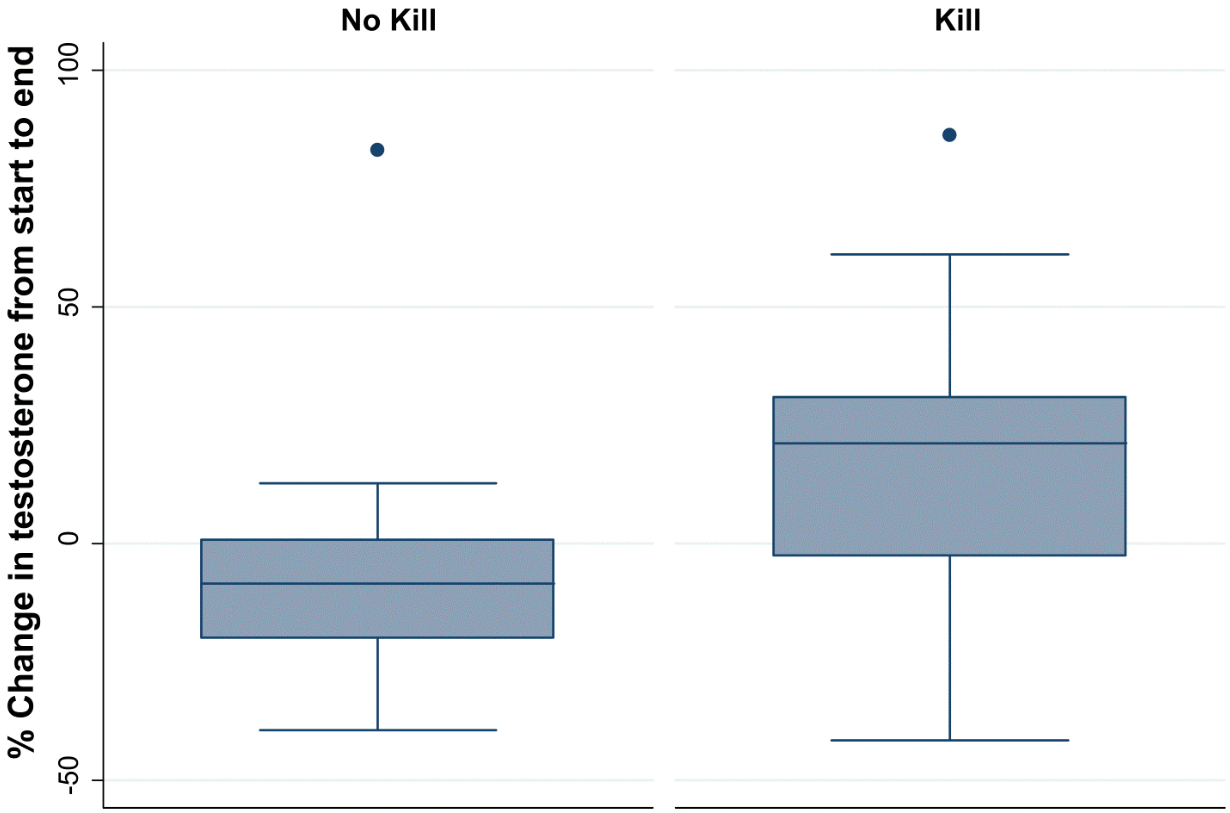
Figure 6.3: Change in testosterone and meat



This figure depicts the average percent change in testosterone from the initial baseline specimen collection to the final specimen collection, for men that returned with meat, and those who returned without acquiring any meat. The meat here could have been killed by the hunter, collected (e.g. tortoise), or killed by a fellow hunter.

Figure 6.4: Change in testosterone and killing

This figure depicts the average percent change in testosterone from the initial baseline specimen collection to the final specimen collection, for men that made a kill during the study.



Chapter 7: Discussion and conclusions

Overview

The research in this dissertation was conducted to better understand the dynamics of acute testosterone change across domains ranging from energetic stress, to direct and indirect male competition, in an evolutionary framework. The studies herein provide novel data on several evolutionarily relevant hormone-behavior-environment interactions.

The first study examined the responsiveness of the hypothalamic-pituitary gonadal (HPG) axis, and testosterone in particular, to energetic stress. A major theoretical basis of male life-history research revolves around the premise that a high-testosterone phenotype provides benefits in competition and subsistence, but is energetically expensive to maintain; given this trade-off, it follows that individuals in poorer energetic situations cannot maintain high levels of testosterone without suffering a net fitness loss. We found evidence that the HPG axis is responsive to acute caloric deficits on a shorter timescale than had previously been reported in the literature. Following an evening fast, morning urinary luteinizing hormone, and waking salivary testosterone decreased, while the urinary clearance rates of testosterone increased, resulting in rapid decreases in circulating testosterone. Consistent with previous studies of longer-term energetic stresses and illness (Cameron 1996; Muehlenbein and Bribiescas 2005; Muehlenbein 2008), this study adds to a growing literature reporting that testosterone production decreases when the energetic environment becomes less advantageous. While the physiological effects testosterone has on muscle tissue and behavior can be reproductively advantageous, these benefits trade off against the negative effects testosterone has on immune function during periods of environmental stress (Folstad and Karter 1992; Muehlenbein and Bribiescas 2005).

The remainder of the studies in this dissertation examined the dynamics of acute testosterone change in an energetically, parasitically and pathogenically stressed population, the Tsimane forager horticulturalists of the Bolivian amazon. Consistent with previous research in non-industrialized populations (Ellison et al. 1989; Bentley et al. 1993; Bribiescas 1996; Ellison et al. 2002; Campbell et al. 2006), we found evidence that Tsimane male testosterone levels were significantly lower than those of US men. Despite significantly lower levels of circulating testosterone, Tsimane exhibited a 30.1% increases in testosterone (Trumble et al. 2012) during physical competition, a relative increase in testosterone similar to those reported for men in industrialized populations (Archer 2006). This suggests that for humans engaged in competitive activity, similar hormone-behavior interactions occur regardless of baseline testosterone. Beyond the importance for understanding human testosterone-behavior dynamics, we propose that once engaged in physical competition, acute increase in testosterone are physiologically beneficial regardless of individual condition.

To examine acute testosterone changes during a physical non-competitive activity, we collected saliva specimens before and after men engaged in tree-chopping, a typical and important horticultural activity. One hour of intense tree-chopping was associated with a 48.6% increase in salivary testosterone. Current theoretical models of paternal investment predict that men with offspring have lower testosterone levels, as males invest in parenting as opposed to mating effort (e.g. (Wingfield et al. 1990; Gray et al. 2004; Gettler et al. 2011)). However, in subsistence contexts where family provisioning largely relies on physical activity, reduced testosterone for men engaging in parenting behavior could actually be detrimental, because it would hamper strength-mediated production strategies necessary to provision their current offspring. The challenge hypothesis framework can still be applied to horticultural behavior; men facing environmental stresses including high parasite and pathogen loads exhibit low levels of baseline testosterone to avoid the energetic and immunosuppressive costs associated with high basal testosterone. Testosterone increases during heavy physical activity, allowing rapid physiological performance enhancement, without the costs associated with consistently high basal testosterone.

With a better understanding of the role of physical activity on testosterone, the focused shifted toward a competitive non-physical activity, soccer penalty kicks. This makes it possible to parse out the testosterone-behavior dynamics of competitive male interactions without potentially confounding effects of physical activity on testosterone. Men took five penalty kicks in the presence of various audiences: no audience, young men, young women, old men, or old women. This made it possible to examine not only the effects of non-physical competition, but also the effect of the audience on testosterone. While testosterone increased 7.8% after taking five penalty kicks, no single audience condition had significantly larger increases in testosterone than any other group.

The final study undertaken as a part of this dissertation takes another look at a physically active food production strategy, hunting. While hunting is a production-related activity, it also contains competitive elements, and like other forms of resource production is associated with male reproductive success, though it has not been possible to identify whether hunting ability increases reproductive success through direct provisioning, direct or indirect reciprocity, social status, or access to more or better quality mates, or some combination of these factors. This study reported a 23.5% increase in testosterone immediately following a kill, and that men returning with meat had larger changes in testosterone over the course of the hunt than those who returned empty handed. Similar to the examination of acute changes in testosterone during penalty kicks, we found no evidence that audience presence played a role in testosterone change, which may have implications for understanding male hunting motivation.

Comparing the results of these studies allows an examination of acute increases and decreases in testosterone under various conditions. As the fasting study demonstrated, the male reproductive axis responds to brief energetic deficits by down regulating testosterone production in young healthy US men. Men in industrialized populations live in evolutionarily novel environments, where they face few energetic constraints, and have low parasite and pathogen loads. Thus subsequent studies examining acute changes in testosterone among Tsimane forager-horticulturalists offer insight into the dynamics of short-term testosterone change in an evolutionary and ecologically relevant environment. These studies were conducted by the same researchers, in the same laboratory, allowing us to make inferences about testosterone dynamics under environmental conditions similar to those faced by males throughout human evolution.

Comparing these results suggests that testosterone increases more during physical activities (soccer, tree-chopping, and hunting) than non-physical activities (penalty kicks and resting), see Table 7.1. This is expected, as previous studies report similar results (e.g., (Archer 2006)) when comparing physical and non-physical competition. One would expect that competition and physical activity would have additive effects, resulting in the largest increases in testosterone. However, a physical, non-competitive activity (tree-chopping) resulted in larger increases in testosterone than a competitive and physical activity (soccer). While our results were initially surprising, soccer and tree-chopping represent different types of physical activity, and there is some evidence that different modes of physical activity result in differential increases in testosterone (Keizer et al. 1989; Jensen et al. 1991; Tremblay et al. 2004). Soccer is a cardiovascularly intensive sport that does not require the same level of muscular involvement as resistance-based physical activities. Tree-chopping combines resistance and cardio exercise modalities. Hunting combines cardiovascularly intensive and muscularly intensive elements, though at different times. Men who encountered an animal expressed rapid increases in testosterone at the time of the kill, which fits within the theoretical framework suggesting that rapid increases in testosterone stimulate and enhance muscular neurophysiology (Tsai and Sapolsky 1996; Crewther et al. 2011). Men returning with meat had to carry out the animals they killed, which required a level of sustained resistance as well as cardiovascular activity.

Theoretical importance

Selective pressures and acute increases in human testosterone

These results raise questions about the evolution of acute testosterone change. Did acute changes in testosterone give males an advantage during male-male competitive interactions, which also augmented muscle physiology beneficial for provisioning in other situations? Or did acute increases in testosterone facilitate muscle physiology; providing a selective advantage which was then co-opted by competitive interactions? Or do acute increases in testosterone arise as a side-effect of a different hormonal-mechanism, such as the fight-or-flight response? Regardless of the initial selective pressures that led to acute increases in testosterone, short-term spikes in testosterone benefit both competitive male-male interactions and physically intensive production strategies, and have since coevolved to benefit reproductive success.

It is important to differentiate between baseline testosterone and the ability to express acute increases in testosterone. Baseline testosterone is thought to play a role in general male behavior, and is an important mediator of muscle mass (Wingfield et al. 1990; Bribiescas 2010). Acute changes in testosterone enhance short-term muscular metabolism (Tsai and Sapolsky 1996), and promote immediate increases in neuromuscular performance (Crewther et al. 2011), which can benefit long-term strength gain following resistance training (Rønnestad et al. 2011). However, the mechanisms responsible for baseline testosterone levels and acute change in testosterone appear to be to be completely separate, as the HPG axis controls baseline testosterone production, and another endocrine mechanism appears responsible for acute changes in testosterone (Flinn et al. 2012). The ability to express acute increases in testosterone is evolutionarily deep, occurring in diverse vertebrate taxa including fishes, reptiles, and mammals (Hirschenhauser and Oliveira 2006). Acute increases in testosterone are not limited to males or competition; female mammals including rodents (Aizawa et al. 2008) and humans (Vingren et al. 2010) express acute increases during non-competitive physical activity, though not at the same magnitude as males.

Males generally have higher baseline circulating testosterone compared to females; circulating testosterone in human males is twenty times higher than that of females (Simpson 2003). Evidence from bird phylogenies suggests that female baseline testosterone levels are driven by positive selection on male testosterone levels (Ketterson et al. 2005; Møller et al. 2005), implying that male-male competition was a key selective pressure in the evolution of baseline testosterone levels. Across taxa, baseline testosterone is a major determining factor in the levels of sexual dimorphism in body size, muscle mass and other sexually discordant somatic features (Bardin and Catterall 1981; Lassek and Gaulin 2009).

Human sexual dimorphism is often used as evidence that male-male competition was the norm throughout human evolution (Plavcan and van Schaik 1997), with chimpanzee-like social structure often thought to be the ancestral social structure (Boesch and Tomasello 1998; Marlowe 2005). However, among other great apes, sexual dimorphism between male and female body size can be higher than 50%, while in humans it is only about 15% (Plavcan 2001; Larsen 2003). While most researchers agree that male-male competition continues to play an important role in mammalian and human evolution (Plavcan and van Schaik 1997; Geary 2000; Plavcan 2001), humans differ significantly from other mammals (Hill et al. 2009). Unlike most mammals, human males invest heavily in offspring (Geary 2000; Winking et al. 2009), which may have played an important role in the evolution of human longevity (Kaplan et al. 2010). In addition to male-male competition, another possibility is that the coevolution of human brain size, feeding niche, and resulting sexual division of labor favored physically intensive male production strategies compared to female strategies (Kaplan et al. 2000; Gurven et al. 2009b). This could potentially have resulted in some selective advantage for larger male body size, resulting in retention of the relatively mild sexual dimorphism seen today. It should be noted that the advantages of acute increases in testosterone benefit both male-male competition and provisioning behavior, and likely coevolved. Additionally, some have argued that paternal provisioning behavior may even be a form of mating effort (Anderson et al. 1999), though not all empirical evidence supports this view (Winking et al., 2009).

If the ability of human males to sustain acute increases in testosterone was initially selected for primarily to facilitate male-male competition, then one might expect the ability to sustain acute increases in testosterone to decrease with age, both because men would be engaged in more parenting and less mating effort, and also because older men have realized fitness and thus fall into the shadow of selection (Kirkwood and Austad 2000). However, if acute changes in testosterone also bestowed a selective advantage for physical activity, then maintenance of this ability in older men might be expected, as provisioning children and grandchildren benefits inclusive fitness even late in life (Kaplan et al. 2010), and thus would be favored by natural selection (Kirkwood and Austad 2000).

The preceding studies found no evidence that age affected the ability to sustain acute increases in testosterone. Other studies examining acute changes in testosterone among older men report that men appear to maintain this ability with age in sexual (Escasa et al. 2011), competitive (Flinn et al. 2012), and physical activities (Vingren et al. 2010), suggesting that the Tsimane are not unusual in their ability to maintain increases in testosterone with increasing age. Studies herein also add to the growing body of research suggesting that men in subsistence populations have a reduced decline in baseline testosterone with age compared to men in industrialized populations (Ellison et al. 2002). Men in industrialized populations appear to reach peak levels of testosterone in their early twenties, and then express declines in testosterone on the order of 1-3% per year (Harman et al. 2001; Uchida et al. 2006; Travison et al. 2007a). Studies of men in non-industrialized populations report lower baseline testosterone, and a shallower rate of testosterone decrease across the lifespan (Bentley et al. 1993; Bribiescas 1996; Ellison et al. 2002; Campbell et al. 2006).

It has been hypothesized that environmental differences in parasites, pathogens, and energetic availability are responsible for these population differences in testosterone production (Ellison et al. 2002). Overall, it appears that as men in industrialized populations age, decreases in testosterone are associated with obesity, heart disease, and diabetes

(Travison et al. 2007b; Yeap 2009), with men who have the lowest levels of testosterone experiencing the highest risk of death (Laughlin et al. 2008; Carrero et al. 2009). In contrast, men in subsistence populations face an environment with higher energetic costs and greater parasitic and pathogenic burdens throughout life, which may constrain their testosterone levels such that they never achieve peak levels like men in industrialized populations.

While the studies herein were not designed to explain the evolution of acute testosterone change, they provide empirical observations on the dynamics of testosterone change in a population of forager-horticulturalists facing environmental pressures more similar to those faced throughout human evolution than is the case in industrial populations. These studies highlight the importance of expanding the current mating-effort and competition-centric focus of testosterone-behavior interactions to include family provisioning and related activities.

Acute changes in vertebrate testosterone and developmental context

These studies provide evidence that males living in environmental settings resulting in reduced baseline testosterone levels can sustain the same relative level of increase in testosterone during physical and competitive interactions. Previous work in animal models suggests that males in poor environments are less likely to engage in aggressive or mate seeking behavior (Neave and Wolfson 2003; Munoz et al. 2010). However, once engaged in an altercation, an acute increase in testosterone would benefit muscle physiology, facilitating escape or fighting ability, which would be beneficial regardless of baseline condition. The conservation of this ability to maintain acute increases in testosterone is found in varied human populations regardless of baseline testosterone and is useful for understanding hormone-behavior interactions.

While not focused on behavior, studies examining progesterone in Bangladeshi women report comparable results with regard to relative changes in hormone levels. Bangladeshi women have significantly lower baseline progesterone levels, yet experience similar relative progesterone fluctuations across the menstrual cycle when compared to US women (O'Connor et al. 2003; Nunez-de la Mora et al. 2007). Baseline adult hormone levels may, in part, be determined by critical periods during development which are thought to prepare the organism for the predicted environment into which they will be born (Nunez-de la Mora et al. 2007). Puberty is thought to be a critical period; women born in Bangladesh who move to the UK prior to puberty have similar adult progesterone levels to women born and raised in the UK, while immigrants that arrive after puberty retain lower progesterone levels throughout life (Nunez-de la Mora et al. 2007). It should be noted that while there is evidence this is the case in baseline progesterone, it does not appear to be the case for all hormones (Nunez-De La Mora et al. 2008), nor has this been tested in human males.

Work with mice suggests that critical periods of development not only influence adult hormone levels, but also hormone receptors in the brain, thus priming brain tissue for future hormone-behavior interactions and associated acute changes in testosterone. Rodents deprived of testosterone during critical developmental periods, including puberty and perinatal development, show reduced behavioral change when exposed to high levels of testosterone during aggressive encounters later in life (Perrin et al. 2008; Paus et al. 2009). Although this research was conducted using rodent models, this research design is useful for understanding variation in hormone-behavior interactions among human populations with different absolute levels of testosterone. Individuals who experience lower levels of testosterone exposure during critical periods like puberty may have testosterone receptors calibrated to respond to smaller absolute levels of testosterone change. Thus, while nutritional and pathogenic environmental factors result in Tsimane men expressing lower baseline testosterone levels compared to US men, we also expect, and observe, that they experience similar levels of relative change in testosterone in response to various behavioral and physical stimuli. This highlights the importance of examining relative changes in testosterone, in addition to absolute population differences in testosterone.

Challenge hypothesis as applied to humans

The challenge hypothesis is a theoretical framework designed to explain variation in avian testosterone levels with regard to mating systems and parenting behavior (Wingfield et al. 1990; Goymann et al. 2007). While later adapted to include acute increases in testosterone during aggressive interactions (Goymann et al. 2007), the original focus of the challenge hypothesis was to understand systematic variation in testosterone, mating system, and paternal care across numerous bird species. While there has been some disagreement over the phylogenetic analysis in the original publication (Hirschenhauser et al. 2003; Adkins-Regan 2005), aspects of the challenge hypothesis have since been applied to numerous other vertebrates (Hirschenhauser and Oliveira 2006), including humans and other primates (Muller and Wrangham 2004; Archer 2006).

The human-centric form of the challenge hypothesis has thus far focused on acute increases in testosterone during competition (e.g., (Archer 2006)), or long-term decreases in testosterone following marriage and fatherhood (Gettler et al. 2011). By focusing narrowly on competitive interactions as the only type of male behavior where increased testosterone can benefit reproductive success, the literature has missed potential reproductive benefits from acute increases in testosterone during other reproductively important activities, such as physically active provisioning strategies. Most competition studies conducted with human males occur in domains that are relatively trivial to overall reproductive success. Men's ability to play soccer, or shoot penalty kicks on goal may offer some tangential social benefits, but are unlikely to have the same level of impact as hunting and horticultural production, which have demonstrated associations with male reproductive success (Smith 2004; Gurven and von Rueden 2006; Gurven and Hill 2009; Mulder and Beheim 2011) by directly provisioning offspring (Winking et al. 2009), or attracting high quality mates (Marlowe 2005; Pillsworth 2008; Gurven et al. 2009b). Challenges that have demonstrated effects on reproductive success are likely subject to stronger selective forces than other competitive activities. Thus, the studies in this dissertation argue for an extension of the challenge hypothesis framework to other activities where acute increases in testosterone benefit male reproductive success, while limiting the energetically and immunological costs of high baseline testosterone phenotypes.

Limitations

Researcher Effect

All studies conducted in Bolivia involved the collection of biological specimens in the presence of an anthropologist. Because the sampling framework required strict adherence to protocols in order to ensure comparability between participants and studies, it was necessary for a trained researcher to collect specimens. While care was taken to show that in the case of resting, researcher presence did not affect testosterone levels (Trumble et al, *in preparation*), there could potentially be interactions between testosterone change, competitive activity, and researcher presence that we were unable to test.

Self-Selection

It should be noted that all of these studies suffered from some level of self-selection, and thus may not be generalizable to all men. The US participants in the first study were young, healthy men recruited from the University of Washington community, which may preclude extending these results to overweight or underweight individuals, or older men. The studies conducted in Bolivia also suffer from self-selection- although these men live in an environment with higher parasite and disease burden, the individuals participating were likely among the healthiest Tsimane men, as they were able to take part in physical activities ranging from soccer, to tree-chopping, to hunting. However, a comparison of the BMIs of soccer match participants and other age-matched Tsimane men found no significant difference between the participants and the overall population, suggesting that these men are not abnormal in terms of BMI. While BMI is a crude measure of health, health information was also collected; 72.5% of men engaged in horticultural activity self-reported some form of illness. Regardless of any self-selection that may have occurred, Tsimane men still had significantly lower levels of testosterone compared to US males, allowing tests of hormone-behavior interactions in a population with low baseline testosterone.

Endocrine mechanism

Another limitation of these studies is our current lack of information on the mechanism responsible for acute changes in testosterone. In humans, basal male testosterone production occurs via the hypothalamic-pituitary-gonadal axis, with testicular testosterone secretion occurring downstream of increases in hypothalamic gonadotropin releasing hormone and pituitary luteinizing hormone (LH) (Nelson 2000). During periods of limited energetic availability, either from decreased caloric intake or increased energetic expenditure, the HPG axis down-regulates testosterone production (Cameron 1996), while simultaneously increasing urinary testosterone clearance rates, resulting in apparently lower circulating testosterone levels (Trumble et al. 2010). While these decreases in testosterone can occur within twelve hours (Cameron 1996; Trumble et al. 2010) acute increases in testosterone occur within 5-15 minutes of a competitive interaction (e.g., (Archer 2006; Pound et al. 2009)). The endocrine pathway responsible for acute increases in human testosterone is unknown (Flinn et al. 2012).

If the mechanism responsible for short term changes in testosterone is under direct control of the HPG axis, then life-history theory predicts that during energetic or immune stress, the increase in testosterone might be diminished as individuals preserve energy for survival. However, if testosterone increases during a challenge via a separate pathway, then spikes in testosterone could occur independently of environmental stressors. The results of the studies in this dissertation suggest that an acute increase in testosterone does occur in energetically and immunologically stressed populations, suggesting that the energetically labile HPG axis is not involved in acute testosterone increases. *In vitro* research also provides evidence suggesting that the HPG axis is not responsible for acute increases in testosterone; injections of synthetic LH do not produce a significant increase in testosterone for at least 45-75 minutes (Veldhuis and Iranmanesh 2004). Additionally, testosterone increases following competitive interactions among females (Bateup et al. 2002; Oliveira et al. 2009; Edwards and Kurlander 2010), indicating that testicles are not necessary for such increases to occur. That said, alternative sources of female testosterone are not discussed in these papers.

One study (*n*=4) hypothesized that decreased blood flow to the liver and kidneys after an intense period of physical activity reduces the amount of testosterone cleared from the body, thereby explaining the acute increase in testosterone (Cadoux-Hudson et al. 1985). However, acute increases in testosterone can occur within minutes, while decreased clearance rates would take a significantly longer period of time to build-up. That said, studies of non-physical male-male competition, including one study completed as a part of this dissertation, report increases in testosterone in the absence of physical activity (Mazur et al. 1992; Oxford et al. 2009), which would indicate that liver clearance is an unlikely culprit.

Studies of human competition have focused largely on salivary testosterone (Archer 2006), which only represents the free portion of circulating testosterone (Granger et al. 2004). Studies have not yet adequately examined whether total testosterone (free and bound) is increasing, just free testosterone is increasing, or if there are changes in the ratio of free and bound testosterone (Figure 7.1).

Another endocrine system known to react on a similar, if somewhat faster time scale than acute testosterone change is the sympathetic nervous system “fight-or-flight” response to stress (Nelson 2000). In humans, a large portion of circulating testosterone is bound with low-affinity to albumin, an important carrier protein for a number of hormones (Nelson 2000). Studies report that catecholamines also bind to albumin (Danon and Sapria 1972). If catecholamines have a higher affinity for albumin than testosterone, then the sudden release of catecholamines as a part of the stress response could result in a dissociation of testosterone from albumin as catecholamines bind to albumin, increasing the portion of circulating free and salivary testosterone, without necessitating novel testosterone production. In humans and baboons, studies find associations between testosterone and catecholamines following competition (Fry et al. 2011) and stress (Sapolsky 1986). Administration of catecholamine antagonists to baboons immediately after darting eliminates increases in testosterone usually seem in the first hour after darting (Sapolsky 1986), suggesting that an increase in catecholamines may be responsible for acute testosterone increases. A recent study found that administration of adrenocorticotrophic hormone (ACTH), a hormone normally released by the anterior pituitary as a part of the initial phase of the stress response, significantly increased adrenal testosterone levels in healthy men (Nakamura et al. 2009), providing additional evidence of linkages between the stress response and acute changes in testosterone. Future studies will examine the role of catecholamines as one potential mechanism for acute increases in testosterone.

Conclusions

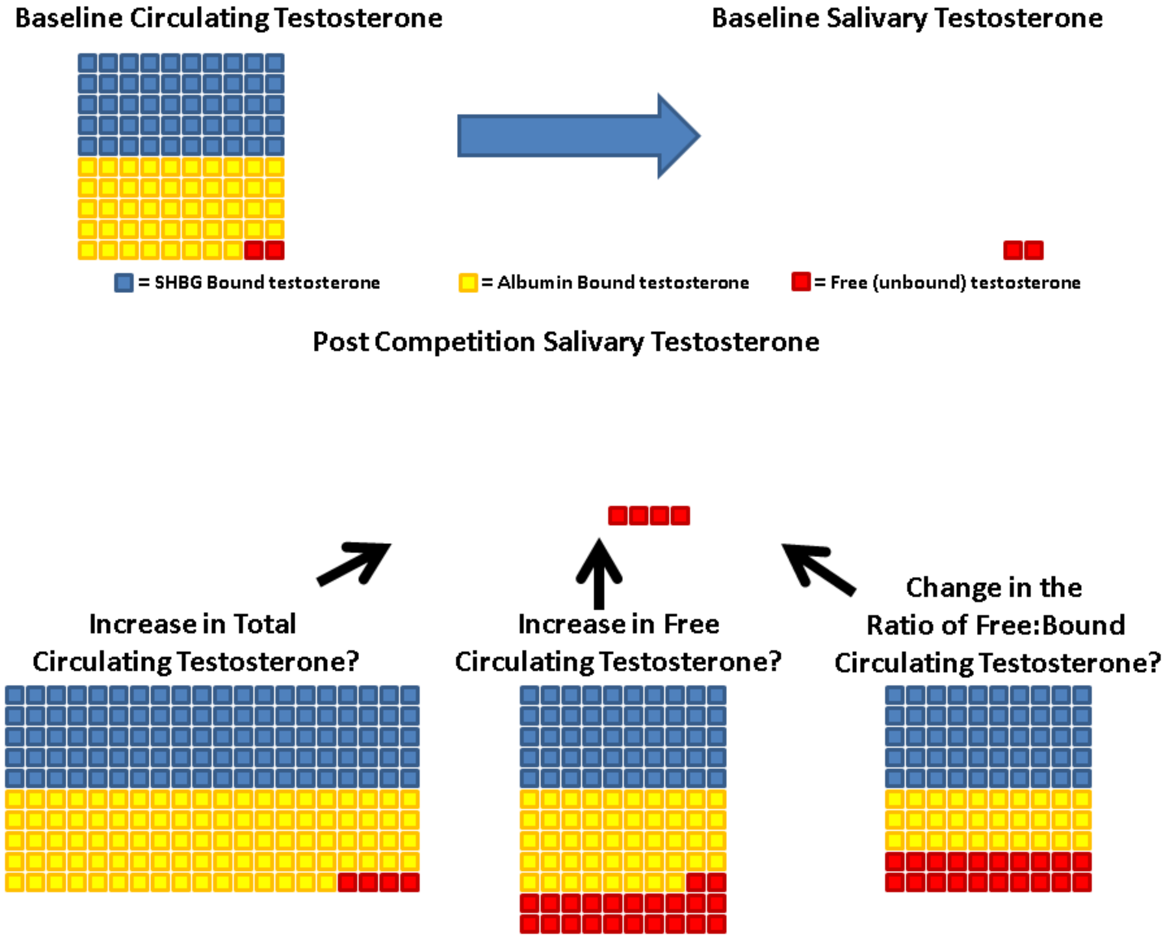
Within the framework of life-history theory, these studies seek to expand the current understanding of how the environment influences hormones, and hormone-behavior interactions. This research reports that the HPG axis reacts more quickly to decreased caloric intake than previously thought in young healthy US males, underscoring the importance of the energetic environment in mediating testosterone. Previous research on male on hormone-behavior interactions had largely focused on male-male competition among well-nourished and healthy young men in industrial populations; this dissertation broadens this research to include immunologically and nutritionally stressed forager horticulturalists, reporting that similar hormone-behavior interactions occur in evolutionarily relevant settings. These studies observed testosterone response to physical and non-physical competition, while also examining non-competitive physical challenges, in order to assess acute changes in testosterone across other fitness enhancing domains, including food production activities. Field and laboratory studies were combined to form a better understanding of the dynamics of acute testosterone change in different immunologic and energetic environments, providing a broader understanding of human biology and adaptive variation.

These studies underscore the importance of moving beyond a one-dimensional emphasis on acute changes in testosterone during competitive interactions, in order to examine and understand the importance of acute testosterone-behavior interactions during other, perhaps more evolutionarily relevant, reproductively important activities, such as resource acquisition. While direct male-male competition can play a role in human adaptation, male reproductive success is mediated by a number of factors. The coevolution of human brain size and food niche shaped the human life-course, placing special emphasis on male provisioning of offspring and grand-offspring. Understanding how selective pressures shaped the dynamics of testosterone response to physical and competitive challenges is central to understanding the evolution of human male life-history.

Table 7.1: Percent change in salivary testosterone in response to competitive, semi-competitive and non-competitive physical and non-physical activities

	Competitive	**Semi-Competitive**	**Non-Competitive**
Physical	Soccer Tournament (*n*=82) **30.1% Increase** **(*p*<0.001)**	Hunting (*n*=31) **23.5% Increase** **(*p*=0.03)**	Tree-Chopping (*n*=49) **48.6% increase** **(*p*<0.001)**
Non-Physical	Penalty Kicks (*n*=73) **7.8% increase** **(*p*=0.069)**		Resting (*n*=20) **4.5% decrease** **(NS)**

Figure 7.1: Potential Changes in free and bound testosterone during competition



This simplified diagram describes how the portion of circulating testosterone that is bound to SHBG, albumin, and free (unbound) testosterone (Vermeulen et al. 1999b) could change. During competitive or physical challenges, salivary (free) testosterone increases. It is not known whether total testosterone is increasing, just free testosterone is increasing, or if the ratio of free to bound testosterone is changing.

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Education

2012	PhD Department of Anthropology, University of Washington
	Co-Advisors: Dr. Kathleen O'Connor and Dr. Eric A. Smith
2009	M.A. Department of Anthropology, University of Washington
2006	B.A. Anthropology and Psychology, University of California Santa Barbara

Awards and Honors

2012	E.E. Hunt Jr. Student Presentation Prize, Human Biology Association
2010	Department Pilot Funding Award
2010	Baldwin Scholarship
2008-2010	NIH/NICHD Predoctoral Fellow, Center for the Studies in Demography and Ecology
2006	Graduated Phi Beta Kappa, University of California Santa Barbara
2006	Graduated with Distinction in Anthropology, University of California Santa Barbara
2006	Lambda Alpha, Anthropology Honor Society, University of California Santa Barbara
2004-2006	Deans Honors, University of California Santa Barbara

Publications

Trumble BC, Cummings DK, von Rueden CR, O'Connor KA, Smith EA, Gurven MD, Kaplan HS. (2012) Physical competition increases testosterone among Amazonian forager-horticulturalists: a test of the "challenge hypothesis". *Proceedings of the Royal Society: B 279 (1739):2907-2912*

O'Connor KA, Brindle E, Shofer J, **Trumble BC**, Aranda JD, Rice K, Carey KD, Tatar M. (2011). The effects of a long termpsychosocial stress on reproductive indicators in the baboon. *The American Journal of Physical Anthropology 145(4):629-38*

Trumble BC, Brindle E, Kupsik M, O'Connor KA. (2010). Responsiveness of the reproductive axis to a single missed evening meal in young adult males. *American Journal of Human Biology 22(6):775-781*

O'Connor KA, Ferrell RJ, Brindle E, Shofer J, **Trumble BC**, Holman DJ, Weinstein M. (2009). Progesterone and ovulation across the stages of the transition to menopause. *Menopause 16(6):1178-1187*

Submitted

O'Connor KA, Hayes-Constant TK, Snipes SA, **Trumble BC**, Goodreau SM, Morrison DM, Shell-Duncan B, Pelman RS. *Qualitative findings from the Health Initiatives for Men (HIM) study: Men thinking about, talking about, and practicing health. Submitted, PLoS ONE.*

In Preparation

Trumble BC, Cummings DK, Holman DJ, O'Connor KA, Gurven MD, Kaplan HS. *Age-independent increases in male salivary testosterone during horticultural activity among Tsimane forager-farmers.*

Trumble BC, Smith EA, O'Connor KA, Kaplan HS, Gurven MD. *Challenge hypothesis and hunting success: male salivary testosterone increases at the time of the kill and upon returning home for successful Tsimane hunters*

Trumble BC, Cummings DK, Stieglitz J, O'Connor KA, Smith EA, Gurven MD, Kaplan HS. *Audience effects and Tsimane male testosterone during a penalty kick shootout.*

Kemmis S, **Trumble BC**, Smith EA, O'Connor KA. *Short-term fasting, hormone levels, and economic game behavior.*

Trumble BC, Holman DJ, Salisbury R. *The hazard of death on Mount Everest: individual success and death over time.*

Conference Presentations

Trumble BC, Smith, EA, O'Connor KA, Gurven, MD. *Challenge hypothesis and hunting success: male salivary testosterone increased at the time of the kill and upon returning home for successful Tsimane hunters.* Human Behavior and Evolution Society Conference (June 2012).

Trumble BC, Cummings DK, Holman DJ, O'Connor KA, Gurven MD, Kaplan HS. *Acute changes in male salivary testosterone in response to intense physical activity among Tsimane forager-horticulturalists.* 36th Annual Meeting of the Human Biology Association (2012).

Trumble BC, Cummings DK, von Rueden CR, O'Connor KA, Smith EA, Gurven MD, Kaplan HS. *Changes in male salivary testosterone in response to competitive soccer matches among the Tsimane of the Bolivian Amazon.* The 35th Annual Meeting of the Human Biology Association (2011).

Blackwell AD, **Trumble BC**, von Rueden CR, Steiglitz J, Cummings D, Emory Thompson M, Snodgrass JJ, Fitzgerald E, Shattuck-Faegre H, Gurven MD, Kaplan HS. *Don't bring your cough to the gun show: Testosterone, immunocompetence, strength and dominance in an Amazonian horticulturalist population.* Human Behavior and Evolution Society Conference (2011).

O'Connor KA, Snipes SA, Hayes Constant TK, Chan Ridley M, Goodreau SM, **Trumble BC**, Morrison DM, Shell-Duncan BK, Guyton AC, Pelman RS. *"Man Up": Qualitative findings from the Health Initiative for Men (HIM) Study.* The Population Association of America Meetings of America (2011).

O'Connor KA, Snipes SA, Hayes Constant TK, Chan Ridley M, Goodreau SM, **Trumble BC**, Morrison DM, Shell-Duncan BK, Guyton AC, Pelman RS. *"Man Up": Qualitative findings from the Health Initiative for Men (HIM) Study.* Western section of the American Urological Association Annual Meeting (2010).

Trumble BC, Holman DJ, Salisbury R. *The hazard of death on Mount Everest: individual success and death over time.* The 79th Annual Meeting of the American Association of Physical Anthropologists (2010).

Trumble BC, Brindle E, Kupsik M, O’Connor KA. *The effect of a single missed evening meal on morning salivary testosterone in young adult males.* The 21st Annual Human Behavior and Evolution Society Conference (2009).

Trumble BC, Brindle E, Kupsik M, O’Connor KA. *The effect of a single missed evening meal on morning salivary testosterone in young adult males.* The 34th Annual Meeting of the Human Biology Association (2009).

Research Development

2010-2011	Field Work- Bolivia: Tsimane Health and Life History Project, Dr. H Kaplan and M Gurven
2009	Laboratory Training: in-house assay development, University of Washington
2009	Ethics Training: University of Washington, Center for Studies in Demography and Ecology
2008	Demographic/Statistical Methods: University of Washington, CSDE
2007	Laboratory Training: Chemoluminescent enzyme immunoassay (CLEIA)
2006	Laboratory Training: Enzyme-linked immunosorbent assay (ELISA)
2006	Research Assistant Training Program: University of Washington

Teaching

Teaching Assistantships

2012	Spring. Teaching Assistant, University of Washington, Biocultural Anthropology 369: Origins and Evolution of Human Diseases.
2012	Winter. Teaching Assistant, University of Washington, Biocultural Anthropology 455: Reproductive Ecology Laboratory Seminar. Dr. Kathleen O’Connor.
2011	Spring. Teaching Assistant, University of Washington, Biocultural Anthropology 455: Reproductive Ecology Laboratory Seminar. Dr. Kathleen O’Connor.
2008	Spring. Teaching Assistant, University of Washington, Biocultural Anthropology 455: Reproductive Ecology Laboratory Seminar. Dr. Kathleen O’Connor.
2007	Winter. Teaching Assistant, University of Washington, Epidemiology 529: Emerging Infections of International Health Importance. Dr. Ann Kimball, and Dr. Carrie Horwitch.

Guest Lectures

2012	*Male Reproductive Endocrinology*. Biocultural Anthropology 455: Reproductive Ecology Laboratory Seminar. Dr. Kathleen O’Connor.
2011	*Challenges to Testosterone: Health and Life-History in Industrial and Forager-Horticulturalist Populations*. Biocultural Anthropology 369: Men’s Health Across the Lifespan. Dr. Kathleen O’Connor

2011	*Male Reproductive Endocrinology*. Biocultural Anthropology 455: Reproductive Ecology Laboratory Seminar. Dr. Kathleen O'Connor.
2011	*The challenges of collecting biological specimens in the field*. Biocultural Anthropology 590: Biomarker Methods Research Group. Dr. Kathleen O'Connor
2010	*Challenges to Testosterone: Health and Life-History in Industrial and Forager-Horticulturalist Populations*. Biocultural Anthropology 369: Men's Health Across the Lifespan. Dr. Kathleen O'Connor
2010	*Male Reproductive Endocrinology*. Biocultural Anthropology 455: Reproductive Ecology Laboratory Seminar. Dr. Kathleen O'Connor.
2009	*Testosterone and Male Health*. Biocultural Anthropology 369: Men's Health Across the Lifespan. Dr. Kathleen O'Connor
2009	*Testosterone and Men*. Biocultural Anthropology 201: Principles of Biological Anthropology. Emily Brunson

Seminars

2012	*Testosterone, energetics, and male life-history*. IGERT Program in Evolutionary Modeling. University of Washington and Washington State University
2010	*The role of energetics and testosterone in male life- history strategy.* Dissertation Colloquium, University of Washington.
2009	*The effect of a single missed evening meal on morning salivary testosterone in young adult males.* Biocultural Anthropology Seminar Series (BASS), University of Washington**.**

Teaching Development

2012	Diversity and Inclusion in Teaching Workshop, University of Washington
2006	Anthropology 599: Effective Teaching of Anthropology, University of Washington.

Academic Service

2012	Manuscript reviewer for *PLoS ONE*
2010-2011	Graduate Student Representative to Faculty
2007-2010	Graduate Activities Fund Committee, University of Washington
2007-2008	Chair of the Graduate Activities Fund Committee, University of Washington

Certifications

2010	Managing Laboratory Chemicals
2010	Fume Hood Training

2008	Hazardous Chemical Waste Disposal
2008	Blood Borne Pathogen Exposure Training
2005	Emergency Medical Technician (Certification-EMT 1)
2005	CPR for the Professional Rescuer
2005	Open Water Dive Certification (PADI)

Languages

Spanish, STATA, Adobe Photoshop, Excel, Visio, Word, Gen5 Data Analysis, MOSIX, MLE.

Media

ScienceNOW, Daily Mail, ScienceDaily, PhysOrg, LiveScience

Professional Affiliations

Tsimane Health and Life History Project

Center for Studies in Demography and Ecology

Human Behavior and Evolution Society

Population Association of America

American Association of Physical Anthropologists

Human Biology Association