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Short-term resource allocation during extensive athletic competition

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Abstract

Objectives: Following predictions from life history theory, we sought to identify acute trade-offs between reproductive effort (as measured by psychological arousal) and somatic maintenance (via functional measures of innate immunity) during conditions of severe energetic imbalance.

Methods: Sixty-six male ultramarathon runners (ages 20 to 37 years) were sampled before and after a lengthy race. Saliva and sera were collected for testosterone and immunological analyses (hemolytic complement activity and bacterial killing ability). Lean body mass was assessed by bioelectrical impedance, and libido was measured using a slideshow of arousing and neutral images.

Results: Following predictions, there was a significant decrease in salivary testosterone levels (109.59 pg/ml versus 97.61 pg/ml, $p < 0.001$) and arousal scores in response to provocative images (5.40 versus 4.89, $p = 0.001$) between pre- and post-race time points. Additionally, participant bacterial killing ability ($p = 0.035$) and hemolytic complement activity ($p = 0.021$) increased between pre- and post-race.

Conclusions: Decreased libido and testosterone with concomitant heightened innate immune responses suggest a shift in energetic priorities away from reproduction and towards maintenance/defense during a period of energetic stress.

Keywords: Life history theory, testosterone, libido, innate immunity

Life history theory predicts the existence of trade-offs between competing physiological functions relating to reproduction, maintenance, defense, growth, and storage under conditions of limited environmental resources (Stearns, 1992; Reznik, Nunney & Tessier, 2000). Individuals that have developed effective mechanisms for both acquisition and optimal allocation of energy in their particular ecological niche are thereby advantaged (Lotka, 1922; Angiletta et al., 2003). According to evolutionary theory, every individual organism should exhibit behavior intended to enhance genetic contributions to subsequent generations. However, a life history strategy involving a greater allocation of resources towards reproduction necessitates a reduction in the resources available for other functions. Consequently, an individual may allocate energy to traits which enhance fecundity and fertility or invest in traits enhancing survivorship.

Fluctuations in the availability of environmental energy influence the relative investment in reproduction versus survivorship. This is exemplified by nutritional infecundability, whereby a negative shift in the relative balance between energy intake and expenditure adversely affects the fecundity in a range of mammalian species (Bronson, 1991; Wade et al., 1996; Mosley, 2012). Despite the pathological connotations, it is likely that conditions of sub-optimal nutrition will be encountered at some point during the lifetime of a mammal, and animals will have been selected to protect functions necessary for survival at the expense of less essential processes. Thus, available energy is partitioned according to a set of priorities that maximize the chance of survival and thereby optimize long term reproductive success (Wade, 1992).

The principle of allocation proposes the existence of negative correlations between investment in reproduction and somatic traits such as maintenance, growth,

or storage (Cody, 1966; Roff, 1992: Zera & Harshman, 2001). Despite the intuitive appeal of a stereotypical life history trade-off featuring a negative relationship between two traits, such negative covariation in traits are frequently absent when phenotypic comparisons are made between individuals within a population (Glazier, 2000). Furthermore, negative correlations are less likely to be reported in a field setting than in a tightly controlled laboratory study. This may be explained by inter-individual variation in resource acquisition exceeding variation in resource allocation (Van Noodwijk and de Jong, 1986).

Identifying quasi-experimental scenarios in which negative correlations are observable in the field would break new ground in the study of human life history trade-offs. While one cannot control individual energy intake in the field without compromising ecological validity, it may be possible to nullify the effect of this variation in resource acquisition by experimentally controlling energy balance. It is not the absolute values of energy intake and expenditure which force the individual to make resource allocation decisions, but the imbalance of the two (Bronson, 1991). Therefore, if the energy deficit is high enough, variation in food intake between individuals will be inconsequential; all individuals will be energetically stressed.

This study uses male participants from running ultramarathons to investigate the relative investment between reproduction and survivorship. Ultramarathons, defined as races lasting over 4 hours (Knez et al., 2006) (but often several days in length). Recent work suggests that high levels of energy expenditure cause metabolic adaptation to reduce total energy output (Pontzer et al. 2016), and the high energetic costs of locomotion necessitated by ultramarathons are expected to necessitate physiological trade-offs. This is due to energy deficit induced by a combination of the high levels of energy expenditure in physiological systems linked

to locomotion, and the lack of opportunity to ingest any significant meals (Knechtle and Bircher, 2005; Knechtle et al., 2005).

Testosterone functions as a physiological regulator, influencing the allocation of energetic resources between life history traits (Muehlenbein and Bribiescas, 2005; Hau, 2007). From a physiological perspective, testosterone plays a significant role in stimulating muscle growth (Kadi, 2008), which is beneficial in both inter-sexual (Lavrakas, 1975; Dixson, 2003; Fan, 2005; Frederick and Haselton, 2007) and intra-sexual (Bribiescas, 2001; Dijkstra and Buunk, 2002) selection. Testosterone is also implicated in behavioral aspects of human male reproductive effort. Sex drive, or libido, is a vitally important motivational force in human behavior (Darwin, 1871; Bancroft, 1988; Ariely and Loewenstein, 2006). In addition to its role in generating sexual motivation (Beach, 1976; Sherwin, 1988; Baumeister et al., 2001), testosterone is important in modulating qualities believed to be beneficial in the male mating effort, such as confidence and assertiveness in social situations (Elias, 1981; Bagatell et al., 1993; Ellison, 2003; Morley, 2003). Consequently, we measured testosterone levels, lean body mass, and libido of participants.

Measures of investment in immune function, a proxy for the body's investment in survivorship, were taken through the use of two functional assays of innate immune responses (bacteria killing assay and hemolytic complement assay). The purpose of the bacteria killing assay is to measure the functional ability of integrative immunological components, including opsonizing proteins and antibodies, to lyse a known quantity of *Echerichia coli* bacteria relative to a positive control (Muehlenbein et al. 2011). The hemolytic complement assay serves to measure the ability of the antibody-dependent pathway of the complement system to lyse pathogens. These assays assess innate immune responses only. While a comprehensive assessment

of immunocompetence would require examination of the adaptive immune response as well, these functional assays are arguably more relevant than simple measures of inflammation (e.g., C-reactive protein or sIgA), and meaningful results have previously been reported in humans and nonhuman primates (Prall and Muehlenbein, 2014; Georgiev et al., 2015; Prall and Muehlenbein, 2015; Prall et al., 2015).

This paper aims to build upon current knowledge of human male energetic investment in reproduction and survivorship through analysis of male ultramarathon runners. While past studies predominantly consider trade-offs acting on a long-term evolutionary scale, the present study employs the conceptual framework provided by life history theory as a reference to consider acute trade-offs. It was hypothesized that markers of investment in reproductive function (Demas et al., 2011) will decrease in magnitude, allowing for an increase in markers indicative of investment in short-term survival. This is due to the high costs of male mating effort (Ellison, 2003) and the suggestion that survival may be prioritized over other processes (Bronson, 1991).

Materials & Methods

Self-reported heterosexual male athletes (total of 66) were recruited at the 2013 North Downs Way 100 (102.6 miles) race, held 10-11th August (http://www.centurionrunning.com/north-downs-way-100-2015/). Athletes received an email explaining the study prior to race day, and were invited to participate. The study was approved by the University of Cambridge Human Biology Research Ethics Committee. The race is a relatively high profile event, with course records of

15:44:39 and 20:10:39 (hr:min:s) for men and women, respectively. Participants had a mean age of 29.4 years (range 20-37 years), and all were of European descent.

Samples and measurements were taken both before and after the race from athletes who completed the full race distance in order to determine the effects of the energetic deficit caused by race participation. Measurements included height, weight, and lean body mass (via bioelectrical impedance analysis). Five ml saliva and 5ml blood serum samples were collected for testosterone measurement and bacteria killing and hemolytic complement assays. While all of the pre-race measurements were taken between 1800 and 2200 hours, the practicalities of varying finish times (ranging from 22:30 hours on the day of race start to 12:00 hours the following day) meant that post-race measurements were not standardized by time. All post-race measurements were collected within 15 minutes of completion of the race course following established methods (Davies and Thompson, 1986; Malarkey et al., 1993; Berg et al., 2008; Lucas et al., 2008; Stuempfle et al., 2010).

Reproductive effort

Testosterone was measured from saliva, rather than serum. Salivary analysis facilitates repeated sampling, enhances subject compliance, and provides reliable data (Dabbs, 1990; Shankar and Dandekar, 2012). Salivary measurements may underestimate testosterone levels; however, this is more problematic in females, and this study sought to track changes in testosterone levels within subjects rather than absolute differences between individuals (Shirtcliff et al., 2002).

A 5ml saliva sample was collected in multiple aliquots using the Salimetrics Saliva Collection Aid (#5016.02). Subjects refrained from eating, drinking, chewing gum, or brushing teeth in the 30 minutes preceding saliva collection. Cotton/polyester swabs were avoided and participants were screened for mouth

injuries such as open sores to prevent blood contamination. Samples were immediately stored at -18°C, and were frozen at -80°C within 48 hours of collection. All samples were analyzed within two weeks of collection, using the DRG Salivary Testosterone ELISA kit (SLV-3013). Pre- and post-race hormone levels were tested for normality, which was confirmed. Intra-assay CV = 6.4%, inter-assay CV = 10.26%. The high and low controls were within established values.

Lean body mass

Lean body mass was measured using bioelectrical impedance analysis (BIA) (BodyStat Quadscan4000). It is appreciated that *in vivo* measurements cannot measure body composition directly, but rather make predictions from other physiological metrics. BIA was chosen because of its speed, simplicity, high precision and suitability for assessing short-term changes in individuals (Johnson et al., 1985; Roubenoff, 1996; Wells and Fewtrell, 2006). In order to avoid the inherent problems of predicting total body water (TBW), regression equations for converting between impedance and TBW were avoided. Instead, the simple index of 1/impedance, which reliably predicts lean mass index (lean mass/height^2), was used (Wells et al., 2007).

Libido

For the purposes of this study, the terms libido and sexual desire are used to refer to desire to partake of sexual activity (Levin, 1994). This attitude is viewed as being ever-present on a continuous scale, and is responsible for inducing a desire for sexual activity upon stimulation (Levin, 1994). A measurement of libido was taken both before and after the ultra-marathon race, using near-nude female images as stimuli. Visual stimuli have been shown to be powerfully sexually provocative for

men (Quiney et al., 1996; Graziottin, 2000; Hietanen and Nummenmaa, 2011). All participants self identified as being heterosexual.

Before the race, subjects practiced using the Self-Assessment Manikin (SAM, these practice scores were not analyzed - Crabbe et al., 2007), a pictogram exhibiting levels of affect on a nine-point scale (Lang, 1980; Hodes et al., 1985). The SAM has gained popularity in such work as it is a low-cost and relatively easy method for quickly determining affective response in many contexts (Bradley and Lang, 1994). Subjects were randomly divided into two groups. The first group was shown a 45-photo slide show (slide show A) before the race and a different 45-photo slide show (slide show B) after the race, and this order was reversed for the second group. Each slide show comprised 15 provocative near-nude female images, and 30 International Affective Picture System (IAPS) images (15 positive valence, 15 negative valence), and were viewed under standardized, private, and relaxed conditions. The order of images within each slide show was pseudo-random such that two photos from the same category (provocative near-nudes, positive and negative) could be viewed consecutively (Smith, 2012). Immediately after each picture was viewed, the participant used the SAM to rate their valence and arousal experienced whilst viewing the picture. Athletes were not asked to rate the same image more than once, to avoid possible development of habituated responses (Rosen, 1973; Freund et al., 1974; Rubin and Henson, 1976; Heiman, 1977; Julien, 1984). See Appendix for further details of this protocol used.

Innate immune function

In vitro bacteria killing assays were used with serum to measure innate immunity. After a test-run to optimize dilutions, serum was diluted 1:12 in L-glutamine supplemented CO_2 Independent Media (Gibco #18045). A single

lyophilized *E. coli* pellet (MicroBiologics Epower Microorganisms #0483E7) was reconstituted in sterile phosphate buffered saline and then diluted into a working solution, which produced approximately 200-300 colonies per 20µl of aliquot. Aliquots of bacteria working solution were added to diluted serum in a microcentrifuge tube, vortexed, and incubated for 30 minutes. After incubation, the samples were spread on trypticase soy agar plates (BD BBL #211043) in triplicate and incubated overnight at 37°C. The number of colonies on each plate the next day were counted, and the percent bacteria killed for each sample relative to a positive control (media and bacteria only) was calculated.

Serum was also used to measure the classical pathway of complement protein activity via a hemolytic complement assay (Sinclair and Lochmiller, 2000; Demas et al., 2011). Following test runs to optimize dilutions, serum was diluted 1:90 and 1:180 in dextrose gelatin veronal buffer (Lonza BioWhittaker #10-539) and pipetted in duplicate onto a round-bottom 96-well plate. Sheep red blood cells (MP Biomedicals #55876) were washed in sterile phosphate buffered saline, and further diluted to 0.6% in veronal buffer. Anti-sheep red blood cell antibodies (Sigma #S1389-1VL) were diluted to 1:40 in veronal buffer. Both the diluted antibodies and sheep red blood cells were then added to each sample well, and the plate was vortexed. Following incubation for 1.5 hours at 37°C, the plate was centrifuged, and the supernatant transferred to a new 96-well round-bottom plate. The absorbance of this supernatant was read at 405nm. Results of this assay are expressed as CH50 units, or the inverse of the dilution predicted to cause 50% hemolysis (Mayer, 1948).

Statistics

Paired samples t-tests were performed to compare pre- and post-race metrics. Associations between baseline (pre-race) metrics were explored using Pearson correlation analysis. All analyses were performed using SPSS v21, and significance set at <0.05. *N* values vary due to some athletes not wishing to participate in certain aspects of the study (e.g., blood testing).

Results

Height and weight

There was a significant decrease in both height (n = 66, pre-race height M =176.9cm, post-race M = 176.4cm, mean change = -0.508cm; 95% confidence interval -.846 – -.169) and weight (n = 66, pre-race M =76.9kg, post-race M = 75.1kg, mean change = -1.83kg; 95% confidence interval -2.30 – -1.37) during the race.

Testosterone

There was a significant decrease in salivary testosterone levels from before (n = 52, M =109.59 pg/ml) to after the race (n = 52, M = 97.61 pg/ml, mean change = -12.0 pg/ml; 95% confidence interval -14.9 – -9.03).

Impedance

There was a significant decrease in bioelectrical impedance from before (n = 66, M =496.80) to after the race (n = 66, M = 454.90, mean change = -41.9; 95% confidence interval -53.3 – -30.5).

There was a significant increase in 1/impedance (1/z) from before (n = 66, M = 0.00204) to after the race (n = 66, M = 0.00223, mean change = 0.000185; 95% confidence interval .000134 - .000235).

Response to visual stimuli

While there was a significant decrease in arousal scores from before (n = 52, M = 5.29) to after the race (n = 52, M = 5.04, mean change = -.248; 95% confidence interval -.389 – -.108), there was no change in either valence (n = 52, before M = 5.37, after M = 5.29, SD = 0.69, mean change = -.0722; 95% confidence interval -.168 - .0236) or dominance (n=52, before M = 5.19, after 5.16, SD = 0.67, mean change = -.0325; 95% confidence interval -.121 - .0556).

Further investigation considered how the three photo subtypes (provocative, positive valence, and negative valence) contributed to the overall decrease in arousal in response to the entire 45-photo slide show. This revealed a significant decrease in arousal scores in response to provocative near-nude images from before (n = 52, M = 5.40) to after the race (n = 52, M = 4.89, mean change = -.501; 95% confidence interval - .782 – -.220). In contrast, there was no significant difference in the arousal scores in response to positive valence images (n = 52, before M = 5.19, after M = 5.10, mean change = -.0833; 95% confidence interval -.230 – .0637) or in response to negative valence images (n = 52, before M = 5.27, after M = 5.11, mean change = -.160; 95% confidence interval -.365 – .0445). The difference in the total arousal score before and after the race can therefore be explained by a decrease in arousal in response to the near-nude provocative images.

Bacteria killing assay

There was a significant increase in bacterial killing ability from before (n = 34, M = 30.0 percent killing) to after the race (M = 41.0 percent killing, mean change = 11.0; 95% confidence interval .847 – 21.2)

Hemolytic complement assay

There was a significant increase in hemolytic complement from before (n = 34, 124.60 CH50) to after the race (n = 34, 139.40 CH50, mean change = 14.9; 95% confidence interval 2.41 – 27.3)

These results are summarized in Table 1. Figure 1 shows percent change in measures of reproductive effort and immune function.

INSERT TABLE 1 AND FIGURE 1 ABOUT HERE

Correlation analyses

Consideration of both baseline (pre-race) and post-race metrics revealed a positive correlation between testosterone and total arousal (pre-race n = 52, r = 0.33, p = 0.017; post-race n = 52, r = 0.35, p = 0.011). This result is unsurprising as testosterone has been implicated in sexual arousal.

The only other significant pre-race correlations came between the subgroups of imagery contributing to the total arousal score, although there was a significant positive correlation between post-race impedance and the provocative imagery sub-set of arousal scores (n = 52, r = 0.31, p = 0.025). We do not propose an explanation for this.

Discussion

It was hypothesized that the energy deficit associated with ultramarathon participation would impose an energetic allocation trade-off, causing an increased investment in short-term survival (as measured by innate immune function) relative to reproductive effort. This hypothesis is supported by the observation of a significant increase in bacteria killing assay and hemolytic complement assay metrics, and a decrease in testosterone and arousal scores.

Evidence from sports medicine and related fields generally support the findings of increased immune function following intense exercise. These findings include temporary increases in the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), granulocyte colony stimulating factor (GCSF), monocyte chemoattractant protein 1 (MCP-1), and other immunological factors (Nieman, 2012). Natural killer (NK) cell function and numbers increase following bouts of exercise as well (Shephard and Shek, 1994; Woods et al., 1999; McFarlin et al., 2008), and lysozyme, an important anti-bacterial component of innate mucosal immunity, shows a significant increase in male runners after two hours of running (Costa et al., 2012). In endurance athletes of both sexes, both leukocyte and lymphocyte counts are increased after an hour of intense exercise (McFarlin et al., 2008). While there are some reports of declining immune measures after exercise (e.g., sIgA), these resuts could be due to a number of different parameters, including sample collection, hydration, and even environmental factors (reviewed in Bishop and Gleeson, 2009).

On the whole, however, strenuous exercise is associated with changes in several inflammatory mediators (e.g., IL-6) which are released from injured and/or contracting muscle cells during intense and prolonged exercise (Petersen and Pedersen, 2005). This leads to local neutrophil and monocyte invasion and phagocytosis of debris from damaged myocytes (Evans and Cannon, 1991; Weight et al., 1991; Northoff et al., 1994). Changes in core body temperature have also been shown to affect leukocyte and neutrophil counts during bouts of exercise, although this effect is also mediated by environmental temperature (Mestre-Alfaro et al., 2012; Sureda et al., 2015). Given the systemic effects of many immune molecules, it may be that localized increases induce or contribute to a general

increase in immune function, as measured here. However, because these same molecules and cells may also be involved in minimizing or repairing the damage caused by intense physical exertion (Tidball, 2005), we maintain that this represents an additional investment in somatic maintenance, rather than a confounding factor. Furthermore, measuring multiple aspects of immune function (assays which do not measure inflammation, i.e., complement activity and bactericidal capabilities) may mitigate the effects of locally produced immune mediators on our results, as different immune responses are often driven by different mechanisms.

Although human males do not face the same energetic challenges as females (i.e., gametogenesis, gestation, and lactation), and energetic investment in spermatogenesis is minimal (Bagatell and Bremner, 1990; Elias, 1992), mating effort is still metabolically demanding. Energy costs include competition and mate attraction, as well as protection of and provisioning for mates and offspring (Muehlenbein and Bribiescas, 2005). While male mating effort may therefore be considered as largely behavioral, it does have important physiological correlates stemming from the need to signal underlying quality (Zahavi, 1975; Ellison, 2003).

The Immunocompetence Handicap Hypothesis (Folstad and Karter, 1992), which builds upon Zahavi's (1975) handicap hypothesis for the evolution of secondary sexual characteristics, suggests that testosterone mediates signals of reproductive status and quality in humans and other animals. In humans, muscle mass provides one such signal (Griggs et al., 1989; Kadi, 2008). Muscularity provides benefits in sexual selection from both inter- and intra-sexual perspectives (Lavrakas, 1975; Bribiescas, 2001; Dijkstra and Buunk, 2002; Frederick and Haselton, 2007; Gallup et al., 2007). However, skeletal muscle mass accounts for approximately 20% of human male basal metabolic rate (Elias, 1992). While this

figure is lower than the percentage of body mass accounted for by muscle (42% for a male, 36% for a female on average) (Marieb and Hoehn, 2010), suggesting that muscle may be a relatively cheap tissue at rest, it is the large size of some muscle groupings and high rates of energy consumption during activation that ensure muscles comprise a significant portion of the human energy budget (McArdle, Katch and Katch, 2001). Skeletal muscle is therefore an expensive tissue to maintain and thereby constrains the amount of energy available for competing physiological functions such as immune responses (Muehlenbein and Bribiescas, 2005). Thus, a highly muscular phenotype, which is dependent on the anabolic effects of testosterone (Bhasin et al., 1996; Tsai and Sapolsky, 1996), presents an energetic handicap.

Additionally, high levels of testosterone have a number of other detrimental health effects, including increased incidence of prostate cancer, oxygen radical production, reduced tissue and organ maintenance, and injury associated with aggressive confrontational behavior (Muehlenbein, 2006; Lassek and Gaulin, 2009). As such, testosterone is an effective mediator of quality signalling due to the significant metabolic costs of a muscular physique (Zahavi, 1975; Graffen, 1990; Andersson, 1994), immunomudulation (Muehlenbein and Bribiescas, 2005; Muehlenbein et al., 2006; Muehlenbein and Watts, 2010), and the negative health effects of high levels of the hormone.

The high energetic demands associated with muscle mass mean that during periods of energetic deficits there may be a suppression of testosterone levels. This leads to reduced somatic reproductive effort through decreasing muscle mass (Bribiescas, 2001) and frequency of behaviors associated with mating. This study has demonstrated a decrease in electrical impedance. Although this could be in part

due to reductions in hydration (Talluri *et al.*, 1999), it is suggested that a breakdown of muscle tissue also contributed to these measurments (Friedl *et al.*, 1994).

Our findings of both increased innate immunity and decreased testosterone levels in runners lends further support to the considerable literature on relationships between testerone and immune function (Bouman, 2005).

This study demonstrated an acute-level trade-off between reproduction and survivorship in ultramarathon runners. Because this is a highly trained and physically active group, these precise results may not be generalizable to the wider population. Such conditioning may buffer our participants from any negative health consequences of such strenuous exercise and nutritional imbalance. On the other hand, it could be argued that endurance running has cross-cultural ecological relevance due to its importance in activities such as persistence hunting, as observed in the Kalarhari in Africa and the Tarahumara tribe of Northern Mexico (Liebenberg, 2006; Pennington, 1963). Indeed, endurance running may have played a role in our evolution (Carrier, 1984; Lieberman et al. 2006, Longman, 2015). We therefore maintain that results in different populations should be broadly comparable, given the universality of general life history theory predictions. Although we have used two immune measures, we have measured only one arm of the immune system, which is not indicative of total immunocompetence. Future avenues of research should include adaptive immune measures and use similar approaches to consider trade-offs between other life history traits (i.e., growth). In addition to controlling for both core and environmental temperature, repeated post-race measures at different intervals could also help distinguish the precise contribution of muscle-produced immunological mediators on increased immune function.

Author contributions

DL, JTS and JCKW designed the study as a whole, with further input from IDS, and DL collected the data. MPM contributed financially to laboratory analyses, and oversaw the laboratory analyses performed by ECS and SPP. DL performed statistical analyses, and all authors edited the manuscript.

Literature Cited

Andersson M. (1994). Sexual selection. Princeton: Princeton University Press.

Angiletta MJ, Wilson RS, Navas CA, James RS. (2003). Tradeoffs and the evolution of thermal reaction norms. Trends Ecol Evol. 18(5): 234-240.

Ariely D, Loewenstein G. (2006). The heat of the moment: the effect of sexual arousal on sexual decision making. J Behav Decis Mak 19:87–98.

Bagatell C, Bremner W. (1990). Sperm counts and reproductive hormones in male marathoners and lean controls. Fertil Steril 53:688–92.

Bagatell C, Matsumoto, Christensen R, Rivier J, Bremner W. (1993). Comparison of a gonadotropin releasing-hormone antagonist plus testosterone (T) versus T alone as potential male contraceptive regimes. J Clin Endocrinol Metab 77:427–32.

Bancroft J. (1988). Sexual desire and the brain. Sex Marital Ther 3:11–27.

Baumeister RF, Catanese KR, Vohs KD. (2001). Is there a gender difference in strength of sex drive? Theoretical views, conceptual distinctions, and a review of relevant evidence. Pers Soc Psychol Rev 5:242–73.

Beach FA. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. Horm Behav 138:105–38.

Berg U, Enqvist JK, Mattsson CM, Carlsson-Skwirut C, Sundberg CJ, Ekblom B, Bang P. (2008). Lack of sex differences in the IGF-IGFBP response to ultra endurance exercise. Scand J Med Sci Sports 18:706–14.

Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunnell R, Tricker A, Sirazi A, Casaburi R. (1996). The effects of supraphysiological doses of testosterone on muscle size and strength in normal men. N Engl J Med 335:1-7.

Bishop NC, Gleeson M. (2009). Acute and chronic effects of exercise on markers of mucosal immunity. Front Biosci 14:4444–4456.

Bouman A. (2005). Sex hormones and the immune response in humans. Human Reprod Update 11:411–423.

Bradley M, Lang PJ. (1994). Measuring emotion: the self-assessment manikin and the semantic differential. J Behav Ther Exp Psychiatry 25:49–59.

Bribiescas R. (2001). Reproductive physiology of the human male: An evolutionary and life history perspective. In: Ellison PT, editor. Reproductive ecology and human evolution. New York:Aldine de Gruyter. p. 107-135.

Brock P, Murdock C, Martin L. (2014). The history of ecoimmunology and its integration with disease ecology. Integr Comp Biol 54:353-362.

Bronson FH. (1991). Mammalian reproductive biology. Chicago:University of Chicago Press.

Carrier DR. (1984). The energetic paradox of human and hominid evolution. Curr Anthropol 25(4):483-95.

Cody M. (1966). A general theory of clutch size. Evolution 20:174–84.

Costa RJS, Fortes MB, Richardson K, Bilzon JLJ, Walsh NP. (2012). The effects of postexercise feeding on saliva antimicrobial proteins. Int J Sport Nutr Exerc Metab 22:184–191.

Crabbe JB, Smith JC, Dishman RK. (2007). Emotional and electroencephalographic responses during affective picture viewing after exercise. Physiol Behav 90:394–404.

Dabbs J. (1990). Salivary testosterone measurements: reliability across hours, days, and weeks. Physiol Behav 48:83–6.

Darwin C. (1871). The descent of man, and selection in relation to sex. New York:Modern Library.

Davies CT, Thompson MW. (1986). Physiological responses to prolonged exercise in ultramarathon athletes. J Appl Physiol 61:611–7.

Demas G, Zysling D, Beechler B, Muehlenbein M, French S. (2011). Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. J Anim Ecol 80:710–30.

Dijkstra P, Buunk BP. (2002). Sex differences in the jealousy-evoking effect of rival characteristics. Eur J Soc Psychol 32:829–52.

Dixson A, Halliwell G, East R, Wignarajah P, Anderson M. (2003). Masculine somatotype and hirsuteness as determinants of sexual attractiveness to women. Arch Sex Behav 32:29–39.

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

Elias M. (1992). Organ and tissue contribution to metabolic rate. In: McKinney J, Tucker H, editors. Energy metabolism: tissue determinents and cellular corollaries. New York:Raven Press. p. 51–79.

Ellison P. (2003). Energetics and reproductive effort. Am J Hum Biol 15:342–51.

Evans WJ, Cannon JG. (1991). The metabolic effects of exercise-induced muscle damage. Exercise Sport Sci Rev 19:99-125.

Fan J, Dai W, Liu F, Wu J. (2005). Visual perception of male body attractiveness. Proc Biol Sci 272:219–26.

Folstad I, Karter A. (1992). Parasites, bright males, and the immunocompetence handicap. Am Nat 139:603–22.

Frederick D, Haselton M. (2007). Why is muscularity sexy? Tests of the fitness indicator hypothesis. Pers Soc Psychol Bull 33:1167–83.

Freund K, Langevin R, Zajac Y. (1974). A note on the erotic arousal value of moving and stationary human forms. Behav Res Ther 12:117–9.

Friedl K, Moore R, Martinez Lopez L, Vogel JA, Askew EW, Marchitelli LJ, Hoyt RW, Gordon CC. (1994) Lower limit of body-fat in healthy active men. J Appl Physiol 77(2):933-940.

Gallup AC, White DD, Gallup GG. (2007). Handgrip strength predicts sexual behavior, body morphology, and aggression in male college students. Evol Hum Behav 28:423–9.

Georgiev A, Muehlenbein M, Prall S, Emery Thompson M, Maestripieri D. (2015). Male quality, dominance rank, and mating success in free-ranging rhesus macaques. Behav Ecol 26:763-772.

Glazier DS. (2000). Trade-offs between reproductive and somatic (storage) investments in animals : a comparative test of the Van Noordwijk and De Jong model. Evol Ecol 13:539–55.

Graffen A. (1990). Biological signals as handicaps. J Theor Biol 144:517–46.

Granger DA, Shirtcliff EA, Booth A, Kivlighan KT, Schwartz EB. (2004). The “trouble” with salivary testosterone. Psychoneuroendocrinology 29:1229–40.

Graziottin A. (2000). Libido: the biologic scenario. Maturitas 34:9–16.

Griggs R, Kingston W, Jozefowicz R, Herr B, Forbes G, Halliday D. (1989). Effect of testosterone on muscle mass and muscle protein synthesis. J Appl Physiol 66:498–503.

Hau M. (2007). Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. Bioessays 29:133–44.

Heiman J. (1977). A psychophysiological exploration of sexual arousal patterns in males and females. Psychophysiology 14:266–74.

Hietanen JK, Nummenmaa L. (2011). The naked truth: the face and body sensitive N170 response is enhanced for nude bodies. PLoS One http://dx.plos.org/10.1371/journal.pone.0024408

Hirshfield M, Tinkle D. (1975). Natural selection and the evolution of reproductive effort. Proc Natl Acad Sci USA 72:2227–31.

Hodes R, Cooke E, Lang PJ. (1985). Individual differences in autonomic response: conditioned association or conditioned fear? Psychophysiology 22:545–60.

Johnson E, Bolonchuk W, Lykken I. (1985). Assessment impedance of fat-free mass using bioelectrical measurements of the human body. J Clin Nutr 41:810–7.

Julien E, Over R. (1984). Male sexual arousal with repeated exposure to erotic stimuli. Arch Sex Behav 13:211–22.

Kadi F. (2008). Cellular and molecular mechanisms responsible for the action of testosterone on human skeletal muscle. A basis for illegal performance enhancement. Br J Pharmacol 154:522–8.

Kaplan H, Gangestad S. (2005). Life History Theory and Evolutionary Psychology. In: Buss D, editor. The handbook of evolutionary psychology. New York: John Wiley. p. 68–95.

Knechtle B, Bircher S. (2005). Changes in body composition during an extreme endurance run. Praxis 94:371–7.

Knechtle B, Enggist A, Jehle T. (2005). Energy turnover at the Race Across AMerica (RAAM) - a case report. Int J Sports Med 26:499–503.

Knez WL, Coombes JS, Jenkins DG. (2006). Ultra-endurance exercise and oxidative damage: implications for cardiovascular health. Sport Med 36:429–41.

Lang PJ. (1980). Behavioral treatment and bio-behavioral assessment: computer applications. In Sidowski JB, Johnson JH, Williams TA, editors.Technology in mental health care delivery systems Norwood:Ablex Publishing. p. 119–37.

Lang PJ, Ohman A, Vaitl D. (1988). The international affective picture system [photographic slides]. Gainesville, Florida.

Lang P, Bradley M, Cuthbert B. (2008). International Affective Picture System (IAPS): affective ratings of pictures and instruction manual. Technical Report A-8. Gainesville:University of Florida.

Lassek WD, Gaulin SJ. (2009). Costs and benefits of fat-free muscle mass in men: relationship to mating success, dietary requirements, and native immunity. Evol Hum Behav 30:322–8.

Lavrakas P. (1975). Female preferences for male physiques. J Res Pers 9:324–34.

Leonard WR, Ulijaszek SJ. (2002). Energetics and evolution: an emerging research domain. Am J Hum Biol 550:547–50.

Leonard WR. (2012). Laboratory and field methods for measuring human energy expenditure. Am J Hum Biol 24:372–84.

Levin R. (1994). Human male sexuality: appetite and arousal, desire and drive. In: Legg C, Boott D, editors. Human appetite: neural and behavioural bases. New York: Oxford Unversity Press. p. 127–64.

Liebenberg L. (2006). Persistence hunting by modern hunter gatherers. Curr Anthropol 46(7):1017-26.

Lieberman DE, Bramble DM, Raichlen DA, Shea JJ. (2006). Brains, brawn, and the evolution of human endurance running capabilities. In: Grine, Fleagle, Richard, Leakey, editors. The First Humans – Origins and early evolution of the genus Homo. Springer.

Longman D, Wells JCK, Stock JT. Can persistence hunting signal male quality? A test considering digit ratio in endurance athletes. PLoS one 10(4), e0121560.

Lotka J. (1922). Contribution to the energetics of evolution. Proc Natl Acad Sci USA 8:147–51.

Lucas SJ, Anglem N, Roberts WS, Anson JG, Palmer CD, Walker RJ, Cook CJ, Cotter JD. (2008). Intensity and physiological strain of competitive ultra-endurance exercise in humans. J Sports Sci 26:477–89.

Malarkey WB, Hall JC, Rice RR, O'Toole ML, Douglas PS, Demers LM, Glaser R. (1993). The influence of age on endocrine responses to ultraendurance stress. J Gerontol 48:M134–9.

Marieb E, Hoehn, K. (2010). Human anatomy & Physiology. Benjamin Cummings, San Francisco.

Mayer M. (1948). Complement and protein fixation. In: Kabat E, Mayer M, editors. Experimental Immunochemistry. Springfield:Charles C. Thomas. p. 100–12.

McArdle W, Katch F, Katch, V. (2001). Exercise physiology: energy, nutrition and human performance. Lippincott, Williams & Williams. Philadelphia, PA.

McFarlin BK, Hutchison AT, Kueht ML. (2008). Knowledge of carbohydrate consumption does not alter natural killer cell activity following an acute bout of high-intensity aerobic exercise. Appl Physiol Nutr Metab 33:1007–1012.

Mestre-Alfaro A, Ferrer MD, Banquells M, Riera J, Drobnic F, Sureda A, Tur JA, Pons A (2012). Body temperature modulates the antioxidant and acute immune responses to exercise. Free Radical Res 46:799-808.

Millet S, Bennett J, Lee K, Hau M, Klasing K. (2007). Quantifying and comparing constitutive immunity across avian species. Dev Comp Immunol 31:188–201.

Morley JE. (2003). Testosterone and behaviour. Clin Geriatr Med 19:605–16.

Mosley W. (Ed) (2012). Nutrition and human reproduction. Springer Science & Business Media.

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

Muehlenbein MP, Bribiescas RG. (2005). Testosterone-mediated immune functions and male life histories. Am J Hum Biol 17:527–58.

Muehlenbein MP, Cogswell FB, James MA, Koterski J, Ludwig GV. (2006). Testosterone correlates with Venezuelan equine encephalitis virus infection in macaques. Virol J 3:19.

Muehlenbein MP, Prall SP, Chester E. (2011). Development of a noninvasive salivary measure of functional immunity in humans. Am J Hum Biol 23:267-268.

Muehlenbein MP, Watts DP. (2010). The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. BioPsychoSocial Med 4:21.

Nieman DC. (2012). Clinical implications of exercise immunology. J Sport Health Sci 1:12–17.

Northoff H, Weinstock C, Berg A. (1994). The cytokine response to strenuous exercise. Int J Sports Med 15:S167-S171.

Petersen AM, Pedersen BK. (2005). The anti-inflammatory effect of exercise. J Appl Physiol 98:1154-1162.

Pennington C. (1063). The Tarahumara of Mexico. Salt Lake City: University of Utah Press.

Pontzer H, Durazo-Arvizu R, Dugas LR, Plange-Rhule J, Bovet P, Forrester TE, Lambert EV, Cooper RS, Schoeller DA, Luke A. (2016). Curr Biol 26(3): 410-417.

Prall S, Muehlenbein M. (2014). Testosterone and immune function in primates: A brief summary with methodological considerations. Int J Primatol 35:805–24.

Prall S, Ambu L, Nathan S, Alsisto S, Ramirez D, Muehlenbein M. (2015). Androgens and innate immunity in rehabilitated semi-captive orangutans (Pongo pygmaeus morio) from Malaysian Borneo. Am J Primatol 77:642-650

Prall S, Muehlenbein M. (2015). Dehydroepiandrosterone and multiple measures of functional immunity in young adults. Am J Hum Biol 27:877-880.

Quiney V, Ketsetzis M, Earls C, Karamanoukian A. (1996). Viewing time as a measure of sexual interest. Ethol Sociobiol 17:341–54.

Reznick D, Nunney L, Tessier A. (2000). Big houses, big cars, superfleas and the costs of reproduction. Trends Ecol Evol 15(10): 421-425.

Roff D. (1992). Evolution of life histories: theory and analysis. New York: Chapman & Hall.

Rosen R. (1973). Suppression of penile tumescence by instrumental conditioning. Psychom Med 15:509–14.

Roubenoff R. (1996). Applications of bioelectrical impedance analysis for body composition to epidemiological studies. Am J Clin Nutr 64:459S – 62S.

Rubin H, Henson D. (1976). Effects of alcohol on male sexual responding. Psychopharmacology 15:366–76.

Shankar AA, Dandekar RC. (2012). Salivary biomarkers – an update. Dent Update 39:566–8.

Shephard RJ, Shek PN. (1994). Potential impact of physical activity and sport on the immune system – a brief review. Br J Sport Med 28:247-255.

Sherwin B. (1988). A comparative analysis of the role of androgen in human male and female sexual behavior: Behavioral specificity, critical thresholds, and sensitivity. Psychobiology 16:416–25.

Shirtcliff EA, Granger DA, Likos A. (2002). Gender differences in the validity of testosterone measured in saliva by immunoassay. Horm Behav 42:62–9.

Sinclair J, Lochmiller R. (2000). The winter immunoenhancement hypothesis: associations among immunity, density, and survival in prairie vole (*Microtus ochrogaster*) populations. Can J Zool 78:254–64.

Smith JC. (2012). Effects of emotional exposure on state anxiety after acute exercise. Med Sci Sports Exerc 45:372–8.

Stearns SC. (1992). The evolution of life histories. Oxford: Oxford Unversity Press.

Stuempfle KJ, Nindl BC, Kamimori GH. (2010). Stress hormone responses to an ultraendurance race in the cold. Wilderness Environ Med 21:22–7.

Sureda A, Mestre-Alfaro A, Banquells M, Riera J, Drobnic F, Camps J, Joven J, Tur JA, Pons A. (2015). Exercise in a hot environment influences plasma anti-inflammatory and antioxidant status in well-trained atheletes. J Therm Biol 47:91-98.

Talluri T, Lietdke R, Evangelisti A, Talluri J, Maggia G. (1999). Fat-free mass qualitative assessment with bioelectric impedance analysis (BIA). Ann Ny Acad Sci 873: 94-98.

Tidball JG. (2005). Inflammatory processes in muscle injury and repair. Am J Physiol Regul Integr Comp Physiol 288:R345-R353.

Tsai LW, Sapolsky RM. (1996). Rapid stimulatory effects of testosterone upon myotubule metabolism and sugar transport, as assessed by silicon microphysiometry. Aggress Behav 22:357-364.

Ulijaszek SJ. (1995). Human energetics in biological anthropology. Cambridge, UK: Cambridge University Press.

Van Noordwijk A, de Jong G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. Am Nat 128:137–42.

Wade GN. (1992). Metabolic fuels and reproduction in female mammals. Neurosci Biobehav Rev 16:235–72.

Wade GN, Schneider JE, Li HY. (1996). Control of fertility by metabolic cues. Am J Physiol 270:E1–19.

Weight LM, Alexander D, Jacobs P. (1991). Strenuous exercise: analogous to the acute-phase response? Clin Sci 81:677-683.

Wells JCK, Fewtrell MS. (2006). Measuring body composition. Arch Dis Child 91:612–7.

Wells JCK, Williams JE, Fewtrell M, Singhal A, Lucas A, Cole TJ. (2007). A simplified approach to analysing bio-electrical impedance data in epidemiological surveys. Int J Obes 31:507–14.

Woods JA, Davis JM, Smith JA, Nieman DC. (1999). Exercise and cellular innate immune function. Med Sci Sports Exerc 31:57-66.

Zahavi A. (1975). Mate selection - a selection for a handicap. J Theor Biol 53:205–14.

Zera AJ, Harshman LG. (2001). The physiology of life history trade-offs in animals. Annu Rev Ecol Evol Syst 32:95–126.

Appendix

Changes in general arousal that may be elicited by such prolonged exercise were controlled for, using the International Affective Picture System (IAPS) (Lang et al., 1988; Lang et al., 2005; Lang et al., 2008). The IAPS is a large collection of color photographs designed for distribution and research as standardized affective materials. The system includes normative ratings of each photograph with respect to valence (pleasure), arousal, and dominance.

A pilot study of 30 subjects (age- and sex-matched to race athletes) was conducted to collect SAM-rated valence and arousal data on 30 provocative near-nude female photographs. These photographs were obtained from a dating website, where visitors had given scores out of 10 to each image. The photographs chosen for the study had a mean score of 8.0 from a sample size of over 2000 website visitors. The pilot study enabled a determination of mean valence (6.45), arousal (5.11) and dominance (5.64). These thirty images were evenly split to make two groups, with equal mean valence, arousal, and dominance (see Table 2 below).

INSERT TABLE 2 ABOUT HERE

Thirty non-sexual positive IAPS images were then selected to match the pilot-derived arousal, valence, and dominance scores as closely as possible. These thirty images were split to make two groups of fifteen (Group A numbers: 1500, 1560, 2075, 2155, 2160, 8090, 5330, 5890, 8220, 7250, 7280, 7291, 7440, 7499, 8021; Group B numbers: 1540, 1590, 2152, 2158, 2224, 8208, 5628, 5990, 8467, 7279, 7289, 7402, 7450, 7660, 8060).

A further thirty non-sexual negative IAPS images were selected with matched arousal and dominance, but with a negative valence (3.39). Again, these thirty images were split to make two groups of fifteen (Group A numbers: 1220, 1274, 2055.1, 2120, 2683, 2692, 2710, 3181, 3212, 3300, 6021, 6200, 6561, 6836, 9101; Group B numbers: 1111, 1275, 2039, 2457, 2691, 2694, 2717, 3160, 3213, 3301, 6022, 6250.1, 6570, 6834, 9102). This design allowed the possibility of detecting any effects the exercise may have on the arousal valuations of both positive and negative pictures. Table 3 shows the average valence, arousal and dominance scores of each image subset.

Independent samples t-tests have confirmed that there were no significant differences between slide show A and slide show B in any of the three metrics (valence, arousal, or dominance). Furthermore, Levene's Test confirmed there were no significant differences in the variances of any variable.

INSERT TABLE 3 ABOUT HERE

	Pre-race	Post-race	Comparison of pre- to post-race	
	Mean (SD)	Mean (SD)	t	p
Height (cm) (*n* = 66)	176.90 (6.69)	176.40 (6.53)	3.00	0.004
Weight (kg) (*n* = 66)	76.90 (8.58)	75.10 (8.26)	7.91	< 0.001
Testosterone (pg/ml) (*n* = 52)	109.60 (17.60)	97.60 (17.50)	8.18	< 0.001
Impedance (*n* = 66)	496.80 (58.90)	454.90 (50.9)	7.32	< 0.001
Valence Total (*n* = 52)	5.36 (0.69)	5.29 (0.69)	1.51	0.136
Arousal Total (*n* = 52)	5.29 (0.86)	5.04 (0.99)	3.55	0.001
Dominance Total (*n* = 52)	5.19 (0.65)	5.16 (0.67)	0.74	0.463
Haemolytic complement assay (CH50) (*n* = 34)	124.6 (30.20)	139.40 (33.30)	2.43	0.021
Bacteria killing	29.98 (36.70)	41.00 (39.60)	2.20	0.035

assay (% killing) (*n* = 34)	

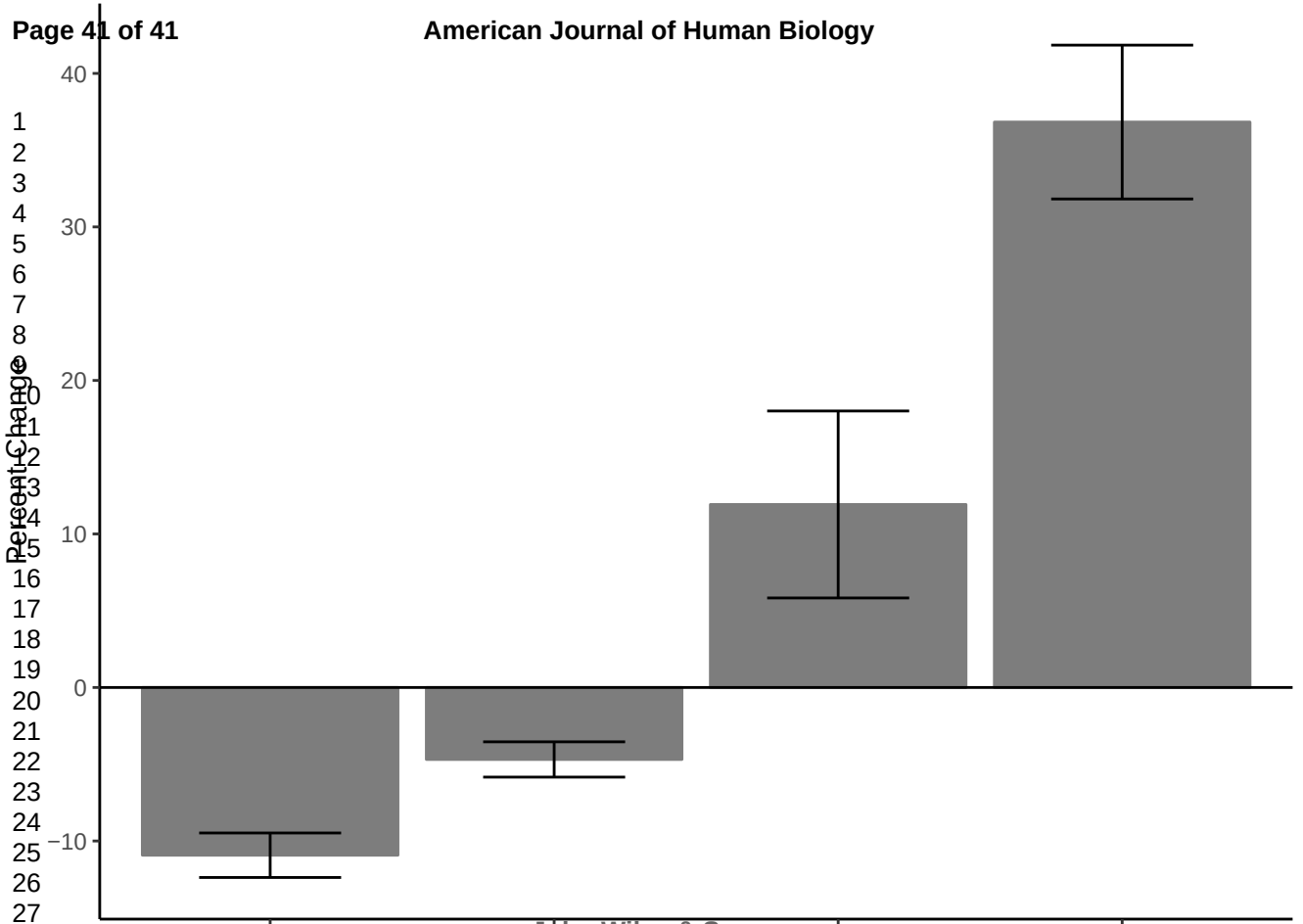
Table 1: Descriptive statistics for pre-race and post-race metrics, with independent t-test comparisons.

Table 2: Average valence, arousal and dominance scores of each image subset.

	Prov A	Prov B	t (p)
Valence	6.49 (0.516)	6.41 (0.739)	0.315 (0.185)
Arousal	5.19 (0.712)	5.02 (0.641)	0.692 (0.420)
Dominance	5.47 (1.070)	5.81 (0.840)	-0.947 (0.192)

Table 3: Mean (standard deviation) valence, arousal and dominance for each image subset, independent t-test (p-value) reported no significant difference between the image subsets. The valence, arousal and dominance scores for the 2 subsets of provocative images (abbreviated below) were obtained through a pilot study.

	Prov A	Prov B	t (p)	IAPS+ A	IAPS+ B	t (p)	IAPS- A	IAPS- B	t (p)
Valence	6.49	6.41	0.315	6.54	6.56	-0.332	3.45	3.33	0.590
	(0.516)	(0.739)	(0.185)	(0.223)	(0.238)	(0.742)	(0.431)	(0.607)	(0.560)
Arousal	5.19	5.02	0.692	5.15	5.07	0.503	5.10	5.20	-0.352
	(0.712)	(0.641)	(0.420)	(0.502)	(0.415)	(0.619)	(0.600)	(0.956)	(0.727)
Dominance	5.47	5.81	-0.947	5.65	6.01	-1.298	4.85	5.01	-0.675
	(1.07)	(0.840)	(.192)	(0.927)	(0.481)	(0.205)	(0.552)	(0.753)	(0.506)


Percent Change
40
30
20
10
0
−10
Testosterone
Arousal
Hemolytic Complement Assay
Bacterial Killing Assay