Eur J Appl Physiol (2012) 112:3765–3773
DOI 10.1007/s00421-012-2356-2

ORIGINAL ARTICLE

Reproductive hormones and interleukin-6 in serious leisure male athletes

Leah Z. FitzGerald · Wendie A. Robbins · James S. Kesner · Lin Xun

Received: 26 October 2011 / Accepted: 14 February 2012 / Published online: 1 March 2012

Abstract Lifestyles associated with different types and intensities of exercise result in improved health including positive changes in chronic low-grade inflammatory biomarkers. Alternatively, some forms of exercise adversely affect reproductive health of men, including changes in circulating reproductive hormones. To explore the associations between exercise intensity and circulating levels of reproductive hormones, and inflammatory analytes in serious leisure athletes (triathletes and cyclists) and recreational athletes. Male athletes 18–60 years old, 16 triathletes, 46 cyclists and 45 recreational athletes, were recruited to provide plasma for the measurement of total testosterone, estradiol, follicular stimulating hormone, luteinizing hormone (LH), sex hormone-binding globulin (SHBG), cortisol, interleukin-6 (IL-6), and interleukin-1β (IL-1β) levels, and calculation of free androgen index (FAI) and the estradiol: SHBG ratio (ESR). Plasma estradiol concentrations were more than two times higher in cyclists than in triathletes and recreational athletes ($p < 0.01$). Testosterone levels were also higher in cyclists than recreational athletes ($p < 0.01$), but not significantly different from triathletes. SHBG levels were higher in triathletes and cyclists than in recreational athletes ($p < 0.01$). LH levels were lower in cyclists than in recreational athletes ($p < 0.05$). IL-6 and IL-1β levels were each two times lower in triathletes than in cyclists ($p < 0.05$) and IL-6 levels were lower in cyclists than in recreational athletes ($p < 0.01$). IL-1β levels were two times lower in triathletes than in cyclists ($p < 0.05$). Circulating estradiol and testosterone levels were elevated in serious leisure male cyclists. This effect is discussed in light in the absence of a substantial concomitant change in gonadotropin levels and other variables.

Keywords Estradiol · Testosterone · Reproductive hormones · Men · Serious leisure athletes · Cytokines · Interleukin

Communicated by William J. Kraemer.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

L. Z. FitzGerald · W. A. Robbins · L. Xun
School of Nursing, University of California, Los Angeles, USA

L. Z. FitzGerald (✉)
UCLA School of Nursing, 700 Tiverton Avenue,
Factor Building 5-137, Los Angeles, CA 90095, USA
e-mail: lfitzger@sonnet.ucla.edu

W. A. Robbins
School of Public Health, University of California,
Los Angeles, USA

J. S. Kesner
Division of Applied Research and Technology,
Centers for Disease Control and Prevention, National Institute
for Occupational Safety and Health, Cincinnati, USA

Introduction

A considerable literature documents the effects of long-term exercise on health (Jankord and Jemiolo 2004). Moderate exercise is associated with enhanced cardiovascular and metabolic function and reduced body fat (Jankord and Jemiolo 2004). Lifestyles associated with high-intensity and high-volume exercise result in positive changes in chronic low-grade inflammatory markers (Lira et al. 2010). Ultra-endurance levels of exercise can manifest beneficial physiological adaptations in the cardiovascular and hema-

topoietic systems and body composition, but can also adversely affect the neuroendocrine system and reproductive health (Izquierdo et al. 2004; Maimoun et al. 2003).

Although most research studying the effects of exercise on reproductive health has focused on female athletes, a growing number of recent studies have looked at male endurance-trained athletes. For example, men exposed to chronic ultra-endurance-training for competition by running or cycling 10–20 h a week have been shown to present as hypogonadal with low basal-resting testosterone levels (Hackney et al. 2005). Literature describing male endocrine status across a range of exercise durations and intensities is sparse, but would be important to better characterize the point at which adverse effects on the neuroendocrine system and male reproductive health might occur.

In humans, studies show that strenuous exercise affects plasma cytokine levels for tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 beta, IL-10, and IL-8 in an intensity and gender dependent manner (Dressendorfer et al. 2002; Edwards et al. 2006; Timmons et al. 2006a). The best characterized cytokine related to exercise is interleukin-6 (IL-6). Physical activity is associated with circulating levels of IL-6, but the relationship appears complex. Chronic exercise is associated with reduced levels of IL-6 (Abramson and Vaccarino 2002), while acute exercise increases circulating levels of IL-6 produced primarily by muscle tissue and, to a much lesser extent, adipose tissue (Pedersen and Febbraio 2005; Sacheck et al. 2006). Elevated plasma levels of IL-6 are associated with body mass index (BMI), percent body fat and increased morbidity (Di Carlo et al. 2004). Both gender (Timmons et al. 2006b) and exercise intensity (Edwards et al. 2006) play roles in this association. Both estrogens and androgens affect the immune system in relationship to exercise, with exercise representing a physical stress challenging homeostasis (Chrousos 2010; Mastorakos and Pavlatou 2005).

The hypothalamic–pituitary–adrenal (HPA) axis, the key limb of the stress system, interacts with the reproductive system at multiple levels, and has potent actions on the immune and inflammatory reaction (Chrousos 2010). Corticotropin-releasing hormone (CRH) is the stimulatory force driving the HPA. Estrogen attenuates infiltration of inflammatory cells into skeletal muscle of rats after exercise or injury and stimulates muscle repair and regeneration (Enns and Tiidus 2010). In mammals estradiol augments the stress response by directly stimulating CRH neurons within the hypothalamic paraventricular nucleus (Chrousos 2010). In contrast, androgens may have mild suppressive effects on the HPA axis and the immune actions (Chrousos 2010). Cortisol demonstrates a complex relationship with exercise and it is an important aspect of the body's immune, inflammatory, and endocrine responses to exercise (Fragala et al. 2011).

To investigate the dynamics of indicators of male reproductive and immune health across a range of exercise, we studied three groups of healthy exercising men. Two groups were serious leisure athletes engaging in different types of exercise (triathletes and cyclists) whose level of exercise was intense, but less than that of ultra-endurance athletes. The third group consisted of recreational male athletes exercising regularly, but at a less vigorous level than the serious leisure athletes. The goal of this study was to assess the effects of different types and intensities of exercise in non-ultra-endurance athletes on circulating levels of reproductive hormones and inflammatory analytes. The specific aims were to: (1) determine if there are differences in reproductive hormone levels among serious leisure cyclists, serious leisure triathletes and recreational athletes, and (2) describe circulating inflammatory analyte levels among exercise groups.

Methods

Study population

Participants were 107 healthy men (age 18–60 years) who were part of a larger study examining the effect of exercise frequency and intensity on plasma IL-6 and bone health (FitzGerald and Carpenter 2007). They included serious leisure cyclists (cycled >8 h/week, $n = 46$), serious leisure triathletes (trained >5 h/week, $n = 16$) and recreational athletes (exercised $\leq$30 min moderate intensity most days; $n = 45$). To be eligible for the study, participants had to be non-smokers, free from co-morbid conditions that would impact inflammatory markers and any known cardiovascular, respiratory, endocrine, metabolic disorders, or conditions that might alter reproductive hormones. All participants reported that they had not taken medications on a regular basis or used steroid and/or hormone supplementation in the past six months. Participants gave written informed consent prior to the investigation. The research protocol was approved by the University of California, Los Angeles, Institutional Review Board for use of Human subjects according to the Helsinki declaration.

Procedure

Age, marital status, race/ethnicity and education level were self-reported (Ware et al. 1996). Alcohol and caffeine intake was ascertained during a medical history interview. Participants were asked the brand name, frequency and duration of use of chamois cream applied to their perineum area; chamois cream is often used by athletes to help prevent chafing and bacterial infections related to saddle sores during cycling.

Body composition

Height and body weight were measured to the nearest 0.25 cm and nearest 0.1 kg, respectively, using a floor model physician's scale/stadiometer. Body mass index (BMI) was calculated (kg/m^2). Blood pressure (BP) was measured after the patient was seated for 10 min using a 9000BPM Vital Signs Monitor ADC Adview Modular Diagnostic Station Base Unit (9000BPM) automatic, non-invasive BP monitor. The arm being used was relaxed, uncovered, and supported at the level of the heart (Pickering et al. 2005). Body composition was measured by dual energy X-ray absorptiometry (GE Lunar DXA/GE Lunar Body Composition Software) and bioelectrical impedance (Bioimpedance Analyzer BIA model 450, Biodynamics Corporation, Seattle WA, USA).

Physical activity assessment

Participants completed the International Physical Assessment Questionnaire (IPAQ) short form to obtain an objective estimate of time spent in different dimensions of physical activity and inactivity during the previous 7 days (http://www.ipaq.ki.se). The form was administered using an interview probe-type format (Zanovec et al. 2009). IPAQ estimates the time spent per week on physical activities of moderate and strong intensity, in different contexts of the daily life: work, transportation, housework, yard work, and leisure. It also estimates time spent in passive activities performed in the seated position. It consists of seven open questions and its information allows for an estimation of the time spent per week in different types and intensities of physical activity and inactivity. IPAQ has good measurement properties for monitoring population levels of physical activity among 18–65-year-old adults in diverse settings (Craig et al. 2003).

Physical activity was measured in metabolic equivalents (MET), the energy expended during rest. One MET equals 3.5 ml O_2/kg/min in adults. Each subject's total physical activity level was calculated and recorded in units of MET-minutes per week (MET-min/week) according to the IPAQ scoring protocol (http://www.ipaq.ki.se) based on each study subject's response. MET-min/wk was computed as: MET level × minutes of activity/day × day/week. These values were converted to MET-hours per week by dividing MET min/week by 60 min/h. Participants were categorized into "low," "moderate," or "vigorous" activity levels based on the standard IPAQ scoring cutoffs and guidelines (Craig et al. 2003). Normative data suggests that MET min/week of 3.3, 4.0 and 8.0 correspond to walking activities, moderate-intensity, and vigorous-intensity.

Biochemical analysis

Participants were fasting (except for water) for 8 h and free of vigorous exercise for a minimum of 24 h prior to sampling. They sat quietly for 10 min prior to blood draw. Single 20 ml blood samples were collected between 10 am and 2 pm. Blood samples to measure total testosterone, estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), sex hormone binding globulin (SHBG), cortisol, IL-6, and IL-1β were collected in tubes containing ethylenediaminetetraacetic acid. Within 1 h of collection, blood samples were centrifuged at 4°C at 3,000*g* for 20 min, and the plasma was harvested, and stored in polypropylene tubes at −80°C until analyses.

Hormone assays

Frozen plasma samples were allowed to thaw at room temperature. Total testosterone, estradiol, FSH, LH and SHBG were determined using chemiluminescent immunometric assays performed by an Immulite 1000® automatic analyzer (Vankrieken 2000). Hormone controls and standards (Siemens Medical Solutions Diagnostics, USA) and plasma pools generated in our laboratory were analyzed for quality control. The intra-assay coefficients of variation were 6.8–13.0% for total testosterone, 6.3–15% for estradiol, 2.3–3.7% for FSH, 4.8–6.5% for LH, and 4.1–7.7% for SHBG. The inter-assay coefficients of variation were 7.7–16.4% for total testosterone, 6.4–16% for estradiol, 5.4–6.7% for FSH, 7.2–26% for LH, and 5.8–13% for SHBG. Duplicate samples with >10% coefficient of variation were re-analyzed. The sensitivity of the assays reported by the manufacturer were 0.52 nmol/L for testosterone, 55 pmol/L for estradiol, 0.1 mIU/mL for FSH, 0.1 mIU/L for LH, and 0.2 nmol/L for SHBG. The estradiol/SHBG ratio (ESR) was calculated as estradiol levels (pmol/L) divided by SHBG levels (nmol/L) (Knochenhauer et al. 1998). Free androgen index (FAI) was calculated as 100× total testosterone levels (nmol/L) divided by SHBG levels (nmol/L) (Vankrieken 2000). The derived values were calculated to evaluate differential binding of estradiol and testosterone to SHBG, respectively. Serum cortisol was analyzed by immunometric assay (Nichols Advantage, San Juan Capistrano, CA, USA) using an established procedure. Validity was determined using inter- and intra-assay with three known control concentrations. The inter-assay coefficients of variation were 5–9% for cortisol. The sensitivity of the assay reported by the manufacturer was 0.5 ug/dL.

Inflammatory analytes

Levels of IL-6 and IL-1β were determined using high-sensitivity enzyme-linked immunosorbent assays (Quantikine HS;

R & D Systems, Minneapolis, MN) with mean minimum detectable dose ranges of 0.011–0.016 pg/ml for IL-6 and 0.023–0.14 pg/ml for IL-1β. IL-1β levels were not measured in recreational athletes. Intra- and interassay coefficients of variations for these assays were less than 10%.

Statistical analysis

All data are expressed as means ± SD for continuous variables and as percentages for categorical/binary variables. Exercise groups were compared with regard to blood pressure, demographic, and lifestyle factors using ANOVA for normally distributed continuous variables. If the distribution was non-normal, variables were log transformed to approximate a normal distribution. Chi-square was used to test for group differences in categorical variables. ANCOVA was used to assess hormone levels and circulating levels of IL-6 among serious leisure cyclists, serious leisure triathletes and recreational athletes with covariates. Student *t* test was used to compare IL-1β between serious leisure cyclists and triathletes. Correlation analysis was used to assess relationships between hormones and inflammatory markers.

To assess potential confounding, the following covariates were considered in the modeling of hormone level and exercise group: age, BMI, percent body fat, blood pressure, alcohol intake, paraben use, caffeine were modeled as continuous variables; education and race were modeled as categorical variables, four categories each. Total alcohol intake was calculated in grams by adding the intake from each alcoholic-beverage unit: 13.2 g for beer; 10.8 g for wine; and 15.1 g for liquor. Caffeine intake was calculated in mg/day on the basis of weekly consumption of coffee, tea, cola, caffeinated energy drinks, and chocolate (Hernandez-Avila et al. 1991) and reported as the equivalent of 8 oz unit/day and converted to mg/day. Use of paraben-containing creams was calculated as a binary yes/no variable and as years of use. Pearson correlation coefficients were computed for continuous variables. For all test statistics, significance was defined as $p \leq 0.05$.

Results

Participant characteristics

The study participants were 107 healthy men, 18–60 years of age, including serious leisure athletes (16 triathletes, 46 cyclists) and 45 recreational athletes. IPAQ ratings of activity were significantly different between the three groups ($p < 0.01$). All cyclists and triathletes rated their activity as "high", while 91% of the recreational athletes rated their activity as "low" or "moderate" (Table 1). Differences among the groups were seen in socio-demographic variables and body composition (Table 1). Cyclists were older than triathletes and recreational athletes ($p < 0.01$), and consumed more caffeine than recreational athletes ($p < 0.05$). Recreational athletes were more racially diverse ($p < 0.01$) and less educated ($p < 0.05$) than the intensely exercising groups. Triathletes were leaner than recreational athletes based on BMI ($p < 0.01$) and percent body fat ($p < 0.05$). Recreational athletes had more lean body mass than either cyclists or triathletes ($p < 0.01$). Intensely exercising athletes had higher systolic and diastolic blood pressure ($p < 0.01$) compared to recreational athletes. Two triathletes (12.5%) and 11 cyclists (23.9%) reported periodic use of anti-inflammatory medication; these rates were not different from each other, but were greater than for recreational athletes who reported no use of anti-inflammatory medications within the past 6 months ($p < 0.01$). Paraben-containing chamois cream was used by more cyclists than triathletes ($p = 0.02$) and not by any recreational athletes. Alcohol use was similar among the three groups.

Reproductive hormones

Plasma estradiol concentrations were more than two times higher ($p < 0.01$) in cyclists than in triathlete and recreational athletes (Table 2). Total testosterone levels were about 50% higher in cyclists than in recreational athletes ($p < 0.01$); triathlete levels were intermediate and not significantly different from either group. LH levels were lower in cyclists than in recreational athletes ($p < 0.05$), while SHBG was significantly higher in both groups of serious leisure athletes ($p < 0.01$) compared to recreational athletes. There were no significant differences among groups for FSH levels, the estradiol:SHBG ratio, the free androgen index and cortisol (Table 2).

Inflammatory markers: IL-6 and IL-1β

Plasma IL-6 levels were four times lower in triathletes and two times lower in cyclists than in recreational athletes ($p < 0.01$), even after controlling for percent body fat ($p < 0.01$), and lower in triathletes than in cyclists ($p < 0.01$) (Table 2). IL-1β levels were two times lower in triathletes than in cyclists ($p < 0.05$). Plasma IL-1β was not measured in recreational athletes. No significant correlations existed between the endocrine measurements and the inflammatory markers (data not shown).

Discussion

The differences found in IPAQ activity ratings among the three groups were expected. However, an unexpected finding was the markedly elevated estradiol levels in the

Table 1 Demographics, physiological parameters and physical activity

	Cyclists ($n = 46$)	Triathletes ($n = 16$)	Recreational athletes ($n = 45$)	p value
Mean (SD)				
Age (years)	40.7 (11.9)^{A}	31.6 (10.8)^{B}	32.6 (10.1)^{B}	<0.01
BMI (kg/m^2)	24.6 (2.6)	23.2 (1.6)^{A}	26.1 (4.8)^{B}	<0.01
Body fat (%)	17.8 (7.4)	14.1 (4.9)^{A}	19.3 (7.2)^{B}	<0.05
Lean body mass (km)	60.40 (5.63)^{A}	59.75 (5.65)^{A}	64.46 (9.19)^{B}	<0.01
Systolic BP (mmHg)	125.5 (9.5)^{A}	128.8 (7.4)^{A}	118.5 (13.5)^{B}	<0.01
Diastolic BP (mmHg)	77.1 (6.4)^{A}	77.1 (5.3)^{A}	68.8 (9.1)^{B}	<0.01
Alcohol (g/week)	32.4 (39.7)	33.2 (31.4)	27.1 (54.9)	NS
Caffeine (mg/day)	317.3 (276)^{A}	227.8 (238)	182.8 (221)^{B}	<0.05
N (%)				
Paraben chamois	22 (47.8)	1 (6.5)	0 (0)	<0.05
Cream use				
Race/ethnicity				<0.01
Black	2 (4.3)	0 (0)	12 (26.7)	
White	33 (71.7)	10 (62.5)	19 (42.2)	
Asian Pacific Islander	7 (15.2)	4 (25)	5 (11.1)	
Hispanic	4 (8.7)	1 (6.3)	9 (20.0)	
Unknown	0 (0)	1 (6.3)	0 (0)	
Highest education				<0.05
High school	0 (0)	0 (0)	8 (17.8)	
Any college	8 (17.4)	5 (31.3)	11 (24.4)	
College graduate	21 (45.7)	5 (31.3)	18 (40.0)	
Any post-graduate	17 (37.0)	6 (37.5)	8 (17.8)	
IPAQ activity grouping				<0.01
Low	0 (0)	0 (0)	17 (37.8)	
Moderate	0 (0)	0 (0)	24 (53.3)	
High	46 (100)	16 (100)	4 (8.9)	

Superscript letters denote significant group differences using, Bonferroni's Post Hoc comparisons, $p < 0.05$

Table 2 Plasma endocrine and inflammatory analyte measurements of male serious leisure and recreational athletes

Variable	Cyclists		Triathletes		Recreational athletes		p value
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	
Estradiol (pmol/L)	44	253.9 (175.9) ^{A}	16	89.4 (29.3)^{B}	43	109 (49.7)^{B}	<0.01
Testosterone (nmol/L)	43	33.0 (12.0)^{A}	16	28.7 (8.5)	38	22.3 (9.6)^{B}	<0.01
SHBG (nmol/L)	45	32.6 (12.1)^{A}	16	32.2 (11.3)^{A}	43	22.9 (12.1)^{B}	<0.01
LH (mIU/mL)	45	2.3 (1.3)^{A}	16	2.9 (1.3)	40	4.0 (1.9)^{B}	<0.05
FSH (mIU/mL)	45	3.4 (1.9)	16	2.9 (1.3)	41	3.8 (1.3)	NS
Estradiol SHBG ratio	44	8.2 (6.8)	16	3.3 (2.1)	43	8.3 (14.9)	NS
Free androgen index	46	94.1 (73)	16	161 (439)	43	218 (485)	NS
Interleukin-6 (pg/ml)	45	0.64 (0.46)^{A}	16	0.31 (0.24)^{B}	36	1.2 (1.1)^{C}	<0.01
Interleukin-1β (pg/ml)	45	0.38 (0.4)	16	0.16 (0.13)	0	–	<0.05
Cortisol (μg/dL)	45	309 (127)	16	292 (97)	42	376 (179)	NS

ANOVA was used to compare the three athlete groups, with superscript letters denoting significant group differences by Bonferroni's Post hoc comparisons, $p < 0.05$. For Interleukin-1β, Student t test was used to compare triathlete and cyclist groups

serious leisure cyclists, to our knowledge, the first demonstration of elevated estradiol levels in any type of male athlete. The mean estradiol level in cyclists (mean age 40 years) exceeded that typically reported for this aged population of healthy men (50–200 pmol/L) and was two times higher than the triathletes and recreational athletes

(Khosla et al. 2002). Testosterone level was also elevated in cyclists compared to recreational athletes, but not statistically higher than in triathletes and fell within the range expected (~10–33 nmol/L) (Bjerner et al. 2009) for healthy men of the age in this study. The testosterone levels in our recreational athletes were consistent with a report of no effect on plasma testosterone in sedentary young men following a 12 week moderate-intensity and low frequency training (Hiruntrakul et al. 2010). While SHBG levels were higher in the endurance athletes than in the recreational athletes, the difference was modest and the values were similar for triathletes and cyclists, even as sex hormone levels were higher in the cyclists. Furthermore, the estradiol:SHBG and free androgen index did not differ significantly among any of the groups.

It seems unlikely that demographic or age differences among the study groups would account for the elevated sex hormones in the cyclists. Estradiol and testosterone generally decline with age in males. Yet cyclists, who had the highest average age in our study, had the highest levels of estradiol and testosterone. Further, differences between the groups in estradiol, total testosterone, and LH remained after controlling for age. The recreational athletes consisted of a larger proportion of black and Hispanic men and a smaller proportion of white men than in either of the intense exercise groups. While racial differences for circulating levels of estradiol and testosterone have been reported in men, it does not appear that the directions of those differences could account for the endocrine effects seen in the current study (Darbre et al. 2004; Dolomie-Fagour et al. 2008; Rohrmann et al. 2007; Stephen et al. 2001).

Lower IL-6 levels in both amateur endurance groups than in recreational athletes are consistent with previous findings of intense physical activity (Sousa e Silva et al. 2010). That IL-6 levels were significantly different between the two serious endurance groups conforms to the notion that both intensity and duration of exercise may affect changes in a range of inflammatory markers (Pedersen and Hoffman-Goetz 2000).

Neuroendocrine response to exercise stress involves activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcriptional regulatory protein, leading to an increased transcription of proinflammatory genes responsible for the expression of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α), IL-1β and IL-6, activating cortisol, and in turn inhibiting NF-κB activation through INF-κB, to eventually shut down this cycle (Mastorakos et al. 2005). Moderate exercise has been associated with lower baseline cortisol levels whereas mild hypercortisolism has been observed in highly trained athletes suggesting an adaptive change to chronic exercise (Wittert et al. 2012).

Circulating estrogens in the male are derived from testicular secretions and peripheral conversion of androgens, mainly in fat tissue (Vanderschueren et al. 2004). Inflammatory cytokines, including IL-6, enhance conversion of androgens to estrogens by stimulating aromatase activity and conversion of estrone to estradiol by stimulating reductive 17β-hydroxyseriod dehydrogenase activity (Park et al. 2005). So, while up-regulation of these enzyme pathways by increased inflammatory cytokine production could partially explain why cyclists have higher estradiol levels than triathletes, it does not explain why their levels are higher than the recreational athletes with the highest IL-6 levels across the exercising groups.

Research suggests that NF-κB may be the mediator of the repression of cytokine expression by testosterone (Li and Verma 2002). Others have shown that androgen receptor activation suppresses NF-κB activity (Libby 2002). Animal studies report short-term exposure to estradiol in vitro decreased the production of proinflammatory cytokines in lipopolysaccharide (LPS)-activated macrophages (Ghisletti et al. 2005). In contrast, others reported that long-term exposure to estradiol in vivo enhanced proinflammatory cytokine production from LPS-activated macrophages (Calippe et al. 2008; Soucy et al. 2005). It is possible that up-regulation of these pathways in relation to long-term exposure to elevated estradiol could partially explain why cyclists have higher IL-1β levels than triathletes; however no causal inference can be made.

Lifestyle, environmental exposure and altered set point of hypothalamus-pituitary gonadal (HPG) axis

It is puzzling that estradiol and testosterone levels were elevated in cyclists without a reduction in FSH levels relative to the other groups, and without a significant change in LH or SHBG relative to triathletes, and only a modest reduction of LH and increase of SHBG compared to the recreational athletes. To explore this finding, we assessed lifestyle and environmental exposures, and altered set point of HPG centers as possible explanations that might interfere with the HPG axis. Some cyclists apply chamois cream to their perineum area to help prevent chafing and bacterial infections related to bicycle saddle sores. The various commercial creams contain a variety of ingredients including lubricants, polymers, oils (jojoba, lanolin, mineral, olive, peppermint, rosewood, soybean, tea tree, St John's wort), vitamins (A, C, D, E), and alcohols. Additionally, some of these creams contain parabens which are anti-microbial preservatives, but also weak estrogen agonists (Frederiksen et al. 2011). In vitro studies demonstrate that parabens bind to estrogen receptors and initiate estrogenic cellular pathways (Darbre et al. 2004). Among the amateur endurance study athletes, of all participants responding to a follow up

questionnaire, 48.5% of cyclists compared to 10% of triathletes reported using paraben-containing chamois cream ($p = 0.03$; Table 1). Among the cyclists, there was a significant dose-dependent increase in estradiol levels with increasing years of chamois cream use for men using the cream for more than 4 years ($p = 0.03$) with notable effect size (partial $\eta^2 = 0.12$).

While it might be tempting to suggest that elevated steroid hormone levels are due to use of performance-enhancing drugs, self-medicating with estrogens are not the sex hormones of choice for this purpose (Brown et al. 2000). Although not on the World Anti-Doping Agency's list of banned substances, caffeine is the most commonly used drug by athletes, consumed for its ergogenic benefits and potential to enhance performance through endurance in cycling and running (Ballard et al. 2010). Surveys reveal that 24–56% of adolescent and middle aged persons use energy drinks, most of which contain caffeine, to enhance athletic performance and weight loss (Ballard et al. 2010). Chronic cola intake has been shown to increase estradiol and testosterone levels in animal studies (Celec and Behuliak 2010). Caffeine increases testosterone levels in animals and humans in response to acute exercise (Kraemer et al. 1990, 2006). Habitually low to moderate caffeine consumption is described as 300 mg per day and high dose is reported as 500 mg per day (Heckman et al. 2011). In the present study, 23.9% of cyclists reported habitually consuming 540–1080 mg caffeine per day which was significantly higher than the 4.8% of recreational athletes who reported habitually consuming 540–1080 mg of caffeine per day, ($p < 0.05$) (Table 1). Significant correlations between caffeine dose and specific hormone levels were not detected in this small data set, but suggest an area for further study.

Finally, we considered the possibility that the set point for HPG centers may have been altered by this serious exercise. The shift in basal body temperature at ovulation is a clear example of the endocrine milieu altering hypothalamic set points. There are examples of exercise also affecting hypothalamic set points, in some cases, with apparent involvement with the endocrine axis. In the human, the hypothalamus controls sympathetic nervous system-mediated thermogenesis with the supra-chiasmatic nucleus (SCN) driving homeothermic temperature re-setting and compensation (Buhr et al. 2010). Trained athletes have lower pituitary sensitivity to glucocorticoids than untrained athletes (Wright et al. 2010), have lower basal core body temperatures, and are more tolerant of high temperatures at exhaustion (McLellan 2001). These traits in amateur endurance trained athletes could represent compensable responses by the hypothalamus to chronic exercise-induced heat stress. Premarin and follicular phase of the estrous cycle can protect rats and mice from heat stress (Chen et al. 2006; Lin et al. 2010; Shen et al. 2008; Uchida et al. 2010; Wright et al. 2010). Additionally, women athletes are more resistant to heat stress during the follicular phase compared to the luteal phase of the menstrual cycle (Cheung et al. 2000). Perhaps, the elevated plasma estradiol levels in the amateur endurance cyclists in the present study reflect a compensatory shift in set point for the HPG axis. Lack of this shift in triathletes may reflect time spent swimming in water (cooling) compared to cyclists who remain in a thermal state compounded by restrictive cycling clothing worn for long periods of time.

Our results of elevated serum testosterone and estradiol levels in cyclists may seem at odds with those who have shown that ultra-endurance athletes are hypogonadal with low circulating levels of testosterone (Hackney et al. 2005; Hackney 2008). However, the training intensity of the men in our study was less than that for the ultra-endurance athletes in those referenced above, yet more intense than for the recreational athletes in our study. We are unable to explain these findings other than to suppose that the mechanism(s) for these endocrine effects might follow a nonlinear dose–response; where high levels of exercise intensity stimulate sex hormone secretion, but then at extremely high 'doses,' the same or different mechanism(s) inhibit secretion of these hormones, perhaps by throttling down the hypothalamic drive. Clearly, more research is needed to confirm and clarify these relationships.

Another unexpected finding in the present study was that systolic blood pressure (SBP) and diastolic blood pressure (DBP) were higher in the cyclists and triathletes than the recreational athletes. SBP significantly correlated with daily caffeine consumption in cyclists ($r = 0.24$, $p = 0.05$), but not in triathletes or recreational athletes. Large epidemiological studies have demonstrated that regular training decreases the BP in normotensive persons and prevents the development of hypertensive disease (Varga-Pinter et al. 2011). A study examining the influence of sport on BP in young men and women reported lower BP readings in dynamic activities (endurance sports, ball games, speed) compared to ball games, however, they do not report elevated BP readings at rest in cyclists (Varga-Pinter et al. 2011).

Strengths of the present study include use of validated measures of physical activity assessment using calculated metabolic equivalents and the use of both medical exam format and self-report to gather information on lifestyle exposure such as caffeine intake and use of chamois creams. In addition, looking at IL-6 in relation to reproductive hormone outcomes in serious leisure male athletes and exercise intensity adds to the literature. A limitation of the research could be the single endocrine measurements. However, single measurements of testosterone provide a good estimate of long-term levels (Vermeulen et al. 1999). A potential problem with a retrospective study design is

that participants have subjected themselves to the rigors of training for years and we are observing the end result. This can potentially add to observed variance in the hormonal responses of these subjects as a result of variations in inter-individual response to exercise training. Further research using a longitudinal study design could address issues of relationships and change over time. The current findings are focused on differences between exercise groups with relatively simple comparative statistics. Further analyses with these data could address more complex issues relating to multivariate differences between groups, the identification of constellations of factors that distinguish these exercise groups, and interrelationships among predictors.

Conclusion

Plasma estradiol and testosterone levels were significantly elevated in serious leisure male cyclists, a finding not previously reported for male athletes. These effects were accompanied by little or no compensatory shift in circulating gonadotropin levels. Although preliminary, these findings warrant further investigation and replication, but suggest that despite well-known positive effects associated with exercise, specific types of exercise may be associated with altered sex hormone levels in men that could affect general health and reproductive wellbeing.

Acknowledgments We would like to acknowledge the research participants, the General Clinical Research Center (GCRC) nursing staff at UCLA for their support and Mauricio Pena for his support in preparing the data for this manuscript.

Conflict of interest The authors are not aware of any conflict of interest.

References

Abramson JL, Vaccarino V (2002) Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults. Arch Intern Med 162(11):1286–1292

Ballard SL, Wellborn-Lim JJ, Clauson K (2010) Effects of commercial energy drink consumption on athletic performance and body composition. Phys Sportsmed 38(1):107–117

Bjerner J, Biernat D, Fosså SD, Bjøro T (2009) Reference intervals for serum testosterone, SHBG, LH and FSH in males from the NORIP project. Scand J Clin Lab Investig 69(8):873–879

Brown GA, Vukovich MD, Reifenrath TA, Uhl NL, Parsons KA, Sharp RL, King DS (2000) Effects of anabolic precursors on serum testosterone concentrations and adaptations to resistance training in young men. Int J Sport Nutr Exerc Metab 10(3):340–359

Buhr ED, Yoo SH, Takahashi JS (2010) Temperature as a universal resetting cue for mammalian circadian oscillators. Science 330(6002):379–385

Calippe B, Douin-Echinard V, Laffargue M, Laurell H, Rana-Poussine V, Pipy B, Guery JC, Bayard F, Arnal JF, Gourdy P (2008) Chronic estradiol administration in vivo promotes the proinflammatory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway. J Immunol 180(12): 7980–7988

Celec P, Behuliak M (2010) Behavioural and endocrine effects of chronic cola intake. J Psychopharmacol 24(10):1569–1572

Chen SH, Chang FM, Niu KC, Lin MY, Lin MT (2006) Resuscitation from experimental heatstroke by estrogen therapy. Crit Care Med 34(4):1113–1118

Cheung SS, McLellan TM, Tenaglia S (2000) The thermophysiology of uncompensable heat stress: physiological manipulations and individual characteristics. Sports Med 29(5):329–359

Chrousos GP (2010) Stress and sex versus immunity and inflammation. Sci Signal 3(143):e36

Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P (2003) International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 35(8):1381–1395

Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ, Pope GS (2004) Concentrations of parabens in human breast tumours. J Appl Toxicol 24(1):5–13

Di Carlo C, Tommaselli GA, Sammartino A, Bifulco G, Nasti A, Nappi C (2004) Serum leptin levels and body composition in postmenopausal women: effects of hormone therapy. Menopause 11:466–473

Dolomie-Fagour L, Gatta B, Nguyen TDT, Corcuff JBt (2008) Bioavailable estradiol in man: relationship with age and testosterone. Clin Chim Acta 398(1–2):145–147

Dressendorfer RH, Petersen SR, Moss Lovshin SE, Hannon JL, Lee SF, Bell GJ (2002) Performance enhancement with maintenance of resting immune status after intensified cycle training. Clin J Sport Med 12(5):301–307

Edwards KM, Burns VE, Ring C, Carroll D (2006) Individual differences in the interleukin-6 response to maximal and submaximal exercise tasks. J Sports Sci 24(8):855–862

Enns DL, Tiidus PM (2010) The influence of estrogen on skeletal muscle: sex matters. Sports Med 40(1):41–58

FitzGerald L, Carpenter C (2007) Bone mineral density results influencing health-related behaviors in male athletes at risk for osteoporosis. J Clin Densitometry 13(3):256–262

Fragala MS, Kraemer WJ, Denegar CR, Maresh CM, Mastro AM, Volek JS (2011) Neuroendocrine–immune interactions and responses to exercise. Sports Med 41(8):621–639

Frederiksen H, Jorgensen N, Andersson AM (2011) Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). J Expo Sci Environ Epidemiol 21(3):262–271

Ghisletti S, Meda C, Maggi A, Vegeto E (2005) 17+-Estradiol inhibits inflammatory gene expression by controlling NFκB intracellular localization. Mol Cell Biol 25(8):2957–2968

Hackney AC (2008) The effects of endurance exercise on the reproductive system of men: the "exercise-hypogonadal" male condition. J Endocrinol Invest 31:932–938

Hackney AC, Moore AW, Brownlee KK (2005) Testosterone and endurance exercise: development of the exercise-hypogonadal male condition. Acta Physiol Hung 92(2):121–137

Heckman MA, Weil J, Gonzalez De Mejia E (2011) Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. J Food Sci 75(3):R77–R89

Hernandez-Avila M, Colditz GA, Stampfer MJ, Rosner B, Speizer FE, Willett WC (1991) Caffeine, moderate alcohol intake, and risk of fractures of the hip and forearm in middle-aged women. Am J Clin Nutr 54:157–163

Hiruntrakul A, Nanagara R, Emasithi A, Borer KT (2010) Effect of endurance exercise on resting testosterone levels in sedentary subjects. Cent Eur J Public Health 18(3):169–172

Izquierdo M, Ibáñez J, Häkkinen K, Kraemer WJ, Ruesta M, Gorostiaga EM (2004) Maximal strength and power, muscle mass, endurance and serum hormones in weightlifters and road cyclists. J Sports Sci 22(5):465–478

Jankord R, Jemiolo B (2004) Influence of physical activity on serum IL-6 and IL-10 levels in healthy older men. Med Sci Sports Exerc 36(6):960–964

Khosla S, Melton LJ, Riggs BL (2002) Estrogen and the male skeleton. J Clin Endocrinol Metab 87(4):1443–1450

Knochenhauer ES, Boots LR, Potter HD, Azziz R (1998) Differential binding of estradiol and testosterone to SHBG. Relation to circulating estradiol levels. J Reprod Med 43(8):665–670

Kraemer WJ, Marchitelli L, Gordon SE, Harman E, Dziados JE, Mello R, Frykman P, McCurry D, Fleck SJ (1990) Hormonal and growth factor responses to heavy resistance exercise protocols. J Appl Physiol 69(4):1442–1450

Kraemer R, Hollander D, Reeves G, Francois M, Ramadan Z, Meeker B, Tryniecki J, Hebert E, Castracane V (2006) Similar hormonal responses to concentric and eccentric muscle actions using relative loading. Eur J Appl Physiol 96(5):551–557

Li Q, Verma IM (2002) NF-[kappa]B regulation in the immune system. Nat Rev Immunol 2(10):725–734

Libby P (2002) Inflammation in atherosclerosis. Nature 420(6917): 868–874

Lin CY, Lin MT, Cheng RT, Chen SH (2010) Testosterone depletion by castration may protect mice from heat-induced multiple organ damage and lethality. J Biomed Biotechnol 2010:485306

Lira F, Rosa J, Pimentel G, Souza H, Caperuto E, Carnevali L, Seelaender M, Damaso A, Oyama L, de Mello M, Santos R (2010) Endotoxin levels correlate positively with a sedentary lifestyle and negatively with highly trained subjects. Lipids Health Dis 9(1):82

Maimoun L, Lumbroso S, Manetta J, Paris F, Leroux JL, Sultan C (2003) Testosterone is significantly reduced in endurance athletes without impact on bone mineral density. Horm Res Paediatr 59(6):285–292

Mastorakos G, Pavlatou M (2005) Exercise as a stress model and the interplay between the hypothalamus–pituitary–adrenal and the hypothalamus–pituitary–thyroid axes. Horm Metab Res 37(09): 577–584

Mastorakos G, Pavlatou M, Diamanti-Kandarakis E, Chrousos GP (2005) Exercise and the stress system. Hormones 4(2):73–89

McLellan TM (2001) The importance of aerobic fitness in determining tolerance to uncompensable heat stress. Comparative biochemistry and physiology—part A: molecular & integrative physiology 128(4):691–700

Park J, Rho HK, Kim KH, Choe SS, Lee YS, Kim JB (2005) Overexpression of glucose-6-phosphate dehydrogenase is associated with lipid dysregulation and insulin resistance in obesity. Mol Cell Biol 25(12):5146–5157

Pedersen BK, Febbraio M (2005) Muscle-derived interleukin-6—a possible link between skeletal muscle, adipose tissue, liver, and brain. Brain Behav Immun 19(5):371–376

Pedersen BK, Hoffman-Goetz L (2000) Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev 80(3):1055–1081

Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz T, Sheps SG, Roccella EJ (2005) Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the subcommittee of professional and public education of the American Heart Association Council on High Blood Pressure Research. Hypertension 45(1):142–161

Rohrmann S, Nelson WG, Rifai N, Brown TR, Dobs A, Kanarek N, Yager JD, Platz EA (2007) Serum estrogen, but not testosterone, levels differ between black and white men in a nationally representative sample of Americans. J Clin Endocrinol Metab 92(7): 2519–2525

Sacheck JM, Cannon JG, Hamada K, Vannier E, Blumberg JB, Roubenoff R (2006) Age-related loss of associations between acute exercise-induced IL-6 and oxidative stress. Am J Physiol Endocrinol Metab 291(2):E340–E349

Shen KH, Lin CH, Chang HK, Chen WC, Chen SH (2008) Premarin can act via estrogen receptors to rescue mice from heatstroke-induced lethality. Shock 30(6):668–674

Soucy G, Boivin G, Labrie F, Rivest S (2005) Estradiol is required for a proper immune response to bacterial and viral pathogens in the female brain. J Immunol 174(10):6391–6398

Sousa e Silva T, Longui CA, Rocha MN, Faria CDC, Melo MR, Faria TG, de Souza JA, Rizzo LV (2010) Prolonged physical training decreases mRNA levels of glucocorticoid receptor and inflammatory genes. Horm Res Paediatr 74(1):6–14

Stephen J, Brufsky A, Weissfeld J, Trump DL, Dyky MA, Hadeed V (2001) Testosterone, sex hormone-binding globulin, and body composition in young adult African American and Caucasian men. Metabolism 50(10):1242–1247

Timmons BW, Tarnopolsky MA, Snider DP, Bar-OR O (2006a) Immunological changes in response to exercise: influence of age, puberty, and gender. Medicine Sci Sports Exerc 38(2):293–304

Timmons BW, Hamadeh MJ, Tarnopolsky MA (2006b) No effect of short-term 17beta-estradiol supplementation in healthy men on systemic inflammatory responses to exercise. Am J Physiol Regul Integr Comp Physiol 291(2):R285–R290

Uchida Y, Kano M, Yasuhara S, Kobayashi A, Tokizawa K, Nagashima K (2010) Estrogen modulates central and peripheral responses to cold in female rats. J Physiol Sci 60(2):151–160

Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C (2004) Androgens and bone. Endocr Rev 25(3):389–425

Vankrieken L (2000) Immulite® reproductive hormone assays, 4th edn. DPC Technical Report. Ref Type: Report

Varga-Pinter B, Horvath P, Kneffel Z, Major Z, Osvath P, Pavlik G (2011) Resting Blood Pressure Values of Adult Athletes. Kidney and blood pressure research 34(6):387–395

Vermeulen A, Verdonck L, Kaufman JM (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84(10):3666–3672

Ware JE, Kosinski MK, Keller SD (1996) A 12-item short-form health survey: construction of scales and preliminary tests of reliability and validity. Med Care 34(3):220–233

Wittert GA, Livesey JH, Espiner EA, Donald RA (2012) Adaptation of the hypothalamopituitary adrenal axis to chronic exercise stress in humans. Med Sci Sports Exerc 28(8):1015–1019

Wright H, Selkirk G, McLellan T (2010) HPA and SAS responses to increasing core temperature during uncompensable exertional heat stress in trained and untrained males. Eur J Appl Physiol 108(5):987–997

Zanovec M, Johnson LG, Marx BD, Keenan MJ, Tuuri G (2009) Self-reported physical activity improves prediction of body fatness in young adults. Med Sci Sports Exerc 41(2):328–335