ORIGINAL RESEARCH

The Impact of an Ultramarathon on Hormonal and Biochemical Parameters in Men

Brian R. Kupchak, PhD, MT; William J. Kraemer, PhD; Martin D. Hoffman, MD; Stephen D. Phinney, MD, PhD; Jeff S. Volek, PhD, RD

From the Human Performance Laboratory, Department of Kinesiology, University of Connecticut, Storrs, CT (Drs Kupchak, Kraemer, and Volek), the Department of Physical Medicine and Rehabilitation, University of California Davis Medical Center, Sacramento, CA (Dr Hoffman); the Department of Veterans Affairs, Northern California Health Care Systems, Mather, CA (Dr Hoffman), and the School of Medicine (Emeritus), University of California, Davis, Davis, CA (Dr Phinney).

Objective.—To examine circulating hormonal responses in men competing in the Western States Endurance Run (WSER, June 23 to 24, 2012): a 161-km trail run that starts in Squaw Valley, CA, and concludes in Auburn, CA.

Methods.—We examined 12 men who completed the WSER. Blood samples were obtained the morning before the race, immediately post-race (IP), and 1 (D1) and 2 (D2) days after the conclusion of the WSER. The hypothalamic-pituitary-testicular (HPT) axis was assessed by measuring testosterone and luteinizing hormone (LH). We also examined sex hormone-binding globulin (SHBG) and cortisol. Biochemical and muscle damage markers were also measured.

Results.—Relative to pre-race, there were significant ($P \leq .05$) decreases in testosterone, LH, and SHBG, whereas cortisol showed a significantly marked elevation at IP. Testosterone, LH, SHBG, and cortisol remained significantly different from pre-race at D1. Additionally, the testosterone to cortisol (T:C) ratio, a marker of anabolism, was decreased at IP and D1. Serum total protein, albumin, and globulin significantly decreased at IP, and remained decreased at D1 and D2. Bilirubin increased significantly IP and D1, whereas alkaline phosphatase decreased at D1 and D2. Creatine kinase, myoglobin, aspartate aminotransferase, and alanine aminotransferase increased at IP, and continued to be significantly elevated at D1 and D2.

Conclusions.—Training for and completing the WSER produced a significant suppression in the HPT axis as seen by decreased levels of testosterone and LH. Additionally, running the WSER continued to influence endocrine function until 2 days after the race. Furthermore, the stress caused by the WSER produced severe muscle damage.

Key words: ultramarathon, testosterone, luteinizing hormone, sex hormone-binding globulin, cortisol, tissue injury

Introduction

Participation in running events such as marathons, Ironman triathlons, and ultramarathons has gained increased popularity. Ultramarathons in particular have become increasingly popular as the number of events and participants has dramatically risen during the last 30 years.¹ The Western States Endurance Run (WSER), one of the oldest and most prestigious 161-km ultramarathons,

involves not only running for a prolonged duration but also exposes the athlete to numerous environmental stressors, including marked fluctuations in temperature and altitude. Thus, the body is exposed to multiple stressors in this competition.

Prolonged strenuous endurance exercise can alter normal physiological processes including induction of severe muscle damage,^{2–4} imbalance in fluid and electrolyte levels,^{5–8} changes in immune function,⁹ increased inflammation,^{4,10} alterations in coagulatory and fibrinolytic systems,³ and increased risk of exertional heat stroke.¹¹ Additionally, long-duration events are known to affect endocrine function, reflected by alterations in the hypothalamic-pituitary-testicular (HPT) axis. Events of this duration and magnitude can suppress gonadal

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Corresponding author: Brian R. Kupchak, PhD, MT, Human Performance Laboratory, Department of Kinesiology, University of Connecticut, 2095 Hillside Road, Unit 1110, Storrs, CT 06269 (e-mail: brian.kupchak@uconn.edu).

function in men as denoted by significant decreases in the anabolic hormone testosterone,^{12–14} an effect that has been shown to last for days after the race.¹⁵

The impact of prolonged endurance events on luteinizing hormone (LH), the main regulator of testosterone, is less consistent. Concentrations of LH are either decreased^{12,14} or remain constant^{15–17} after prolonged endurance events, an effect potentially related to the pulsatile nature of LH and the highly individual response to the exercise stress. In addition, prolonged strenuous exercise decreases sex hormone-binding globulin (SHBG),^{14,18} the primary carrier of testosterone. The physiological stress produced by endurance events greatly increases hypopituitary-adrenal stress as measured by cortisol concentrations,^{13,19} possibly serving as a mechanism to increase blood sugar levels, suppress the immune system, and to assist in fat and protein metabolism.^{20,21}

To date, it appears that only one study has examined how an ultramarathon affects anabolic hormonal response,¹³ and so the recovery of endocrine responses after an ultramarathon remains to be determined. In the present study our primary purpose was to examine the hormonal responses in men competing in the WSER and determine the time course of recovery after the event. A secondary purpose was to document changes in markers of tissue function or injury compared with data obtained in previous endurance events. The present study complements our prior report on findings that running the WSER activated the coagulation and fibrinolytic systems.³ We hypothesized that completion of the WSER would suppress the HPT axis, with the stress produced from running this ultraendurance event delaying the recovery of the endocrine system.

Methods

SUBJECTS

Subjects were recruited from 381 entrants in the 2012 WSER, who were invited to participate in the study during the registration and information meetings and medical symposium held 2 days before the race. To be considered for the study, participants were males between the ages of 21 and 70 years who had completed an ultramarathon previously. In total, 22 healthy men from various parts of the United States volunteered to be subjects. All participants were non-tobacco users and reported no history of cardiovascular disease. Exclusion criteria included the following: 1) no reported use of cholesterol lowering or blood pressure medications; 2) no reported use of anticoagulant medications (eg, coumadin); 3) never had been diagnosed with liver, kidney, blood, or gastrointestinal disease or severe metabolic or

endocrine disorders; and 4) no reported use of hormonal substances including testosterone, anabolic steroids, or growth hormones. The Institutional Review Board for use of human subjects in research at the University of Connecticut approved this study. All subjects provided written informed consent after having the study risks and benefits carefully explained to them.

Subjects also completed training logs, which were used to determine how many kilometers each subject ran in the preceding 4 weeks before the WSER. Additionally, subjects completed food history questionnaires to estimate nutrient intakes before, during, and after the WSER.

ANTHROPOMETRICS

Body composition was determined via skinfold measurements obtained from the subject's right side (chest, abdomen, and thigh) using calibrated calipers (Harpenden Skinfold Caliper; Body Care Direct, Southam, Warwickshire, England). Body fat percentage was estimated using a 3-site skinfold equation²² with the mean of 3 measurements used in the equation. Body mass was obtained with subjects wearing running clothes and sneakers on a digital scale (model 349KLX; Health o Meter, Bridgeview, IL).

SETTING

The WSER is a 161-km (100.2 miles) trail run that follows the Western States Trail. Beginning in Squaw Valley, CA, the course traverses the Sierra Nevada mountain range and ends in Auburn, CA. During the race, runners climb approximately 6000 m (maximum elevation: 2655 m) and descend about 7000 m through the Granite Chief Wilderness and canyons of California Gold Country. The 39th WSER started at 0500 h on June 23, 2012, and had a 30-hour time limit for completion. During the race, nearby temperatures ranged across the course from approximately 1°C (34°F) to 28°C (82°F).

BLOOD COLLECTION

Prerace blood samples were obtained after an overnight fast 20 hours before the start of the race (0700–1000 h; June 22, 2012). Additional blood samples were collected immediately postrace (IP; within 10 minutes of finishing the race) and 1 (D1) and 2 (D2) days after race completion (corresponding to 51–54 hours and 75–78 hours from the start of the race, respectively [0700–1000 h; June 25 and 26]). Similar to prerace, D1 and D2 blood samples were both obtained after an overnight fast between 0700 h and 1000 h. At each time, 20 mL of whole blood was obtained from an antecubital vein and

aliquoted into individual vacutainers containing no additive or EDTA. Tubes were centrifuged at 1500g for 15 minutes, and serum or plasma was separated into individual cryovials, and then stored and shipped on dry ice. On arrival at the University of Connecticut Human Performance Laboratory, the specimens were subsequently placed in a -80°C ultralow freezer until biochemical analysis occurred.

BIOCHEMICAL ANALYSIS

Total creatine kinase, total protein (TP), albumin, globulin, bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured from serum by an automated clinical analyzer (Quest Diagnostics, Willimantic, CT). Myoglobin was measured from EDTA-plasma in duplicate via enzyme-linked immunosorbency assay (ELISA; CALBiotech, Spring Valley, CA). The mean intraassay coefficients of variation (CVs) were 6.2% and interassay CVs were 9.0%.

Cortisol, total testosterone, and LH were measured in duplicate by ELISA from serum (CALBiotech). The intraassay CVs were 3.9%, 4.8%, and 3.2% and the interassay CVs were 7.7%, 5.8%, and 3.9% for cortisol, testosterone, and LH, respectively. SHBG was determined from serum (ALPCO Diagnostics, Salem, NH) by ELISA. The intraassay CV was 8.1% and interassay CV was 10.2%. All ELISAs were performed on a Versamax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at the appropriate wavelength for that particular assay.

STATISTICAL ANALYSES

Data are presented as means and 95% CI. Using the nQuery Advisor software (Statistical Solutions, Saugus, MA), we found that an n size of 12 for each variable was adequate to defend the 0.05 alpha level of significance with a Cohen probability level between 0.73 and 0.82 for each dependent variable. Changes in study variables from prerace were evaluated using one-way repeated-measures analysis of variance (ANOVA) followed by Tukey's post-hoc test if appropriate. Data were tested for normal distribution using the Kolmogorov-Smirnov test and homogeneity of variance using Levene's test before ANOVA analysis. If data failed these tests, they were log transformed and reanalyzed for normality and homogeneity of variance. Furthermore, the Friedman test and Wilcoxon signed-rank test (nonparametric analysis) were performed on the database to confirm that type II experimental error did not dwell in the results with the n size we used. Pearson product-moment correlations were used to examine whether any relationships existed

in the data. Statistical analyses were performed using SPSS software (version 20.0; SPSS Inc, Chicago, IL). Significance was set at $P \leq .05$ for all analyses.

Results

Twelve men completed the WSER; their physical (age, body mass, % body fat) and performance (weekly training and finishing times) characteristics are presented in Table 1. Subjects consumed an average of 4836 kcal (95% CI, 3795–5878) during the WSER, and 2957 kcal (95% CI, 2316–3597) per day 2 days after the event according to their food questionnaire recall.

Testosterone, SHBG, and LH were used to examine gonadal responses to the race. Compared with prerace levels (14.9 nmol/L; 8 of 12 participants below lower reference limit),²³ serum testosterone concentrations decreased at IP (0.73-fold; $P = .002$) and D1 (0.85-fold; $P = .018$; Figure 1A). Testosterone concentrations at D2 remained lower; however, this did not reach statistical significance. Serum LH concentrations decreased from Pre at both IP (0.34-fold; $P = .001$) and D1 (0.65-fold; $P = .014$) and were normalized by D2 (Figure 1B). Similarly, serum SHBG values changed by 0.83-fold ($P < .001$) and 0.92-fold ($P = .031$) at IP and D1, respectively, returning to prerace levels by D2 (Figure 1C).

Serum cortisol concentrations were markedly increased at IP relative to Pre (4.34-fold; $P < .001$) and remained elevated at D1 (1.40-fold; $P = .013$; Figure 1D). The testosterone to cortisol (T:C) ratio decreased at IP (0.18-fold; $P < .001$) and remained below prerace levels at D1 (0.63-fold; $P = .007$; Figure 1E). Individual postrace concentrations of testosterone, LH, SHBG, cortisol, and T:C ratio, along with finishing times and time of day the race was finished, are presented in Table 2. No correlations were found between immediate postrace endocrine markers and the time of day the subjects finished the event (Table 2).

As previously reported (using a data subset),³ marked muscle damage occurred in men completing the WSER as evidenced by dramatic increases in serum CK and

Table 1. Subject characteristics and physical performance

Variables	Mean (95% CI)
Age (years)	45.9 (38.3–53.5)
Body mass (kg)	72.6 (67.7–77.4)
% Body fat	10.2 (8.6–11.9)
Training (km/wk)	98.7 (81.9–115.5)
Finish time (hours)	25.08 (22.53–27.62)

Values are means and 95% CI (in parentheses); $n = 12$.

Figure 1. Testosterone (A), luteinizing hormone (B), sex hormone-binding globulin (C), cortisol (D), and testosterone to cortisol ratio (E) before and after an ultramarathon. Values are mean $\pm$ 95% CI ($n = 12$). ^a $P < .05$ vs Pre; ^b $P < .01$ vs Pre; ^c $P < .001$ vs Pre. Pre, prerace; IP, immediately posttrace; D1, 51 to 54 hours from start of race; D2, 75 to 78 hours from start of race; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; Test:Cort, testosterone to cortisol ratio.

myoglobin at IP (179.4-fold and 110.8-fold for CK and myoglobin, respectively; $P < .001$). At IP, CK ranged from 7436 U/L to 64,600 U/L whereas myoglobin concentrations were between 50.4 nmol/L and 188.1 nmol/L. Both markers remained elevated at D1 (64.7-fold and 7.0-fold for CK and myoglobin, respectively; $P < .001$) and D2 (30.4-fold and 2.2-fold for CK and myoglobin, respectively; $P < .01$; Table 3).

Serum concentrations of TP (0.90-fold), albumin (0.93-fold), and globulin (0.85-fold) were decreased ($P < .01$) at IP, and remained below prerace levels at

D1 (0.84-fold, 0.85-fold, and 0.83-fold for TP, albumin, and globulin, respectively; $P < .001$) and D2 (0.87-fold, 0.86-fold, and 0.87-fold for TP, albumin, and globulin, respectively; $P < .001$; Table 3). The albumin to globulin ratio increased ($P < .004$) IP by 1.09-fold, and returned to prerace levels at D1. The product of normal heme metabolism, bilirubin, increased ($P < .001$) from prerace values at IP (1.61-fold) and remained elevated at D1 (1.34-fold; $P < .033$; Table 3).

Serum ALP was unaffected at IP; however, concentrations decreased at both D1 ($P < .003$) and D2 ($P < .014$)

Table 2. Individual runner hormonal values immediately posttrace

Subject	Age (y)	Race time (h)	TOD (h:min)	T (nmol/L)	LH (IU/L)	SHBG (nmol/L)	C (nmol/L)	T:C ratio
Runner 1	62	29.56	10:33	5.0	1.15	28.2	1811	0.003
Runner 2	42	28.74	09:44	7.6	1.42	19.9	739	0.010
Runner 3	53	23.85	04:51	5.6	2.29	28.9	2696	0.002
Runner 4	45	26.97	07:58	13.0	2.59	27.8	2174	0.006
Runner 5	37	18.39	23:23	8.3	0.95	33.3	2612	0.003
Runner 6	56	24.81	05:48	9.2	3.31	17.9	1300	0.007
Runner 7	34	19.31	00:18	8.8	1.21	26.1	2277	0.004
Runner 8	60	28.19	09:11	10.2	1.02	18.4	1234	0.008
Runner 9	65	29.46	10:27	21.4	1.19	29.0	1632	0.013
Runner 10	27	27.56	08:33	9.8	1.62	16.1	1489	0.007
Runner 11	26	16.89	21:53	21.0	0.98	35.0	1488	0.014
Runner 12	44	27.20	08:12	10.2	0.72	31.6	1662	0.006

TOD, time of day finished; T, testosterone; LH, lutenizing hormone; SHBG, sex hormone-binding globulin; C, cortisol; T/C, testosterone/cortisol ratio.

by 0.83-fold and 0.85-fold, respectively. Disturbance in tissue injury, as evidenced by marked increases ($P < .001$) in serum AST and ALT, occurred after completion of the WSER (Table 3). Compared with prerace levels, levels of AST and ALT were increased at IP (22.7-fold and 5.0-fold for AST and ALT, respectively), and remained elevated at D1 (14.8-fold and 5.5-fold for AST and ALT, respectively) and D2 (9.8-fold and 5.2-fold for AST and ALT, respectively). Serum AST and ALT measured at IP ranged from 253 U/L to 1068 U/L and 43 U/L to 184 U/L, respectively (Table 3). Compared with baseline, changes in IP concentrations of AST were positively correlated with changes in CK ($r = .838$; $P = .001$) and myoglobin ($r = .584$; $P = 0.040$).

Discussion

As previously noted, ultraendurance events have become increasingly popular in the last 30 years, with competitors pushing their bodies to their physiological limit. Thus, it is important to gain further insights into the effects of such competitions to better manage the athlete's preparation physically and medically and make sports medicine professionals aware of potential issues with such stress. The WSER is a 161-km run through the potentially harsh environmental conditions of the Sierra Nevada mountain range, subjecting competitors to substantial and multiple stress vectors. In the present study, this stress is exemplified by marked alterations in the HPT axis (ie, decreased testosterone,

Table 3. Biochemical markers before and after an ultramarathon

Marker	Pre	IP	D1	D2
CK (U/L)	114 (95–134)	20,484 ^c (11,714–29,253)	7389 ^c (4117–10,660)	3470 ^c (1733–5206)
Myoglobin (nmol/L)	1.1 (0.9–1.2)	118.5 ^c (93.5–143.5)	7.4 ^c (4.5–10.4)	2.4 ^b (1.6–3.1)
Total protein (g/dL)	7.2 (7.1–7.4)	6.5 ^b (6.3–6.8)	6.1 ^c (5.9–6.3)	6.3 ^c (6.1–6.5)
Albumin (g/dL)	4.7 (4.6–4.9)	4.4 ^b (4.2–4.6)	4.0 ^c (3.9–4.1)	4.1 ^c (4.0–4.2)
Globulin (g/dL)	2.5 (2.4–2.6)	2.1 ^c (2.0–2.3)	2.1 ^c (2.0–2.2)	2.2 ^c (2.1–2.3)
A:G ratio	1.9 (1.8–2.0)	2.1 ^b (2.0–2.2)	2.0 (1.9–2.1)	1.9 (1.8–2.0)
Bilirubin (mg/dL)	0.83 (0.68–0.99)	1.34 ^c (1.17–1.52)	1.12 ^a (0.88–1.35)	0.78 (0.65–0.91)
ALP (U/L)	61 (50–72)	61 (54–68)	51 ^b (45–56)	52 ^a (46–58)
AST (U/L)	25 (23–28)	575 ^c (440–710)	376 ^c (279–473)	248 ^c (169–327)
ALT (U/L)	21 (19–23)	104 ^c (81–127)	115 ^c (86–145)	110 ^c (76–144)

Values are means and 95% CI (in parentheses); $n = 12$.

Pre, prerace; IP, immediately posttrace; D1, 51 to 54 hours from start of race; D2, 75 to 78 hours from start of race; CK, creatine kinase; A:G, albumin to globulin ratio; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

^a $P < .05$ vs Pre.

^b $P < .01$ vs Pre.

^c $P < .001$ vs Pre.

LH, and SHBG) in conjunction with an increased catabolic state (ie, increased cortisol and decreased testosterone to cortisol ratio). Additionally, considerable alterations in tissue function and injury parameters occurred with completion of the WSER, as shown by decreases in TP and albumin as well as substantial and prolonged increases in bilirubin, AST, and ALT concentrations. These findings complement previous data from both men and women runners who demonstrated activation of both the coagulation and fibrinolytic systems after the WSER, placing a major stress on renal function.³

The impact of the WSER on the HPT axis was examined by measuring serum levels of testosterone and LH. Prerace concentrations of testosterone were low, but were still within reference limits (14 to 28 nmol/L; however, 8 of the 12 subjects did have values less than the lower reference limit).²³ The low testosterone concentrations exhibited by these athletes may be a result of chronic endurance exercise training, a finding in accordance with previous research.¹³ Additionally, basal concentrations of LH were within the normal range (3 to 25 U/L),²³ and thus, the failure of LH signaling, which might be thought to increase with decreasing testosterone concentrations, may reflect HPT abnormalities. This is consistent with both Wheeler et al²⁴ and Hackney et al²⁵ showing this phenomenon in endurance-trained athletes. However, without direct LH pulse analysis it is difficult in this investigation to determine whether it is the pulse generator that is the issue for the low testosterone values or a problem at the level of the testes as to fluid volume flow. Concentrations of both testosterone and LH declined immediately posttrace, which could be attributed to energy deficiency²⁶ caused by sustained running of the WSER. In addition, it has been suggested that prolonged exercise may cause a drop in testosterone because of a decrease in testicular blood flow,²⁶ or that the decline is the result of an increase in androgen utilization to repair tissues.²⁷ Furthermore, the low LH concentrations may be explained by the increase in β -endorphins after the stress produced from the WSER.^{29,30} This is an agreement with previous marathon studies.^{31,32} Additionally, we observed both hormones remained depressed at D1, but returned to baseline levels 75 to 78 hours from the start of the race. Mechanistically, this phenomenon in these highly trained endurance athletes may be explained by reduced gonadotropin-releasing hormone (GnRH) from the hypothalamus,³³ reduced sensitivity of the anterior pituitary receptor to GnRH, or the failure of the pituitary to produce LH.³⁴ However, again with no pulse analysis of LH seen in our study, these data should be interpreted with caution.

Consequences of a low testosterone brought on by extended and repeated bouts of endurance training may have both positive and negative physiological implications for the endurance runner. One negative physiological effect of low testosterone is that there is a decrease in spermatogenesis, thereby affecting reproduction.^{35,36} Another detrimental consequence of hypotestosterone production is that it has been shown to cause low bone mineral density levels in athletes.^{36,37} However, there are some desirable physiological adaptations of having low testosterone levels. Hackney et al³⁸ suggest this positive adaptation is a benefit to the ultraendurance runner by limiting protein synthesis and muscle mass development, which would hurt the ultraendurance runner by carrying excess and unnecessary muscle mass. However, without a progressive resistance training program, testosterone utilization beyond normal muscle cell homeostasis would not be able to stimulate added hypertrophy or body mass. Some researchers have also suggested that a low testosterone level may provide a favorable environment to mobilize amino acids away from protein synthesis and redirect them to gluconeogenesis.^{39,40} In addition, some studies have found a low testosterone concentration may have cardiovascular benefits,⁴¹ as well as increase high-density lipoprotein in men.⁴² Again, such benefits remain highly speculative because of the importance of normal homeostatic concentrations of testosterone in mediating a host of metabolic and cellular signaling systems for normal health and development.⁴³

We are aware of 2 studies that assessed the effect of prolonged endurance exercise on SHBG, which is the primary carrier of both testosterone and estradiol in the blood and is thought to play a leading role in the response of testosterone. Both studies examined marathon runners^{14,18} and showed decreases in SHBG concentrations immediately posttrace. Our observations of decreased SHBG concentrations immediately and 1 day after completion of the WSER concur with these previous findings. One explanation of decreased SHBG concentrations could be a byproduct of protein loss,⁴⁴ as SHBG has a long half-life. Additionally, the decrease in SHBG concentration reduced the total pool of testosterone available for release as free hormone. However, it has become well known that the free hormone availability is regulated by the total quantity of hormone available from all bound proteins.

Cortisol, examined in the present study as a marker of the stress placed on athletes running the WSER, increases immediately after completion of endurance events.^{12,15,19,45,46} We showed a 4.3-fold increase in cortisol concentrations, which was markedly greater than previous reports in marathon,^{15,19,32,45} Ironman

triathlon,⁴⁶ and ultramarathon^{12,47} finishers. This exaggerated increase in cortisol could be the result of not just the duration of the race⁴⁸ but also the eccentric downhill running (totaled 7000 m), change in altitude (165 to 2655 m),⁴⁹ lack of sleep,⁵⁰ energy deficiency,²⁸ and dealing with temperatures that varied from 1°C to 28°C.^{48,51,52} Recovery cortisol concentrations at D1 declined from immediate postrace values but were still significantly elevated from prerace levels. This is in contrast to marathons^{15,19} and Ironman triathlons,⁴⁶ in which, surprisingly, cortisol concentrations returned to baseline or below 1 day postrace. Such data indicate a potential dichotomy of ultraendurance events. However, our results were similar to previous data obtained during a 180-km ultramarathon.⁴⁷ At IP, the high concentrations of cortisol were inversely related to the decrease of testosterone, suggesting a separation in the 2 axes. Similarly, Kraemer et al¹³ reported this phenomenon during a 160-km ultramarathon in Alaska (now called the Susitna 100), which was not constituent to testosterone reduction and gonadal function.

Although highly general in nature of overall anabolic and catabolic signaling, we also examined the effect of the WSER on the testosterone to cortisol ratio, which is a gross indicator of anabolic–catabolic balance.⁵³ The T:C ratio decreased immediately postrace, which is in agreement with findings from an Ironman triathlon,⁴⁵ suggesting that the body is in a catabolic state on completion of demanding endurance events. The state of catabolism was still evident at D1, in contrast to an Ironman triathlon,⁴⁶ with the T:C ratio returning to baseline levels by D2. Decreases in the T:C ratio can be explained by the stress brought on by the duration and environmental factors of the WSER beyond the distance raced. Additionally, the continued catabolic state at D1 supports the attenuated repair of muscle damage induced by the WSER as documented by our extremely high CK and myoglobin concentrations.³

Most of the endocrine parameters exhibit diurnal variation,⁵⁴ which introduces discretion in trying to understand our immediate postrace results, as blood was collected over a far-ranging time span between 2000 h (June 23) and 1030 h (June 24). Even though this was the case, we believe the stress created by the WSER mostly contributed to the dramatic changes seen with our endocrine parameters and that they were not produced by circadian rhythm variation. (There were no correlations found between IP hormonal values and the time of day the subjects finished.) The magnitude of change was well beyond what could be explained by normal circadian undulation. Furthermore, typical clinical ranges for such hormones are inclusive of the span of circadian changes as well.

In the present study we also sought to compare changes in tissue function and injury variables with other endurance exercise events. Albumin is the main protein of human plasma and is synthesized solely in the liver. Consistent with previous studies,^{55–57} albumin concentrations decreased immediately after the WSER and remained lower during recovery (D1 and D2). As expected owing to the decline of albumin, TP levels were also decreased from baseline at IP, D1, and D2. These alterations likely reflect a catabolic state attributable to acute malnutrition placed on the body by completing the WSER^{58,59} and imply that either proteins are being used to repair muscle and tissues or there is a decrease in protein synthesis.⁵⁵

Total bilirubin, which represents erythrocyte destruction and physiological turnover, rose immediately postrace and returned to baseline concentrations at D2. This response was similar to previous studies examining a 100-km ultramarathon race⁶⁰ and an Ironman triathlon.⁵⁶ The elevated bilirubin concentrations may signify increased intravascular hemolysis as a result of repetitive foot strikes, destruction of erythrocyte and degradation of hemoglobin because of high osmotic stress, and high oxygen utilization inducing upregulation of free radicals into the circulation.^{61,62}

The enzyme ALP is primarily located in the lining of the bile duct of the liver, and elevated concentrations are indicative of bile duct obstruction or infiltrative diseases of the liver. In our study, ALP levels remained unchanged immediately postrace but were lower at D1 and D2 compared with prerace. This finding is in contrast to other endurance studies, which showed either no changes^{63,64} or an increase in postrace concentrations of ALP.^{56,65,66} The low ALP levels found in our study may reflect protein deficiency or malnutrition resulting from the extreme length and time of the WSER.⁶⁷

The transaminases AST and ALT are located abundantly in the liver. However, AST is also present in significant concentrations in cardiac and skeletal muscle, kidneys, brain, and red blood cells. Clinically increased levels of AST could indicate hepatitis, hemolytic anemia, heart failure, musculoskeletal disease, or muscle damage, whereas ALT is present predominately in liver, but can also be found in smaller concentrations in kidneys, heart, and skeletal muscle, and is a more specific indicator of liver inflammation. Both enzymes at baseline exhibited concentrations above reference limits (AST and ALT, 0–35 U/L),²³ which may indicate chronic liver damage caused by continuous long-term strenuous exercise.⁵⁵ As expected, AST was elevated immediately after completion of the WSER, which is consistent based on findings after other endurance events.^{10,55–57,63,66,68–70} Because AST is located in the mitochondria, increased

levels in response to endurance exercise reflect destruction of muscle cells leading to AST release into the circulation.⁵⁸ In support, the change of AST was positively correlated with the change in muscle damage markers, CK and myoglobin. The magnitude by which the levels of AST increased was in contrast to that of other ultramarathons,^{57,70} a finding potentially explained by the prolonged duration of the WSER as well as the 7000 m of downhill eccentric running. AST levels remained elevated at D1 and D2, which mirrored the pattern of change seen after Ironman triathlons^{50,51} and ultramarathons.^{10,57,70} The continued elevation of AST suggests muscle permeability remains increased, allowing enzymes to leak into circulation.⁴¹

Parallel to AST, ALT concentrations increased immediately postrace and remained elevated at D1 and D2. Our findings are consistent in magnitude and duration with previous results from a 24-hour ultramarathon held in Soo-Chow, China.⁵⁷ However, most studies examining endurance events, such as marathons,⁶⁶ triathlons,^{55,56,65} or even a 100-km ultramarathon,⁶⁰ either showed no change or only a moderate increase in ALT concentrations. Thus, the exaggerated and prolonged increase in circulating ALT can be explained by the duration of the race and the eccentric damage caused by running downhill from the terrain of the WSER course. Even though ALT is a specific marker for liver injury, our findings are indicative of significant muscle damage and minor liver damage.^{10,71,72} However, in a clinical population these extremely high values of AST and ALT may indicate acute cell liver necrosis brought on by viral infections, drugs, toxins, alcohol, or ischemia.^{59,73,74}

The WSER attracts runners because of its historical importance as the premier trail run of this distance and its challenging conditions.¹⁶ Yet, because of a cap on the number of runners allowed to participate, fewer than 400 runners can gain entry into the event each year. The harsh conditions of the WSER are unlike the conditions of other ultramarathons in which biomarker studies have been performed where the race terrain was flat, oval, recreational trails^{10,70} or a track.⁵⁷ Thus, the exaggerated response of the endocrine and biochemical markers seen during this study appears to be reflective of the combination of extreme stressors associated with conditions of the WSER.

Given the rise in popularity of participating in ultra-endurance events, it is warranted that medical professionals become aware of potential physiological issues that occur from this type of challenge. This study found extremely high levels of both myoglobin and CK, which might indicate the onset of rhabdomyolysis and should be looked at with caution. However, CK values of more

than 20,000 U/L have been documented when running this type of event, which are common and seldom results in detrimental consequences in healthy participants.² Additionally, an emphasis on screening for liver function should be taken into account by individual participants to rule out any hepatic disorder or disease. Even though hepatic enzyme values were elevated in our participants, the values are proportional to the volume of exercise and have little effect in a healthy population, but in a clinical population this event would place a high degree of stress on the liver and may lead to liver damage.

In conclusion, completing the WSER produced suppression in the HPT axis in men, as seen by decreased levels of testosterone and LH. Additionally, running the WSER further affected endocrine function, which did not recover until 2 days after the race. Baseline data indicate that training for the WSER may have lowered testosterone concentrations to hypogonadal levels. Furthermore, the stress caused by the WSER produced severe muscle damage, but probably did not cause hepatic damage. Therefore, these findings reflect the potential differential impact of ultraendurance training and racing in men and provide new insights into such races with multiple stressor contributions.

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