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IMMUNE FUNCTION RESPONSES TO ULTRAMARATHON RACE COMPETITION

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Abstract

Introduction: This article summaries key exercise immunology findings from five years of research on 350 athletes in the 160-km Western States Endurance Run (WSER), a point-to-point trail run in the Sierra Nevada Mountains of northern California.

Methods: WSER athletes provided pre- and post-race blood, urine, and saliva samples, and these were assayed for immune function, inflammation, and oxidative stress outcome measures. Subjects also provided upper respiratory tract infection (URTI), training, and demographic data.

Results: Several key findings from an analysis of the entire 5-year dataset revealed the following:

1. One out of four runners reported sickness during the 2-week period after the 160-km race. Post-race values for granulocyte oxidative burst activity were reduced 50% compared to pre-race. Of all outcome measures, only low post-race sIgA secretion rate was significantly linked to URTI.

2. Variance in plasma cytokine changes during the race was large between WSER finishers, with no male-to-female differences. The cytokine profile measured indicates a strong anti-inflammatory response.

3. Plasma cytokine perturbations were linked to muscle damage and inflammation. Unexpectedly, cytokine variance was unrelated to oxidative stress.

4. Despite the widespread use of ibuprofen by WSER athletes, no beneficial effects were measured for reducing RPE, muscle damage, or DOMS. To the contrary, ibuprofen use was linked to mild endotoxemia, kidney dysfunction, and systemic inflammation.

Conclusions: In general, the WSER data indicate that competitors experience significant immune system stress, reflecting the physiological trauma experienced by the athletes. Long term effects on health have not yet been ascertained.

Key words: *cytokines, running, immune function, ibuprofen, oxidative stress, inflammation*

Introduction

Athletes participating in prolonged and intensive exercise such as marathon and ultramarathon race events experience acute physiological stress reflected by muscle microtrauma, oxidative stress, and systemic inflammation (1-3). Concomitant with these stressors are widespread perturbations in innate and adaptive immunity including decreases in natural killer (NK) cell cytotoxic activity, granulocyte respiratory burst activity, nasal and salivary IgA (sIgA) secretion, delayed type hypersensitivity, and mitogen-induced lymphocyte proliferation, as well as extensive alterations in circulating immune cell populations (4-7). This period of decreased host protection is often followed by elevated rates of upper respiratory tract infections in the athletes 1-2 weeks after competition (8,9). Efforts to link the immunological perturbations with increased infection risks have however, been relatively unsuccessful (10).

The immunology of prolonged and heavy exertion of five hours duration and longer is relatively unstud-

Table 1. *Subject characteristics for 350 competitors in the 160-km Western States Endurance Run (males = 80%; females = 20%) (mean±SE)*

Variable	Males (N=279)	Females (N=71)	*P*-Value
Age (years)	47.1±0.5	43.5±0.8	0.001
Stature (m)	1.78±0.004	1.63±0.007	<0.001
Body mass (kg)	73.0±0.5	55.3±0.7	<0.001
Body mass index ($kg \cdot m^2$)	22.9±0.1	20.7±0.2	<0.001
Body composition (%)	10.7±0.4	18.9±0.6	<0.001
Training distance (km/wk)	81.2±1.6	85.2±3.1	0.169
Years of training	15.6±0.5	13.5±1.0	0.087
Ultramarathon races run	39.2±2.9	29.0±4.7	0.067
Race time (h)	25.9±0.2	27.4±0.4	0.002

ied compared to exercise bouts of shorter duration including marathons (11,12). This paper summarizes data collected from 350 ultra-marathon runners during a five-year period at the 160-km Western States Endurance Run (WSER). Portions of these data have been published in a series of papers, but this paper is a collective analysis and summary of the entire data set (2,13-22).

Table 1 summarizes subject characteristics for all male and female subjects in these studies. In general, the data indicate that these runners (age range, 19 to 69 years) were deeply committed to training for and competing in ultra-marathons. A stepwise, multiple regression model showed that the 160-km WSER race time (h) could be predicted by this equation: 12.57 + (0.126 x age) + (2.56 x gender) + (0.285 x BMI) – (0.01931 x km/week) (R^2 = 0.23) (male gender=1, female=2). Thus race time for a 20 yr old male with a 22.0 BMI, and a training distance of 130 km/wk would be estimated at 21.4 h compared to 28.5 h for a 55 year-old male with a 27 BMI and 65 km/wk training distance.

The 160-km western states endurance run

To enter one of the five WSER studies, subjects must have qualified for the 160-km WSER (held in late June each year) by completing a 160-km race in less than 24 h, or a 100-km race in 12-13 h, depending on age.

The 160-km Western States Endurance Run is a point-to-point trail run in the Sierra Nevada Mountains of northern California, and is regarded as one of the most arduous organized running events in the United States (17). The race starts at Squaw Valley, California (1.890 m altitude), and finishes at Auburn, California (366 m). The trail race course ascends 777 m to Emigrant Pass (2.668 m, the highest point) within the first 7 km and then passes through remote and rugged territory to Auburn. The total altitude gain and loss during the race is 5.500 m and 6.700 m, respectively. The race starts at 5:00 am, and runners have to reach the finish line within 30 hours to be eligible for an award. Up to half of the trail is traveled by some runners at night. A staff of over 1.300 volunteers supports the runners and works 26 aid stations, including 11 medical check points.

Major findings from the wser research projects

This chapter will review four key findings from the WSER research projects:

1. Twenty-four percent of ultra-marathoners studied reported upper respiratory tract infections (URTI) during the two-week period following the 160-km WSER. The best predictor of URTI was a low-post WSER salivary IgA secretion rate.
2. Plasma cytokine levels rose to high levels during the WSER, with the greatest fold increases occurring for IL-6 (~130x), IL-10 (~30x), G-CSF (~25x), IL-8 (~9x), and IL-1ra (~7x).
3. Increases in plasma cytokine levels during the WSER were highly variable between runners, with the largest increases measured in those experiencing the greatest muscle damage (CPK) and inflammation (CRP). Surprisingly, oxidative stress during the WSER was modest and not related to elevations in plasma cytokines.
4. Ibuprofen use was common among WSER competitors (70%). However, data indicate that ibuprofen users compared to nonusers experienced the same degree of muscle damage and soreness, and was linked to more endotoxemia, inflammation, kidney dysfunction, and higher plasma cytokines.

Upper respiratory tract infections

The majority of WSER endurance athletes reported that they felt protected from URTI compared to their sedentary peers. As depicted in Figure 1, 81% of WSER athletes surveyed claimed lower URTI rates in response to this question: "Compared to others who do no run or exercise do you feel that you generally have ___ episodes of sickness with the common cold or flu? However, this training-related protection from URTI was diminished during the two-week period following the summer 160-km race, with 24% reporting URTI (19). This rate is higher than the 13% recorded during the week following the Los Angeles Marathon held during late winter (23).

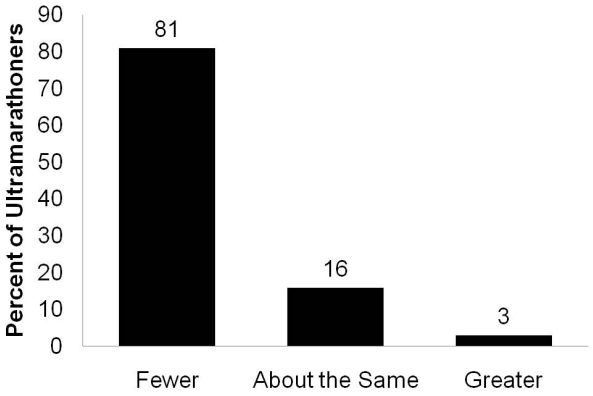

Fig 1. *Survey responses by WSER athletes in response to this question: "Compared to others who do no run or exercise do you feel that you generally have ___ episodes of sickness with the common cold or flu?*

Prolonged and intensive exertion causes numerous changes in immunity that reflect physiologic stress and immunosuppression, and an increased risk of URTI (2,8,23-25). There are multiple ways to measure immune dysfunction in endurance athletes following competitive marathon and ultramarathon race events. At the WSER, samples collected from the athletes had to be processed and stabilized for analysis later on at distant laboratories. Outcome measures that worked

within this context included blood leukocyte and lymphocyte subset cell counts, granulocyte oxidative burst activity, salivary IgA output, plasma cytokine levels, and blood leukocyte cytokine mRNA expression. This paper will summarize some of the important discoveries of immune dysfunction in WSER athletes. For example, in a subset of the WSER athletes, granulocyte oxidative burst activity was decreased by ~50% following the 160-km race (14) (Figure 2).

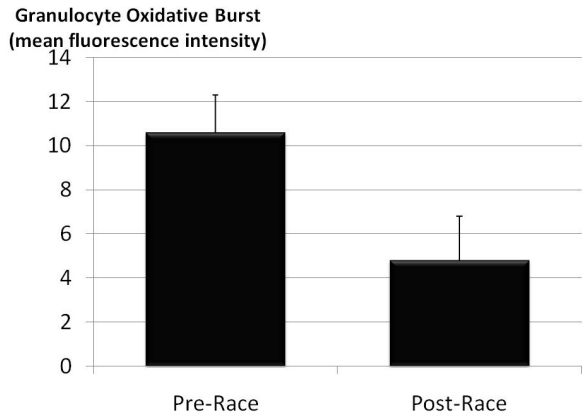

Fig 2. *Pre- and post-WSER levels of granulocyte oxidative burst activity*

Salivary IgA secretion rate has emerged as a potential marker of increased infection risk in endurance athletes (26-28). The secretory immune system of the mucosal tissues of the upper respiratory tract is considered the first barrier to colonization by pathogens, with IgA the major effecter of host defense. Individuals with selective IgA deficiency experience a high incidence of URTI, and a significant relationship between sIgA concentration and URTI incidence has been reported (29). Thus monitoring of mucosal immune parameters during critical periods of training and competition has been promoted as useful in predicting URTI risk in athletes (28).

Following intensive and prolonged exercise of more than 90 minutes duration by endurance athletes, the total sIgA output decreases in athletes. This was first reported by Tomasi et al. (30) in eight elite Nordic skiers following a national cross-country ski race competition, and later confirmed in studies of cyclers, marathon runners, triathletes, and other athletes (25,31,32). Steerenberg et al. (33) reported that salivary flow rate and total sIgA output (but not sIgA concentration) were reduced in 42 triathletes following an Olympic-distance triathlon race event. Little or no change in sIgA output has been measured in athletes following low-to-moderate intensity exercise of an intermittent nature (e.g., tennis drills or soccer play), indicating that the combination of high intensity and prolonged duration is necessary before significant decreases in this immune parameter are measured.

Salivary IgA secretion rate decreased by nearly half in the WSER ultramarathon runners following the 160-km race (14,20). Nearly one in four runners reported an URTI episode during the two week period following the race, and the decrease in sIgA secretion rate was significantly greater in these runners (54%) compared to those not reporting URTI (31%) (20). It is doubtful, however, that sIgA output alone can be used to predict URTI at the individual athlete level. If a 50% decrease in sIgA secretion rate is used as a threshold level indicating increased risk of post-race URTI, about half of non-URTI subjects would have been classified at risk (false positives) compared to about two-thirds of those reporting URTI (true positives), with an overall predictive value of 55% (see Figure 3). These proportions indicate that sIgA output is more useful at the group compared to the individual level, and that other factors need to be discovered and combined with sIgA before URTI risk can be predicted for individual athletes. In this group of 350 WSER athletes, however, none of the demographic, training, or immune factors differed between URTI and non-URTI groups, indicating that the search for other predictive factors may be complex.

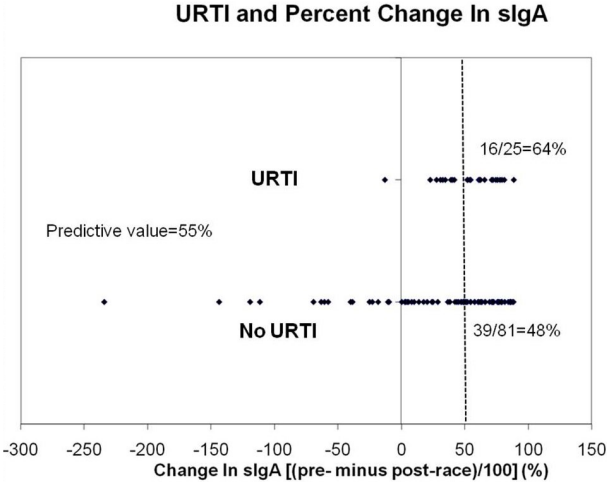

Fig 3. *Scatterplot comparison of the percentage change in salivary IgA output in athletes*

Plasma Cytokine Levels

Plasma concentrations of pro- and anti-inflammatory cytokines increase during prolonged and heavy exertion including interleukin (IL)-6, IL-10, IL-8, IL-1ra, granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 beta (MIP-1β), tumor necrosis factor-alpha (TNF-α), and macrophage migration inhibitory factor (MIF) (2,19,34).

Primary signaling mechanisms for cytokine gene expression during exercise are poorly understood, but data suggest that nitric oxide production is one key

regulator (35,36). Other potential triggers of cytokine release during extreme exercise include leakage of endoxtoxins (lipopolysaccharide or LPS) from the intestines, elevation in catecholamines and cortisol, high core body temperature, glycogen deficiency and other metabolic demands, muscle damage and inflammation, and oxidative stress (19,36).

At the 160-km WSER, the greatest fold-increase was experienced pre-to-post race for IL-6 (~130-fold) followed by IL-10 (30-fold), granulocyte colony-stimulating factor (G-CSF) (25-fold), IL-8 (9-fold), IL-1ra (7-fold), monocyte chemotactic protein 1 (MCP-1) (3-fold), macrophage inflammatory protein 1 beta (MIP-1β) (2-fold), macrophage migration inhibitory factor 1 (MIF-1) (1.5-fold), and tumor necrosis factor alpha (TNF-α) (1.3-fold) (2,17-19,21). See Figure 4. However, as shown in Figure 5, there was a large individual-to-individual variation in the change of plasma cytokine levels pre-to-post WSER. No difference in plasma cytokine changes was measured between male and female runners.

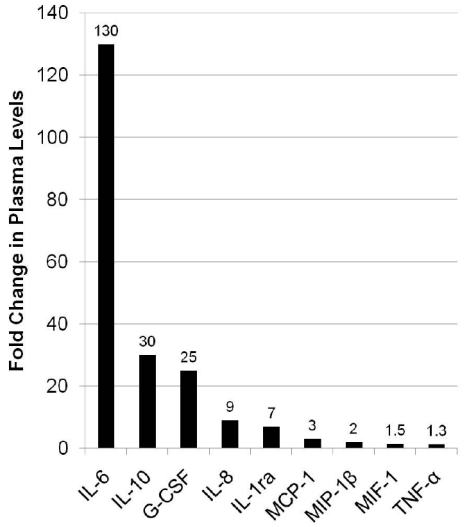

Fig 4. *Pre-to-post WSER fold changes in plasma cytokine levels*

Predictors of Variance in Plasma Cytokine Changes

This large variation in plasma cytokine response to running 160-km was unexpected. Ultramarathon competition is associated with significant muscle cell damage (37). Additional analysis of the entire dataset revealed that athletes with the greatest degree of muscle damage and inflammation (as measured by serum creatine kinase, CK, and C-reactive protein, CRP, respectively) experienced the highest post-race plasma levels for most of these cytokines (2,18,21). Figure 6 shows the large increases in CK (a) and CRP (b) that occurred during the WSER, and Figure 7 shows the scatterplot relationships of the change in IL-6 with

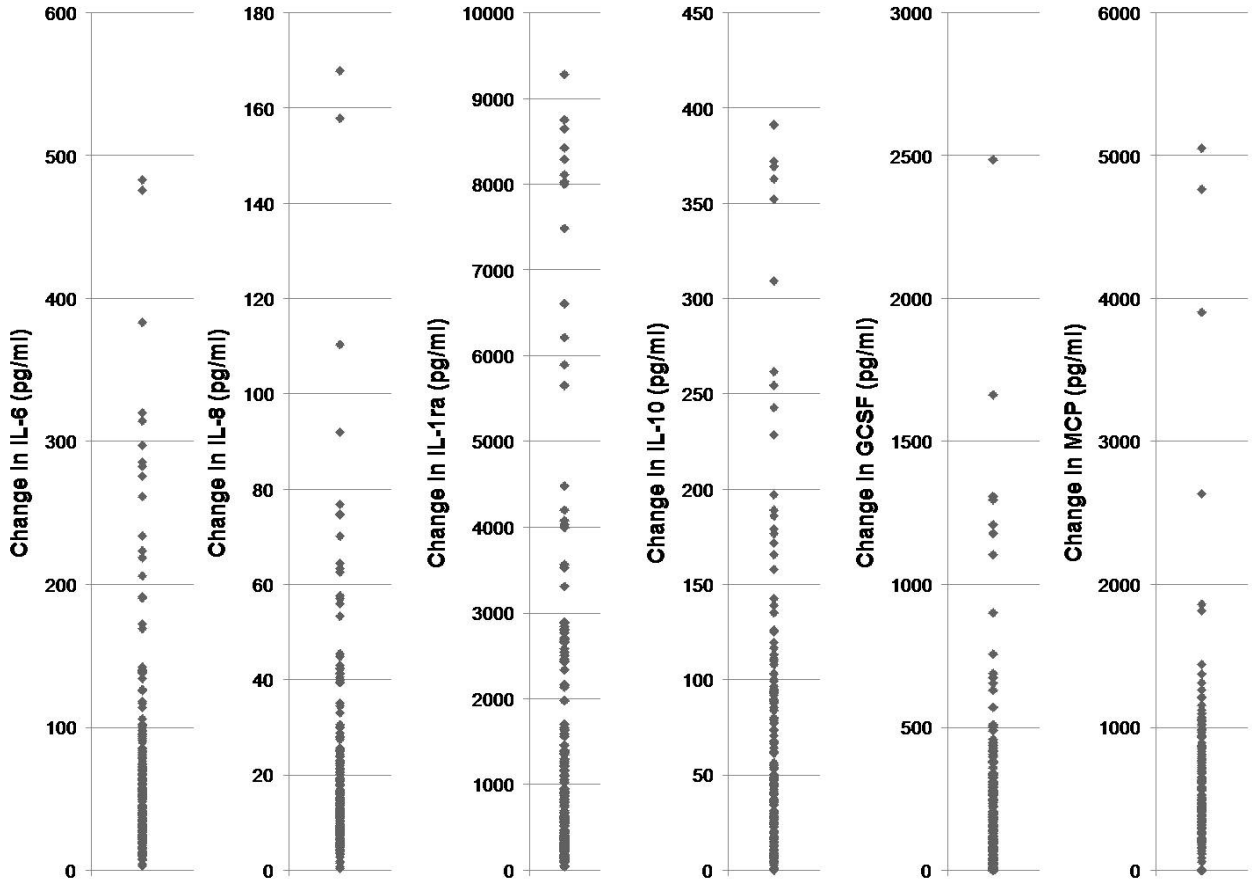

Fig 5. *Scatterplots of change in plasma levels of six cytokines in ultramarathon runners competing in the 160-km WSER*

CK (a) and G-CSF with CRP (b). During the week following the WSER, self-reported delayed onset of muscle soreness (DOMS) also showed modest positive correlations with many of the cytokines changes experienced during the race (18,21).

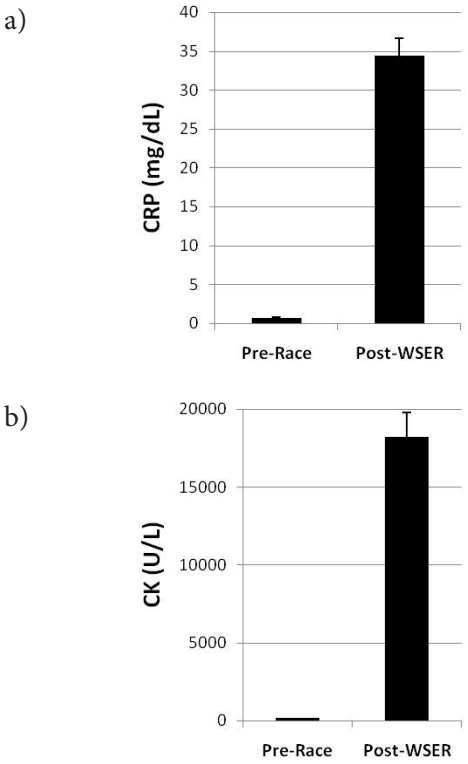

Fig 6. *Pre- and post-WSER serum levels of C-reactive protein (CRP) (a) and creatine kinase (CK) (b)*

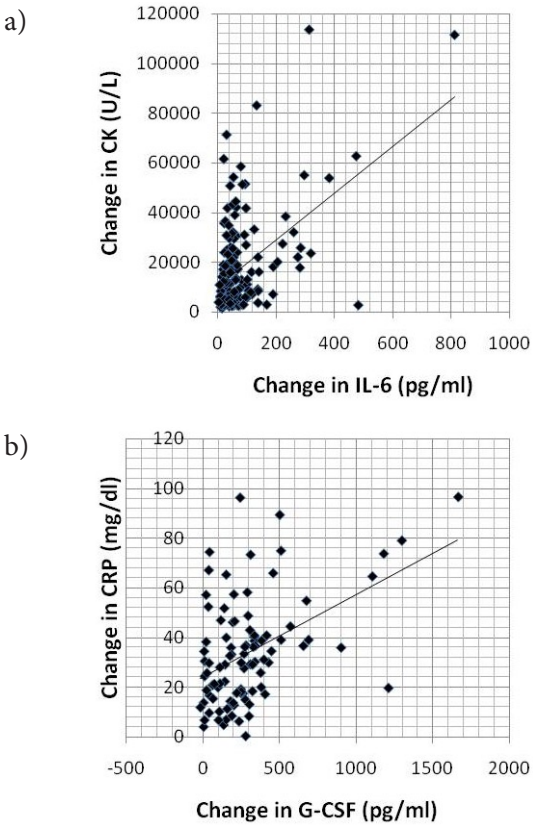

Fig 7. *Scatterplots of change in plasma IL-6 with serum CK (a), and change in plasma G-CSF and serum CRP (b)*

Contrary to expectations, the oxidative stress experienced by the WSER athletes was modest (Figure 8a) and poorly correlated with cytokine, CK, and CRP changes (15,16,22). Figure 8b shows the low correlation between change in plasma IL-6 and F2-isoprostanes (r=-0.010, *P*=0.918). Small but significant correlations between changes in plasma F2-isoprostanes and IL-10 (r=0.21, *P*=0.024), MCP (r=0.31, *P*=0.003), and IL-1ra (r=0.18, *P*=0.045) were measured. In general, however, the large variance in plasma cytokine levels had little relationship to oxidative stress. Acute physical activity is typically marked by an identifiable oxidative stress within the blood plasma, and is accompanied by changes in blood redox status (22,28). However, the endogenous anti-oxidant enzyme defenses of the WSER athletes appear capable of keeping oxidative stress at low levels during 160-km of race competition through mountainous terrain and undue environmental stress.

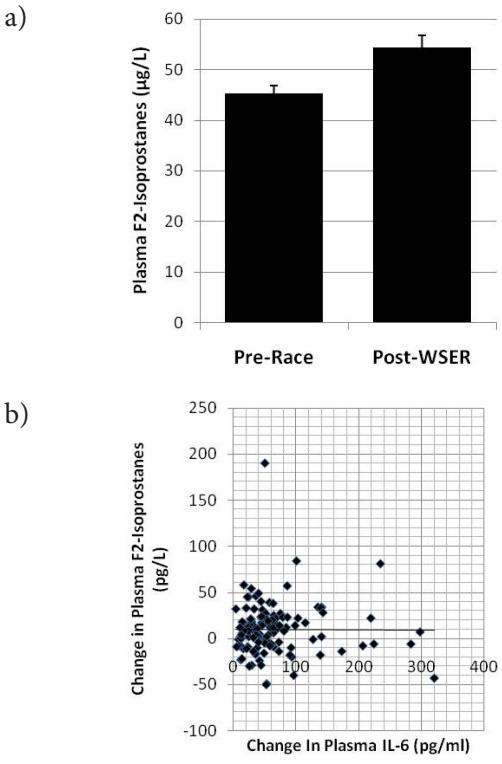

Fig 8. *Pre- and post-WSER plasma levels of F2-isoprostanes (a), and scatterplot relationship between pre-to-post race change in plasma IL-6 and F2-isoprostanes (b)*

Exercise-induced increases in cytokines are produced by multiple cell types both within and outside the immune system (39-41). Several laboratory studies indicate that IL-6, IL-8, IL-1β, and TNF-α mRNA content is increased within post-exercise muscle biopsy samples, with the greatest fold increases measured for IL-6 and IL-8 mRNA (34). Laboratory studies also indicate that blood leukocytes may provide produce IL-8, IL-10, and IL-1ra during sustained exercise (19). Figure 9 shows that blood leukocyte IL-10 mRNA

increased to high levels during the race in contrast to a sharp decrease in leukocyte IL-8 mRNA (2). The marked decrease in leukocyte IL-8 and increase in IL-10 mRNA expression are notable findings that differ from the mild increases measured in the laboratory setting, and suggest an attempt by the immune system to limit polymorphonuclear cell adherence, degranulation, respiratory burst activity, and inflammation when the athlete is already experiencing significant muscle cell damage (2,19).

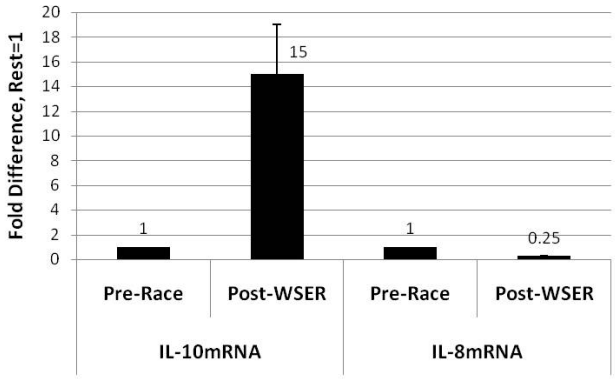

Fig 9. *Fold change in blood leukocyte mRNA for IL-10 (a), and IL-8 (b)*

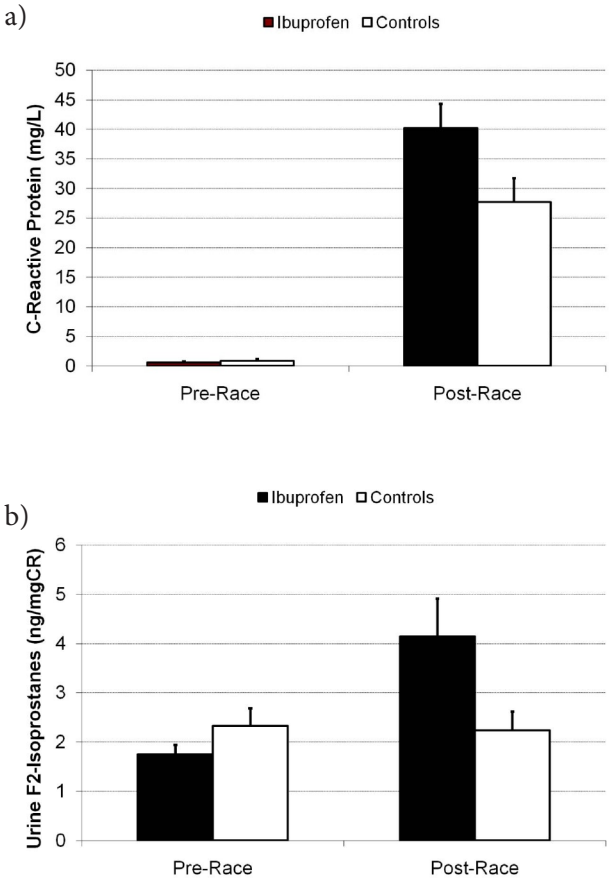

Fig 10. *Comparison of pre- and post-race levels of serum CRP (a), and urine F2-isoprostanes (b) in ibuprofen users and non-ibuprofen users (controls)*

Negative Effects of Ibuprofen Use

Ibuprofen was the most common medication used by WSER runners, with seven in ten reporting use during the race (18,21). Ibuprofen users (600 mg and 1,200 mg ibuprofen the day before and during the race, respectively) and nonusers were compared pre- and post-WSER for inflammatory parameters, plasma cytokines, urine creatinine, and LPS. Ibuprofen use was associated with 25% to 88% higher plasma levels of seven cytokines, and significant elevations in blood neutrophils counts and serum CRP (Figure 10a), urine F2-isoprostanes (Figure 10b), alanine and asparate aminotransferase (ALT, AST), and blood urea nitrogen (BUN) (15,16,18,21). Pre- and post-race plasma LPS combined was 106% higher in the ibuprofen compared to control athletes, and was positively correlated with CRP, cortisol, and three of eight cytokines measured in this study. No differences in race time, ratings of perceived exertion (RPE), gastrointestinal discomfort, muscle damage, or perceptions of muscle soreness were found between ibuprofen and control groups.

Significant increases in plasma LPS have been reported in athletes following prolonged exercise in some studies (42,43), but in most studies increases are small or absent (44-46). When it does occur, exercise-induced endotoxemia has been hypothesized to be related to splanchnic ischemia and hyperthermia (47). Cortisol tended to increase more in the ibuprofen compared to control group, and was correlated with change in six of eight cytokines. Thus group differences in plasma cytokine levels may have been partly related to cortisol influences. Farquhar et al. (48) have shown that ibuprofen ingestion has a small but significant effect in decreasing glomerular filtration rate during exercise. Thus clearance of plasma cytokines by the kidney may have been decreased in athletes using ibuprofen, as supported by the urine creatinine and BUN data. Post-race serum ALT and AST levels were significantly higher in athletes using ibuprofen compared to controls. These data indicate the potential for higher muscle and liver cell enzyme release with ibuprofen use. Significant, positive relationships between CK and most cytokines measured in the ibuprofen users was probably related to several factors including elevated LPS, a tendency for higher cortisol levels, decreased kidney clearance, increased muscle and liver cell release of enzymes, and other unmeasured parameters.

Ibuprofen use did not attenuate plasma CK levels or post-race DOMS in the WSER athletes. A majority of other investigators have also reported no beneficial effect ibuprofen or other NSAIDS in alleviating muscle soreness and damage after contraction-induced muscle injury (49-52). Thus the high prevalence of ibuprofen use by ultramarathon athletes appears to have few if any physiological or performance benefits (18,21).

Conclusions

The 160-km WSER is an arduous mountain race in California reserved for the top echelon of ultramarathon athletes. Over a span of five years, 350 athletes have been studied, with immunity, inflammation, and oxidative stress measures obtained from pre- and post-race blood, urine, and saliva samples. Subjects also provided URTI, training, and demographic data. Several key findings from an analysis of the entire 5-year dataset revealed the following:

1. One out of four runners reported sickness during the 2-week period after the 160-km race. Post-race values for granulocyte oxidative burst activity were reduced 50% compared to pre-race. Of all outcome measures, only low post-race sIgA secretion rate was significantly linked to URTI. However, the predictive value was too low to be of use at the individual level.
2. The variance in plasma cytokine changes during the race was large between WSER finishers, with no male-to-female differences. The cytokine profile measured indicates a strong anti-inflammatory response.
3. The most important link to the level of plasma cytokine perturbations was muscle damage and inflammation. Athletes with the greatest serum CK and CRP levels experienced the highest post-race plasma cytokine levels. Unexpectedly, cytokine variance was unrelated to oxidative stress. Post-race blood leukocyte mRNA expression for IL-10 was unusually high in comparison to very low values for IL-8 reflecting the immune system's effort to dampen inflammation.
4. Despite the widespread use of ibuprofen by WSER athletes, no beneficial effects were measured for reducing RPE, muscle damage, or DOMS. To the contrary, ibuprofen use was linked to mild endotoxemia, kidney dysfunction, and systemic inflammation.

In general, the WSER data indicate that competitors experience significant immune system stress, reflecting the physiological trauma experienced by the athletes. The immune and cytokine perturbations measured in ultramarathon runners at the WSER are comparable to those measured in marathon runners after running 42.2-km marathon races, but the long duration of the race means that the physiologic and immune stress is sustained for 5-10 times longer. Some of WSER athletes have competed in more than 100 ultramarathon races, and the long-term impact on health and disease remains to be determined. To reduce the pain of running 160-km in the Sierra Nevadas, the majority of WSER athletes use ibuprofen. However, every indication is that ibuprofen amplifies inflammation and oxidative stress while providing no relief from exercise effort or muscle damage and soreness.

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Authors' contribution
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection