

Ibuprofen use, endotoxemia, inflammation, and plasma cytokines during ultramarathon competition

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Abstract

The primary purpose of this study was to measure the influence of ibuprofen use during the 160-km Western States Endurance Run on endotoxemia, inflammation, and plasma cytokines. Subjects included 29 ultramarathoners who consumed 600 and 1200 mg ibuprofen the day before and on race day, respectively, and 25 controls that competed in the race but avoided ibuprofen and all other medications. Blood and urine samples were collected the morning prior to and immediately following the race, and subjects recorded muscle soreness during the week following the race using a 10-point Likert scale (DOMS). Race time ($25.8 \pm .6$ and $25.6 \pm .8$ h, respectively) and ratings of perceived exertion (RPE, 6–20 scale) ($14.6 \pm .4$ and $14.5 \pm .2$, respectively) did not differ significantly between ibuprofen users and nonusers. Ibuprofen use compared to nonuse was linked to a smaller increase in urine creatinine ($P = .038$), higher plasma levels of lipopolysaccharide (group effect, $P = .042$), and greater increases (pre-to-post race) in serum C-reactive protein and plasma cytokine levels for interleukin (IL)-6, IL-10, IL-8, IL-1ra, granulocyte colony-stimulating factor, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1 beta, but not tumor necrosis factor alpha. Post-race DOMS and serum creatine kinase levels did not differ significantly between ibuprofen users and nonusers ($20,621 \pm 3565$ and $13,886 \pm 3068$ $\mu\text{cal/L}$, respectively, $P = .163$). In conclusion, ibuprofen use compared to nonuse by athletes competing in a 160-km race did not alter muscle damage or soreness, and was related to elevated indicators of endotoxemia and inflammation.

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1. Introduction

Athletes competing in marathons and ultramarathons experience large increases in plasma cytokine levels including interleukin (IL)-6, IL-10, IL-1ra, IL-8, granulocyte colony-stimulating factor or G-CSF, monocyte chemoattractant

protein 1 or MCP-1, and macrophage inflammatory protein 1 beta or MIP-1 β (Nieman et al., 2001, 2002, 2003, 2005; Ostrowski et al., 2000; Suzuki et al., 2000, 2002).

Exercise-induced increases in these cytokines vary substantially between athletes (Nieman et al., 2001, 2005). Potential triggers of cytokine release during exercise include leakage of endotoxins (lipopolysaccharide or LPS) from the intestines during exercise, elevation in catecholamines and cortisol, high core body temperature, glycogen deficiency, and other metabolic demands, oxidative

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stress, and muscle damage (Bruunsgaard et al., 1997; Camus et al., 1997, 1998; Jeukendrup et al., 2000; Nieman et al., 2001, 2002, 2003, 2005; Ostrowski et al., 2000; Steensberg, 2003; Steinacker et al., 2004; Suzuki et al., 2000, 2002).

We recently reported that muscle damage, perceptions of muscle soreness, and plasma levels of several inflammatory cytokines were positively correlated in ultramarathon athletes following a 160-km race event (Nieman et al., 2005). Unexpectedly, pre-to-post race changes in IL-6, IL-8, G-CSF, MCP-1, and MIP-1 β were two to three times greater in runners reporting use of non-steroidal anti-inflammatory drugs (NSAIDs) during the race compared to nonusers. Plasma creatine kinase (CK) levels and post-race delayed onset of muscle soreness (DOMS) did not differ between NSAID users and nonusers. A majority of other investigators have also reported no beneficial effect of NSAIDs in alleviating muscle soreness and damage after contraction-induced muscle injury (Donnelly et al., 1990; Peterson et al., 2003; Trappe et al., 2002).

We are unaware of other published studies indicating elevated cytokines in NSAID users compared to nonusers following ultramarathons. Increased gastrointestinal permeability and translocation of bacterial lipopolysaccharide (LPS) from the intestines into the circulation (i.e., endotoxemia) due to splanchnic ischemia and hyperthermia have been reported in athletes following prolonged endurance events (Bosenberg et al., 1988; Brock-Utne et al., 1988; Jeukendrup et al., 2000; Lambert, 2004; Lambert et al., 2001; Van Nieuwenhoven et al., 2004). Gastrointestinal permeability is amplified in marathoners using ibuprofen and other NSAIDs compared to nonusers (Ryan et al., 1996; Smetanka et al., 1999). Camus et al. (1998) demonstrated a relationship between exercise-induced endotoxemia and TNF- α . Thus NSAID use during ultramarathons may augment cytokine increases by inducing endotoxemia, but these relationships have not yet been verified using appropriate research designs. Ibuprofen ingestion has a small but significant effect in decreasing glomerular filtration rate during exercise suggesting that kidney clearance of various factors including cytokines is reduced (Farquhar et al., 1999).

In our previous study showing a linkage between ibuprofen use and elevated plasma cytokines, the ibuprofen dose was not controlled or reported, and the major objective was to establish a relationship between muscle damage and post-race cytokine levels (Nieman et al., 2005). As a follow-up to this study, we designed a study in which athletes used 600 and 1200 mg ibuprofen the day before and during the race, respectively, and compared inflammatory parameters, plasma cytokines, urine creatinine, and LPS with nonusers. We hypothesized that ibuprofen use during a 160-km race would augment endotoxemia leading to increased systemic inflammation and plasma cytokine levels.

2. Methods

2.1. Subjects and race description

Sixty-three experienced male and female ultramarathoners from the 2005 Western States 100 Mile Endurance Run were recruited and provided pre-race blood and urine samples. Athletes were placed into ibuprofen ($n=33$) and control groups ($n=30$) based on their historical use during training and competition, and their willingness to use or avoid ibuprofen before and during the race. Permission for a randomized, placebo controlled research design was not granted by the race medical board because of ethical concerns regarding compliance in athletes suffering from pain during the latter stages of the race. Fifty-four subjects ($n=29$ in the ibuprofen group, $n=25$ for controls) completed the race and provided post-race blood and urine samples. Informed consent was obtained from each subject, and the experimental procedures were approved by the institutional review board of Appalachian State University. To enter the study, subjects must have completed a 160-km race, and qualified for the 2004 160-km Western States Endurance Run. To qualify for the Western States Endurance Run, runners must have completed 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. The race starts at Squaw Valley, California (1890 m altitude), and finishes at Auburn, California (366 m). The trail race course ascends 777 m to Emigrant Pass (2668 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 5500 and 6700 m, respectively. The race starts at 5:00 am, and runners must reach the finish line within 30 h to be eligible for an award. Up to half of the trail may be traveled by some runners at night.

2.2. Research design

Subjects provided urine and blood samples during registration, held the morning before the race, and 5–15 min post-race. Urine and plasma samples were transported on dry ice and then stored at -80°C until analysis. Pre-race body mass and percent body fat (via 3-site skinfolds) were measured, and subjects filled in a questionnaire on basic demographics and training history during training. Subjects in the ibuprofen group ingested 600 mg (three 200 mg tablets) during the afternoon prior to the race, and 1200 mg on race day (six 200 mg tablets, with one taken pre-race and one approximately every four hours thereafter). Subjects in the control group avoided use of all medications including ibuprofen, and subjects in the ibuprofen group avoided all other medication use. On race day, body mass was measured at the 90-km aid station (Michigan Bluff, 1220 m) and within 5–15 min post-race at Auburn. Subjects completed a post-race questionnaire indicating adherence to the research design. Subjects consumed food and beverages ad libitum during the race.

2.3. Blood cell counts, diagnostic chemistry panel, C-reactive protein, creatine phosphokinase, and urine creatinine

Blood samples were drawn from an antecubital vein with subjects in the seated position. Complete blood counts and differentials were measured using a Coulter STKS instrument (Coulter Electronics, Inc., Hialeah, FL). The comprehensive diagnostic chemistry panel, C-reactive protein (CRP), and creatine phosphokinase (CK) were measured in a clinical laboratory using an LX-20 clinical analyzer (Beckman, Brea, Calif., USA). Urinary creatinine concentration was measured by the sodium picrate method with an AutoAnalyzer II (Technicon, Tarrytown, NY). Plasma volume changes were estimated using the method of Dill and Costill (1974).

2.4. Cytokine measurements

Total plasma concentrations of interleukin-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10),

granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 beta (MIP-1 β), and tumor necrosis factor alpha (TNF- α) were determined using quantitative sandwich ELISA kits provided by R&D Systems, Inc. (Minneapolis, MN). All samples and provided standards were analyzed in duplicate. High sensitivity kits were used to analyze TNF- α and pre-race samples of IL-6 and G-CSF. The minimum detectable concentration of IL-1ra was <22 pg ml⁻¹, IL-6 <.70 pg ml⁻¹, IL-6 (high sensitivity) <.094 pg ml⁻¹, IL-8 <10 pg ml⁻¹, IL-10 <3.9 pg ml⁻¹, G-CSF <20.0 pg ml⁻¹, G-CSF (high sensitivity) <.80 pg ml⁻¹, MCP-1 <5.0 pg ml⁻¹, MIP-1 β <11.0 pg ml⁻¹, and TNF- α <.12 pg ml⁻¹. Due to the lack of high-sensitivity kits for IL-8 and IL-10, we extrapolated data below the minimum detectable level using a software program suited to this task. When applicable, pre- and post-exercise samples were analyzed on the same assay plate to decrease inter-kit assay variability.

2.5. LPS and cortisol

Plasma bacterial endotoxin was measured using the *Limulus* amoebocyte lysate (LAL) assay (KQCL, Cambrex, Walkersville, MD). The method uses a kinetic LAL assay yielding data as endotoxin units (Eu ml⁻¹), which reflect the biological activity of the bacterial endotoxins rather than sample concentrations in plasma. Briefly, 100 μ l of each plasma sample was diluted in 200 μ l of β -G-Blocker (Cambrex, Walkersville, MD) to eliminate the possibility of false positives. β -1,3 glucans present in plasma may result in an LAL response that is independent of the endotoxin mediated response. Samples were further diluted with 100 μ l of pyrogen free water (PFW) to give a final dilution of 1:4. Samples were then placed in a water bath at 75 °C for 15 min. Standard endotoxin was dissolved in PFW yielding a concentration of 50 Eu ml⁻¹, 1:10 serial dilutions were prepared in pyrogen free tubes to give 5.0, .5, .05, and .005 Eu ml⁻¹. One hundred microliters of each standard and sample were placed in duplicate wells in a microplate and placed in the holder of the microplate reader, which had been previously warmed to 37 °C for 10 min. One hundred microliters of freshly prepared kinetic-QCL reagent was placed in each well. The assay was then read at 150 s intervals for a total of 40 reads at a wavelength of 405 nm and temperature of 37 °C. Using Softmax Pro software (Sunnyvale, CA) the absorbance was monitored throughout the assay. Using the initial absorbance reading of each well as its own blank, the time required for the absorbance to increase .200 absorbance units was determined. A log/log linear correlation of the reaction time of each standard with its corresponding endotoxin concentration was generated from which the endotoxin concentration from each sample was determined.

Plasma concentrations of cortisol were determined using competitive solid-phase ¹²⁵I radioimmunoassay (RIA) technique (Diagnostic Products Corporation, Los Angeles, CA) with cortisol-specific Ab coated tubes (Coat-A-Count tubes). Intra-assay (CV_{intra}) and inter-assay (CV_{inter}) coefficients of variation were 4.5% and 5%, respectively. Assay sensitivity was 5.5 nmol/L (.2 μ g/dl).

2.6. Delayed onset of muscle soreness

Subjects recorded muscle soreness following the race, and during the week following the race using a 10-point Likert scale (Smith et al., 1993). Runners were asked to supply a number that best described any general feeling of painful, sore, aching leg muscles using this scale, 1 (no soreness), 2.5 (dull, vague ache), 4 (slight soreness), 5.5 (more than slight soreness), 7 (sore), 8.5 (very sore), and 10 (unbearably sore).

2.7. Gastrointestinal discomfort scale

During collection of the blood sample post-race, subjects were asked to indicate a rating of gastrointestinal discomfort using a 10-point Likert scale for symptoms experienced at 50, 90, 125 km, and at the finish line. Runners were asked to supply a number that best described any feelings of stomach and intestinal discomfort including stomach upset, nausea, bloating, heartburn, throatburn, or similar symptoms using this scale, 1 (no dis-

comfort), 2.5 (vague discomfort), 4 (slight discomfort), 5.5 (more than slight discomfort), 7 (great discomfort), 8.5 (very great discomfort), and 10 (unbearable discomfort).

2.8. Rating of perceived exertion

During the pre-race blood sampling session, subjects were provided a hand-held laminated RPE scale (6–20) with instructions to rate their perceived exertion at aid stations located at 40, 90, 125, 150, and 160 km (finish line). During collection of the blood sample post-race, subjects were asked to supply a number that best described intensity of effort, stress, discomfort, and/or fatigue at the listed points on the race course using this scale, 7 (very, very light), 9 (very light), 11 (fairly light), 13 (somewhat hard), 15 (hard), 17 (very hard), and 19 (very, very hard).

2.9. Statistical analysis

Data are expressed as means \pm SE, and analyzed using a 2 \times 2 repeated measures ANOVA. DOMS data were analyzed using a 2 \times 8 repeated measures ANOVA. Changes from pre-race to post-race values were calculated and compared between ibuprofen and control groups using student's *t* tests, with significance set at $P \leq .05$. Comparisons between genders were conducted using student's *t* tests. Pearson product-moment correlations were used to test the relationship between changes in measured outcomes, with an emphasis on CK, DOMS, LPS, and cytokines.

3. Results

Fifty-four of 63 subjects completed the 160-km race event. Air temperature was 10 °C at the start of the race (Squaw Valley, CA), 25 °C by 2:00 pm (Michigan Bluff), 15 °C by 12:00 am (race finish, Auburn, CA), and 11 °C by 10:00 am. The average humidity during the last half of the race was 56%. Subject characteristics for ibuprofen and control groups are compared in Table 1, and indicate no significant differences in age, body composition, training and racing history, and race time. Male ($n=43$) and female ($n=11$) runners did not differ significantly in race time (25.4 \pm .6 vs 27.2 \pm .76 h, respectively, $P=.141$) or any of the other variables measured in this study except for those related to body mass and composition. Thus male and female runners were combined for this data analysis.

Plasma volume did not change appreciably, and did not differ significantly between groups ($-1.6 \pm .4\%$ and $-1.2 \pm .3\%$, respectively, $P=.381$), and body mass was maintained near pre-race levels for both groups (Table 1).

Plasma LPS did not change in either group (time effect, $P=.225$, group \times time interaction effect, $P=.378$) (Fig. 1). A significant group effect ($P=.042$) indicated higher plasma LPS levels in the ibuprofen compared to control group when combining pre- and post-race data. LPS tended to be higher pre-race ($P=.078$) in the ibuprofen compared to control group. Pre-race cytokine/chemokine levels and all other measures were not different between groups except for IL-1ra ($P=.009$).

The increase in blood leukocytes and neutrophils, but not lymphocytes and monocytes, was significantly lower in the ibuprofen compared to control groups (Table 2).

Table 1
Subject characteristics in ibuprofen ($n = 29$) and control ($n = 25$) groups (means \pm SE; range)

Variable	Ibuprofen group	Control group	P-value
Age (year)	47.9 \pm 1.4	46.8 \pm 2.1	.657
Height (m)	1.75 \pm .02	1.77 \pm .02	.274
Pre-race body mass (kg)	70.8 \pm 2.1	70.7 \pm 2.0	.978
90-km (kg)	70.0 \pm 2.1	71.1 \pm 1.8	.691
160-km (kg)	70.5 \pm 2.1	71.0 \pm 1.7	.851
Body fat (%)	17.8 \pm 1.2	16.1 \pm 1.1	.294
Running history (year)	16.0 \pm 1.7	16.5 \pm 1.5	.825
Ultramarathons raced (number)	50.6 \pm 12.5	41.3 \pm 5.7	.505
Running distance (km/week)	87.1 \pm 6.7	76.3 \pm 6.2	.244
Race time, 160-km (h)	25.8 \pm .6	25.6 \pm .8	.863

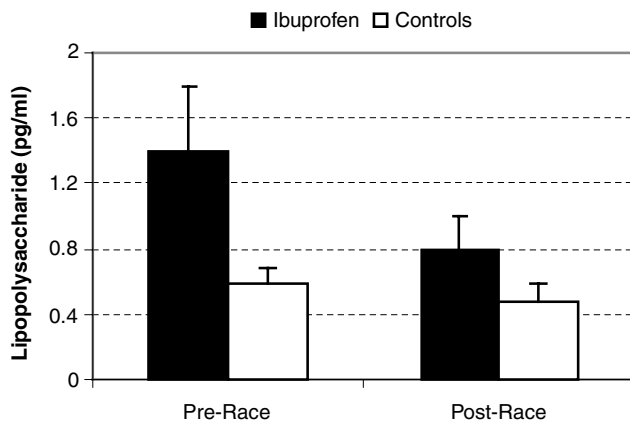


Fig. 1. Plasma bacterial LPS in ibuprofen users and nonusers before and after the 160-km Western States Endurance Run (group effect, $P = .042$; time effect, $P = .225$; group \times time effect, $P = .378$).

Pre-to-post-race increases in serum cortisol did not differ significantly between ibuprofen and control groups (Table 3). Of the variables included in the comprehensive diagnostic chemistry panel, significantly greater increases were measured in the ibuprofen compared to control group for C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (Table 3). No significant group differences were measured for pre-to-post race changes in creatine kinase (CK) (Table 3).

Pre-to-post race increases in IL-6, IL-10, G-CSF, IL-1ra, IL-8, MCP-1, and MIP-1 β , but not TNF- α , were significantly greater in the ibuprofen compared to control group (Table 4). Groups did not differ in ratings for DOMS on race day or the week after the race (group \times time interaction, $P = .634$) (Fig. 2). Average ratings for gastrointestinal discomfort (mean of four ratings) did not differ significantly between ibuprofen and control groups ($3.2 \pm .4$ and $2.8 \pm .3$, $P = .398$, respectively). Average RPE (mean of five ratings) did not differ significantly between ibuprofen and control groups ($14.6 \pm .4$ and $14.5 \pm .2$, respectively, $P = .726$). The increase in urine creatinine concentration was significantly lower in ibuprofen users compared to nonusers (group effect, $P = .003$; time effect, $P < .001$; group \times time effect, $P = .038$) (Fig. 3).

Mean LPS (pre- and post-race average, all subjects combined) was significantly correlated with post-race values for serum CRP ($r = .35$, $P = .009$), plasma cortisol ($r = .35$, $P = .009$), IL-8 ($r = .28$, $P = .039$), IL-10 ($r = .368$, $P = .006$), and MCP-1 ($r = .292$, $P = .032$), but not with post-race levels for IL-6, G-CSF, IL-1ra, MIP-1 β , TNF- α , GI discomfort, or RPE. Significant correlations were measured (all subjects combined) between pre-to-post race changes in CK and ALT ($r = .94$, $P < .001$), AST ($r = .96$, $P < .001$), CRP ($r = .40$, $P = .003$), IL-6 ($r = .45$, $P = .001$), G-CSF ($r = .42$, $P = .002$), MCP-1 ($r = .55$, $P < .001$), IL-10 ($r = .37$, $P = .007$), IL-8 ($r = .27$, $P = .047$), and IL-1ra ($r = .27$,

Table 2
Pre- and post-race (160-km) leukocyte subset blood counts in ibuprofen ($n = 29$) and control ($n = 25$) groups (means \pm SE)

Variable ($10^9/l$)	Pre-race	Post-race	Interaction effect, P-value
Total leukocytes			
Ibuprofen	5.67 \pm .28	15.1 \pm .8*	.036
Controls	5.78 \pm .30	13.3 \pm .7	
Neutrophils			
Ibuprofen	3.51 \pm .25	12.2 \pm .8*	.018
Controls	3.66 \pm .25	10.1 \pm .5	
Lymphocytes			
Ibuprofen	1.59 \pm .09	1.39 \pm .09	.200
Controls	1.50 \pm .08	1.45 \pm .09	
Monocytes			
Ibuprofen	.44 \pm .03	1.15 \pm .08	.415
Controls	.46 \pm .03	1.09 \pm .07	

* $P < .05$, change from pre-race in ibuprofen compared to control group.

Table 3
Pre- and post-race (160-km) serum cortisol, C-reactive protein, creatine kinase, and diagnostic chemistries in ibuprofen ($n=29$) and control ($n=25$) groups (means \pm SE)

Variable	Pre-race	160-km	Interaction effect, P -value
Cortisol (nmol/L)			
Ibuprofen	350 \pm 24	788 \pm 71	.153
Controls	393 \pm 17	679 \pm 62	
C-reactive protein (mg/L)			
Ibuprofen	.57 \pm .12	40.2 \pm 4.1*	.031
Controls	.81 \pm .29	27.7 \pm 4.0	
Creatine kinase (μkat/L)			
Ibuprofen	2.12 \pm .18	344 \pm 59	.163
Controls	2.62 \pm .30	231 \pm 51	
Alanine aminotransferase (μkat/L)			
Ibuprofen	.46 \pm .03	2.52 \pm .37*	.027
Controls	.49 \pm .04	1.53 \pm .21	
Aspartate aminotransferase (μkat/L)			
Ibuprofen	.54 \pm .03	10.8 \pm 1.9*	.039
Controls	.50 \pm .03	5.97 \pm 1.13	
Blood urea nitrogen (mmol/L)			
Ibuprofen	4.50 \pm .25	11.3 \pm .8*	.037
Controls	4.68 \pm .32	9.46 \pm .57	

* $P < .05$, change from pre-race in ibuprofen compared to control group.

$P = .047$), but not with MIP-1 β or TNF- α . The correlation between pre- to post-race change in cortisol (all subjects combined) was significantly correlated with changes in all measured cytokines ($r = .29-.72$, $P = .033$ to $P < .001$) except for MCP ($r = .10$, $P = .455$) and TNF- α ($r = -.16$, $P = .230$), neutrophil counts ($r = .50$, $P < .001$), and BUN ($r = .03$, $P = .016$), but not CRP ($r = .05$, $P = .700$), ALT ($r = .10$, $P = .478$), or ALK ($r = .14$, $P = .311$).

4. Discussion

Ibuprofen use compared to nonuse the day before (600 mg) and during (1200 mg) a 160-km race by ultramarathon athletes was associated with 25–88% higher plasma levels of seven cytokines, and significant elevations in blood neutrophils counts and serum CRP, ALT, AST, and BUN. 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, RPE, gastrointestinal discomfort, muscle damage, or perceptions of muscle soreness were found between ibuprofen and control groups.

We were not able to conduct a double-blinded, placebo controlled study due to ethical concerns by the 160-km Western States Endurance Run medical board. Athletes were divided into ibuprofen and control groups based on their historical use during both training and competition, and the willingness of the experimental group to ingest ibuprofen according to the prescribed dosage pattern. Unexpectedly, pre-race LPS levels tended to be higher in the ibuprofen compared to control group even though the pre-race blood sample was taken prior to ibuprofen ingestion.

Table 4
Pre- and post-race (160-km) plasma cytokine levels in ibuprofen ($n=29$) and control ($n=25$) groups (means \pm SE)

Variable (pg/ml)	Pre-race	160-km	Interaction effect, P -value
IL-6			
Ibuprofen	.86 \pm .11	75.0 \pm 11.8*	.034
Controls	.94 \pm .14	45.7 \pm 6.1	
IL-10			
Ibuprofen	1.52 \pm .09	125 \pm 20.4*	.039
Controls	1.34 \pm .10	69.5 \pm 14.8	
G-CSF			
Ibuprofen	14.1 \pm 4.2	460 \pm 50*	.001
Controls	16.8 \pm 2.5	265 \pm 17	
IL-1ra			
Ibuprofen	233 \pm 27	1381 \pm 188*	.020
Controls	156 \pm 8	734 \pm 129	
IL-8			
Ibuprofen	3.80 \pm .56	20.0 \pm 1.5*	.001
Controls	3.35 \pm .54	12.6 \pm 1.3	
MCP-1			
Ibuprofen	300 \pm 19	993 \pm 52*	.029
Controls	302 \pm 24	795 \pm 65	
MIP-1β			
Ibuprofen	30.0 \pm 3.7	80.7 \pm 8.5*	.012
Controls	29.2 \pm 2.2	57.1 \pm 4.6	
TNF-α			
Ibuprofen	1.16 \pm .21	2.01 \pm .50	.132
Controls	1.49 \pm .37	1.63 \pm .32	

* $P < .05$, change from pre-race in ibuprofen compared to control group.

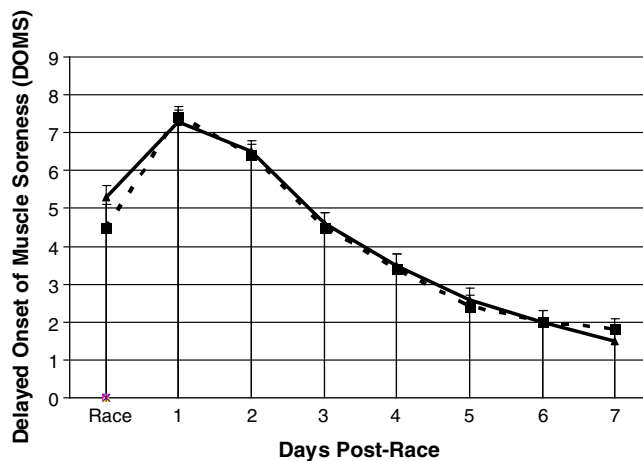


Fig. 2. Delayed onset of muscle soreness in ibuprofen users and nonusers during the week following the 160-km Western States Endurance Run (group \times time effect, $P = .634$). Ibuprofen group, solid line with triangular marks; controls, dotted line with square marks.

The half-life of ibuprofen is approximately five hours, but habitual use of ibuprofen during training may have induced chronic alterations (e.g., deleterious effects on the gastrointestinal tract) that influenced our results.

Within this context, our data indicate that ibuprofen use by ultramarathon athletes does not diminish muscle damage or perceptions of muscle soreness, and promotes mild endotoxemia, systemic inflammation, and elevations in

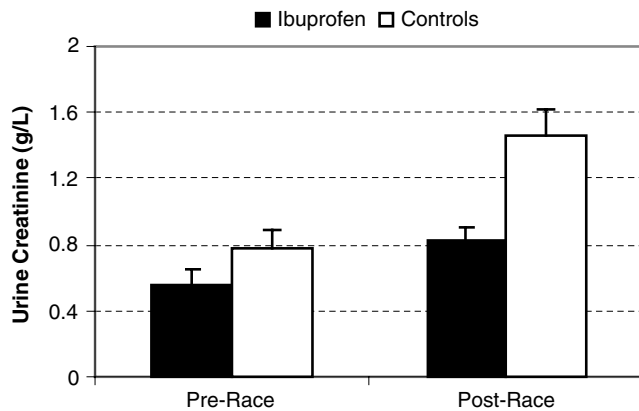


Fig. 3. Urine creatinine concentrations in ibuprofen users and nonusers before and after the 160-km Western States Endurance Run (group effect, $P = .003$; time effect, $P < .001$; group \times time effect, $P = .038$).

plasma cytokines following a 160-km race event. Gastrointestinal permeability is amplified in marathoners using ibuprofen and other NSAIDs compared to nonusers (Lambert et al., 2001; Ryan et al., 1996; Smetanka et al., 1999), but we are unaware of other studies showing that ibuprofen use during training and competition induces mild endotoxemia and systemic inflammation. Significant increases in plasma LPS have been reported in athletes following prolonged exercise in some studies (Bosenberg et al., 1988; Brock-Utne et al., 1988), but in most studies increases are small or absent as confirmed by our data (Camus et al., 1997, 1998; Jeukendrup et al., 2000). When it does occur, exercise-induced endotoxemia has been hypothesized to be related to splanchnic ischemia and hyperthermia (Lambert, 2004). Discrepancies between studies could be due to several factors including environmental conditions, the type of LPS assay used, and whether or not NSAID use by the athletes was controlled (Jeukendrup et al., 2000). It should be noted that environmental conditions in the 2005 160-km Western States Endurance Run were unusually mild, and higher post-race LPS levels may have occurred if the athletes had experienced greater heat stress.

In agreement with data previously published by our research team, ibuprofen use was linked to elevations in plasma cytokines, although this time dosage was controlled (Nieman et al., 2005). We are unaware of other published studies indicating elevated cytokines in NSAID users compared to nonusers following prolonged exertion. Camus et al. (1998) demonstrated a relationship between exercise-induced endotoxemia and TNF- α , but this was not confirmed in a study by Jeukendrup et al. (2000). We showed modest relationships between LPS and plasma IL-8, IL-10, MCP-1, serum CRP, and plasma cortisol, but not with five other cytokines measured in this study. Despite a trend for higher pre-race LPS levels in the ibuprofen compared to control group, none of the measured variables in this study except for IL-1ra differed between groups. These data suggest that higher plasma LPS before and during extreme exertion is just one factor explaining the elevated post-race plasma cytokine levels in ibuprofen users compared to nonusers. 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. (1999) 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. We also showed significant, positive relationships between CK and six of eight cytokines in both this study and a previous investigation (Nieman et al., 2005). Thus the elevation of plasma cytokines in the ibuprofen users is 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 our subjects. 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 (Donnelly et al., 1990; Peterson et al., 2003; Pizza et al., 1999; Trappe et al., 2002). Thus the high prevalence of ibuprofen use by ultramarathon athletes appears to have few if any physiological or performance benefits (Nieman et al., 2005).

In summary, ibuprofen use by athletes the day before and during participation in a 160-km race event was linked to significant increases in blood indicators of systemic inflammation including CRP and seven cytokines compared to controls. Plasma LPS did not increase in either group during the 160-km race, but was modestly elevated pre- and post-race in the ibuprofen group. Elevated post-race serum ALT, AST, and BUN levels, and lower urine creatinine levels in ibuprofen users suggest a higher release of muscle and liver cell enzymes and a slight disturbance of kidney function compared to controls. These ibuprofen-related effects occurred despite no group differences in race time, RPE, or ratings of gastrointestinal discomfort. Ibuprofen use provided no benefit for alleviation of muscle damage or post-race perceptions of muscle soreness.

This study was not placebo controlled or double blinded due to ethical concerns raised by the race directors. We did not control or measure ibuprofen usage during the weeks leading into the race, and unexpectedly measured a tendency for higher pre-race LPS levels despite the short half-life of ibuprofen. Thus further research is warranted using a stronger research design under laboratory conditions to determine what mechanisms best explain the elevated post-race cytokine levels in the ibuprofen users. This study has identified several potential factors that should be measured in future studies including changes in plasma LPS and cortisol levels, altered kidney clearance, and increased muscle and liver cell release of enzymes.

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