A comparison of cytokine responses during prolonged cycling in normal and hot environmental conditions

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Purpose: Components of immune function are affected by physical activity in an adverse environment. The purpose of this study was to compare plasma differences in inflammatory cytokines including tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), in addition to the stress hormone cortisol, during prolonged cycling under normal and hot environmental conditions in elite cyclists.

Methods and design: Six trained elite male cyclists (27 ± 8 years; 75.5 ± 4 kg; maximum oxygen uptake [VO2max] = 66 ± 6 mL/kg/min, mean ± SD). The cyclists biked for 2.5 h at their prescribed 60% maximum exercise workload (Wmax) or 75% VO2max either in an environmental chamber set at 15°C and 40% relative humidity (NEUTRAL) or at 35°C and 40% relative humidity (HOT). The cyclists were given 4 mL of water/kg body weight every 15 min under both conditions.

Results: Total cortisol concentrations were elevated (P < 0.05) immediately postexercise and 12 h postexercise in both the NEUTRAL and HOT conditions. TNF-α concentrations were only significantly (P = 0.045) elevated postexercise in HOT conditions. During the HOT conditions, a significant (P = 0.006 and 0.007, respectively) difference in IL-6 was seen immediately after and 12 h postexercise. During the NEUTRAL condition, IL-6 was only significantly elevated postexercise (P < 0.05).

Conclusions: Heat exposure during a long bout of exercise is sufficient to elicit stress response in elite cyclists. However, the degree of release of anti-inflammatory and proinflammatory cytokines might be related to several factors that include the athlete’s fitness level, hydration status, exercise intensity, and length of exposure to hot environments.

Keywords: cytokines, inflammation, heat, exercise, performance

Introduction
It is understood that the components of immune function are affected by physical activity in an adverse environment. Although light physical activity even in the presence of adverse environmental conditions might promote a beneficial immune response, highly intense or prolonged exercise and/or heat stress might actually elicit an immunosuppressed response similar to trauma or inflammation, which can in turn increase susceptibility to viral infections. For example, during prolonged exercise, inflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), are produced and released by macrophages and lymphocytes. It has also been demonstrated that heat stress affects the physiological response by secreting catecholamines and cortisol. Catecholamines demarginate leukocytes and in turn cortisol causes cells to migrate to...
lymphoid tissue. These responses have been associated with decreased performance in elite cyclists.

Although there have been several studies examining the effects of nutritional supplementation on cytokine release in elite athletes, studies that have observed the effects of adverse environmental conditions at exercise bouts over 120 min are few. The benefits of utilizing exercise studies are two-fold, in that physical exercise and training evoke a subclinical inflammatory response, therefore making it an acceptable model for observing the immune response in humans. In addition, as heat stress and prolonged exercise can negatively affect performance in elite athletes, it is important to determine the magnitude of heat influence in athlete’s performance and ways in which training can be modified, performance salvaged, and viral susceptibility decreased based on an understanding of inflammatory and immune responses. It is important to gain an understanding of cytokine release during prolonged exercise in hot environmental conditions, because the amount of inflammatory response might be much higher than in neutral conditions.

Therefore, the purpose of this study was to compare plasma differences in inflammatory cytokines TNF-α and IL-6, in addition to the stress hormone cortisol, during prolonged cycling under normal and hot environmental conditions in elite cyclists.

Methods

Subjects

Six trained elite male cyclists (27 ± 8 years; 75.5 ± 4 kg; maximum oxygen uptake [VO2max] = 66 ± 6 mL/kg/min, mean ± SD) volunteered to participate in this study. Subject anthropometric data are summarized in Table 1. The study was conducted in the winter months to minimize any natural heat acclimatization. Additionally, subjects were in the preseason of their training cycle since their first competition was going to take place 2 months after testing. Informed consent was obtained from each subject, and the experimental procedures were in accordance with the policy statements review board of the University of West Florida (UWF).

Table 1 Descriptive data for anthropometric measurements of subjects (N = 6)

| Anthropometric measure | Mean   | SE    |
|------------------------|--------|-------|
| Weight (kg)            | 75.58  | 1.99  |
| Height (m)             | 1.75   | 0.04  |
| BMI (kg/m²)            | 23.90  | 1.12  |
| BF %                   | 14.60  | 1.22  |

Abbreviations: SE, standard error; BMI, body mass index; BF, body fat.

Procedures

Two to 3 weeks before the first test session, subjects reported to the UWF Exercise Physiology Laboratory for a study orientation; medical history assessment; anthropometric measures, which included height, weight, body mass index, and body fat composition via Lange skinfold calipers; and a cardiorespiratory fitness, or VO2max, test. The cyclists brought their own bikes, which were set up at the laboratory’s CompuTrainer™ PRO Model 8001 trainers (RacerMate, Seattle, WA), or cycling computer to complete their VO2max tests. A graded maximal protocol used previously by Nieman et al that started at a resistance of 150 W and was increased by 25 W every 2 min was used to determine VO2max. Oxygen uptake, ventilation (VE), heart rate (HR), maximum HR (HRmax), maximum exercise workload (Wmax), and respiratory exchange ratio (RER) were measured using a MAX-II System (AEI Technologies, Inc, Naperville, IL).

During the orientation session, subjects were educated on avoiding additional supplements or ergogenic aids, and a list of these aids was supplied to them. Subjects agreed to avoid the use of these ergogenic aids including vitamins (above 100% of daily recommended allowances), nutritional supplements, herbs, or medications known to affect immune system for 1 week before test sessions. Subjects submitted a weekly nutritional log during the week of the tests.

For this crossover study, subjects came into the laboratory for two randomized, counterbalanced test sessions 1 week apart. Subjects reported to the laboratory at 1 pm on each test session day not having ingested any solid food for 3 h prior to testing. In order to control caloric intake during test day and have consistency among sessions, 2 h before tests session, subjects ingested 16 kcal/kg of Boost Plus (Mead Johnson Nutritional, Evansville, IN), a standardized liquid meal that exceeds daily value recommendations for all vitamins and minerals. During the sessions, the cyclist biked for 2.5 h at his own predicted 60% Wmax or 75% VO2max in an environmental chamber set at both 15°C and 40% relative humidity (NEUTRAL) or 35°C and 40% relative humidity (HOT). In order to monitor intensity throughout the trials, power output (W) and HR were recorded every 10 min and if subjects fell below their individual prescribed intensity, they were asked to increase their efforts. The cyclists were given 4 mL of water per kg body weight every 15 min under both conditions. Metabolic measurements (RER, HR, and VE), and power output (W) were taken every 15 min in order to verify workload. Blood samples were collected ~30 min prior to the test session (pre-exercise), immediately postexercise, and 12 h postexercise. Body weight for differences in
dehydration was assessed post-test sessions. Plasma volume changes due to dehydration were determined according to Dill and Costill.9

Subjects rested for 10 min in the seated position, and blood samples were drawn from the antecubital vein into heparinized tubes. The collection tubes were immediately centrifuged and plasma samples frozen at –80°C until analysis. Plasma cortisol was assayed by using an immunoassay kit (ALPCO Diagnostics™, Salem, NH). Total plasma concentrations of TNF-α and IL-6 measurements were determined using quantitative sandwich enzyme-linked immunoassay kits provided by ALPCO Diagnostics™. All samples and provided standards were analyzed in duplicate. An ultrasensitive kit was used to analyze IL-6. Cytokine concentrations were determined by using linear regression from a standard curve which was provided in standards in the kit (ALPCO Diagnostics™).

**Statistical analysis**

Descriptive data are expressed as means ± standard errors (SE). Cytokines and cortisol hormone values were analyzed by using a 2 (HOT vs NEUTRAL environment) × 3 (pre-exercise, immediately postexercise, and 12 h postexercise measurements) repeated-measurements analyses of variance (ANOVA). The level of significance to reject the null hypothesis was set at \( P < 0.05 \).

**Results**

All of the subjects maintained their own prescribed intensity for HR and power output (Watts) during the testing sessions (Table 2). A plasma volume decrease of 5.1% + 6.2% was observed postexercise in the HOT environment. No significant differences (\( P > 0.05 \)) in body weight were found pre- and postexercise in both conditions (Table 3). Cytokine responses to NEUTRAL and HOT environments are summarized in Table 4.

**Table 2** Mean HR, RER, VE, and power output for subjects during exercise in NEUTRAL or HOT conditions

| Condition | Variable | Mean | SE  |
|-----------|----------|------|-----|
| NEUTRAL   | HR       | 159  | 3.2 |
|           | RER      | 0.87 | 0.01|
|           | VE (L/min)| 138.2| 2.6 |
|           | Power output (W)| 171  | 10.9|
| HOT       | HR       | 160  | 2.3 |
|           | RER      | 0.89 | 0.00|
|           | VE (L/min)| 139.3| 2.5 |
|           | Power output (W)| 172  | 9.8 |

**Table 3** Body weight differences prior and immediately following exercise in NEUTRAL or HOT conditions

| Conditions    | Mean | SE  | \( P \) value |
|---------------|------|-----|---------------|
| NEUTRAL preweight (kg) | 76.1 | 1.9 | 0.083 |
| NEUTRAL postweight (kg)  | 75.4 | 2.1 |     |
| HOT preweight (kg)     | 76.0 | 2.3 | 0.056 |
| HOT postweight (kg)    | 75.3 | 2.3 |     |

**Note:** Statistical significance was set at \( P < 0.05 \).

**Abbreviation:** SE, standard error.

A significant main effect for time was found for cortisol concentrations. Cortisol concentrations were higher postexercise when compared to pre-exercise in both conditions (\( F(1,5) = 23.72; P = 0.002; \eta^2 = 0.83 \)). No significant main effect for condition (\( F(1,5) = 0.060; P = 0.816; \eta^2 = 0.57 \)) and no significant interaction (time × condition) regarding cortisol concentrations were found (\( F(1,5) = 1.734; P = 0.242; \eta^2 = 0.76 \)).

A significant main effect for time was observed in TNF-α (\( F(1,5) = 7.268; P = 0.031; \eta^2 = 0.69 \)), while no significant main effect for condition (\( F(1,5) = 0.578; P = 0.481; \eta^2 = 0.29 \)) or interaction (condition × time) (\( F(1,5) = 0.220; P = 0.682; \eta^2 = 0.53 \)) for TNF-α concentrations were observed.

A significant main effect for time (\( F(1,5) = 19.69; P = 0.003; \eta^2 = 0.80 \)) was observed for IL-6. No significant main effect was observed for condition (\( F(1,5) = 0.578; P = 0.481; \eta^2 = 0.34 \)), and no significant interaction (time × condition) was observed for IL-6 levels (\( F(1,5) = 3.963; P = 0.087; \eta^2 = 0.44 \)).

**Discussion**

For the present study, blood cortisol levels in cyclists biking for 2.5 h at 60% \( W_{\text{max}} \) and 75% \( V_{\text{O2max}} \) increased postexercise in both a NEUTRAL and HOT environment. These findings confirm previous studies where conditions of stress and heat elevate certain inflammatory markers and cytokines.10 Brenner et al4 stated that cortisol values generally increase when exposed to a stressor, in this case, exercise and heat. Moreover, cortisol has a long half-life and therefore, peak levels might be reached well into the recovery period. In a similar study by Niess et al,10 runners performed a 60-min run at 28°C and 50% humidity at 75% \( V_{\text{O2max}} \). Plasma cortisol was only elevated 0.5 and 3 h postexercise in the heat when compared to the same exercise bout performed at 15°C and 50% humidity. We can speculate that Niess et al only observed an increase of cortisol after the acute bout of exercise in the heat and none during neutral conditions because the exercise protocol was not long enough to elicit
cortisol release during neutral environmental conditions. Literature suggests that a rise in core temperature by at least 1.2°C will elicit a rise in plasma cortisol levels. In the present study, a bout of 2.5 h might have been sufficient to elicit an inflammatory response during neutral conditions in these cyclists.

In the present study, an increase of TNF-α was only observed after exercise in the HOT condition. Similar to other studies, our results exhibited an increase in TNF-α levels postexercise in the HOT condition, whereas during the NEUTRAL condition, circulating levels of TNF-α did not increase. Additionally, similar to the study by Selkirk et al, this increase in TNF-α is relatively low compared to levels observed in individuals suffering from heat stroke. Based on those studies, we can speculate that the relatively low increase in TNF-α observed during the HOT conditions and the lack of it during the NEUTRAL conditions could be partly due to TNF-α kinetics and its rapid clearance from the circulation of these well-trained cyclists. Moreover, this subclinical increase in TNF-α during the HOT condition and the lack of its significant increased levels during the NEUTRAL conditions are accompanied by a parallel increase in IL-6, better demonstrated during the NEUTRAL conditions. According to several researchers, IL-6 has been found to increase with strenuous exercise, causing a direct inhibition of TNF-α production. It has been observed that slight increases of TNF-α are accompanied by major plasma level increases of IL-6, which is what the present study confirmed by the significant increase of plasma levels of IL-6 present in cyclists after their exercise bouts, both in NEUTRAL and HOT conditions. Athletes in the present study were highly conditioned and most were active US marines. Therefore, we can speculate that they were well adapted by intense exercise in neutral conditions resulting in a rapid clearance of TNF-α in the circulation during the NEUTRAL conditions.

Plasma levels of IL-6 were significantly higher postexercise in both NEUTRAL and HOT conditions. The increase of IL-6 after the end of exercise concurs with several other studies where heat exposure tends to stimulate the release of IL-6. IL-6 has both anti-inflammatory and pro-inflammatory properties. In addition, it has been suggested that IL-6 serves as a glucoregulatory hormone. Contracting skeletal muscle is responsible for releases of IL-6 when muscle glycogen levels fall during exercise. It is difficult to prove whether IL-6 played an anti-inflammatory role or acted as a glucoregulatory hormone in the present study. It can be speculated that IL-6 served as a glucoregulatory hormone since judging from the RERs observed, cyclists were highly relying on carbohydrates during the 2.5-h exercise bout. On the other hand, IL-6 could also have served as an anti-inflammatory hormone. The more pronounced changes in plasma IL-6 compared to plasma TNF-α suggest that anti-inflammatory responses may prevail over inflammatory responses after a 2.5-h exercise bout in elite cyclists. Further and more specific testing with a larger sample population is warranted to clarify whether IL-6 served as a glucoregulatory or as an anti-inflammatory agent in the present study.

The present study has several limitations that need to be addressed. The small sample size did not allow us to directly observe differences between conditions. Moreover, the results of this study might not be reflective of a ‘true’ average response because of the small sample size. The cyclists were of elite caliber, and most enrolled on the marine corps and involved in a demanding training and competition schedule. Therefore, recruitment and commitment to our study of such high caliber athletes were difficult. We consider our data to be important as a ‘pilot’ study, and future research with larger sample sizes is warranted.

**Conclusion**

In conclusion, heat exposure during a long bout of exercise is sufficient to elicit stress response in elite cyclists.

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**Table 4** Cortisol, TNF-α, and plasma IL-6 concentrations before, after, and 12 h after 2.5 h of cycling at 60% \( W_{\text{max}} \) and 75% \( V\text{O}_{\text{2max}} \) either in NEUTRAL or HOT conditions

| Condition | Pre | Post | 12 h post |
|-----------|-----|------|----------|
| Cortisol (nmol/L) | 730.4 ± 52.0 | 1213.7 ± 32.7 | 878.1 ± 44.7 |
| TNF-α (pg/mL) | 32.0 ± 22.1 | 33.9 ± 21.7 | 32.2 ± 22.0 |
| IL-6 (pg/mL) | 0.1 ± 0.0 | 3.4 ± 0.9 | 0.9 ± 0.4 |

**HOT**

| Condition | Pre | Post | 12 h post |
|-----------|-----|------|----------|
| Cortisol (nmol/L) | 791.4 ± 121.5 | 1109.1 ± 69.7 | 872.3 ± 100.8 |
| TNF-α (pg/mL) | 33.3 ± 23.8 | 35.6 ± 24.3 | 34.5 ± 24.0 |
| IL-6 (pg/mL) | 0.3 ± 0.1 | 1.7 ± 0.2 | 0.9 ± 0.1 |

**Abbreviations:** Pre, prior to; Post, immediately following; 12 h post, 12 h into recovery; NEUTRAL, an environmental chamber set at 15°C and 40% relative humidity; HOT, an environmental chamber set at 35°C and 40% relative humidity; SE, standard error.
However, the degree of release of anti-inflammatory and pro-inflammatory cytokines might be related to several factors that need to be precisely controlled in order to better understand the mechanism of these inflammatory markers during exercise in the heat. Such factors include the athlete’s fitness level, hydration status, exercise intensity, and length of exposure to hot environments. Future studies with a larger sample size are warranted.

Disclosure
All authors have participated substantially in the submitted work and have reviewed and approved the final version of the work and take responsibility for its content. The submitted manuscript is original and unpublished material, except in abstract form or oral report, and is not under consideration by another journal. All authors report no conflicts of interest in this work.

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