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## Exercise-Induced Interleukin-6 and Metabolic Responses in Hot, Temperate, and Cold Conditions

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# Exercise-Induced Interleukin-6 and Metabolic Responses in Hot, Temperate, and Cold Conditions

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## Abstract

The purpose of this study was to determine the effects of exercise in hot, cold, and temperate environments on plasma interleukin-6 (IL-6). Eleven recreationally trained males (age =  $25 \pm 4$  years, height =  $178 \pm 5$  cm, weight =  $79.4 \pm 13.5$  kg, body fat =  $14.7 \pm 3.6\%$ ,  $\text{VO}_2$  peak =  $54.6 \pm 11.5$  ml  $\text{kg}^{-1}$   $\text{min}^{-1}$ ) performed a 1 hr cycling bout in hot ( $33^\circ\text{C}$ ), cold ( $7^\circ\text{C}$ ), and temperate ( $20^\circ\text{C}$ ) environments at 60% of  $W_{\text{max}}$  followed by 3 hr of supine recovery in temperate conditions. Expired gases were measured every 15 min during exercise and once every hour during recovery. Heart rate was continuously measured throughout the trials. Blood samples were obtained from the antecubital vein pre-exercise, immediately post-exercise, and 3 hr post-exercise. Blood samples were analyzed for plasma concentrations of IL-6 using a commercial ELISA kit. Plasma IL-6 concentrations were significantly higher immediately post-exercise ( $14.8 \pm 1.6$  pg  $\text{ml}^{-1}$ ,  $p = 0.008$ ) and 3 hr post-exercise ( $14.8 \pm 0.9$  pg  $\text{ml}^{-1}$ ,  $p = 0.018$ ) compared to pre-exercise ( $11.4 \pm 2.4$  pg  $\text{ml}^{-1}$ ), across all trials. There were no differences in plasma IL-6 concentrations ( $p = 0.207$ ) between temperature conditions. Oxygen consumption and heart rate were higher and respiratory exchange ratio was lower in the hot compared to other conditions ( $p < 0.05$ ). These data indicate that the temperature in which exercise occurs does not affect acute plasma IL-6 response despite differences in metabolic state.

*Keywords:* cytokines, myokines, heat, cold, exercise, inflammation

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## Introduction

An effective method to treat and prevent a multitude of metabolic and low-grade inflammatory chronic diseases is through regular exercise (Booth, Roberts, & Laye, 2012). During exercise, a cascade of myokines are released from skeletal muscle that mediate the health benefits and protect against diseases including, but not limited to, obesity, cardiovascular disease, type 2 diabetes, rheumatoid arthritis, and cancer (Pedersen & Febbraio, 2008). The most prevalent myokine produced during exercise is interleukin-6 (IL-6) (Pedersen, 2000). IL-6 is a complex myokine that has multiple roles, such as stimulating the immune system, maintaining metabolic homeostasis, and acting with both pro- and anti-inflammatory properties (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011).

Acute increases in IL-6 increase the oxidation of fat and uptake of glucose, and exert anti-inflammatory effects (Pedersen & Febbraio, 2008). Conversely, chronic increases or overproduction of IL-6 can lead to fatigue, depression, and low-grade fever (Fonseca, Santos, Canhao, & Choy, 2009). Additionally, those with high levels of IL-6 are 2–5 times more likely to have a heart attack, stroke, or other cardiovascular event (Cesari et al., 2003). The pro- and anti-inflammatory roles of IL-6 make it an interesting target to investigate disease protection of exercise and negative consequences of a sedentary lifestyle.

Exercise is a physical stress that stimulates many hormonal and immunological responses (Fischer, 2006). Contracting skeletal muscle has been shown to produce IL-6 during exercise (Pedersen & Febbraio, 2008). Plasma IL-6 levels have been shown to increase with exercise dependent on duration, mode, and intensity. Peak plasma concentrations occur immediately post-exercise or shortly thereafter with a rapid decrease to pre-exercise levels (Fischer, 2006). The acute increases in IL-6 concentrations from exercise have typically been classified as inducing an anti-inflammatory environment known as the acute-phase response (Heinrich, Castell, & Andus, 1990). Thus, IL-6 may be an indicator of overall acute cellular stress and possibly influence recovery (Lee et al., 2012).

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The response of IL-6 to different temperature environments has been investigated but much discrepancy still exists. Heat exposure (42 °C) has been shown to elicit an increased stress response that increases the expression of IL-6 mRNA in mouse skeletal muscle (Welc et al., 2012). When endurance cycling exercise was performed in a hot (35 °C) and room temperature (15 °C) environment, the circulating plasma IL-6 levels were higher following exercise, but no effect of temperature was observed (Cosio-Lima, Desai, Schuler, Keck, & Scheeler, 2011). The response of IL-6 when exercise is performed in a cold environment is also not clearly defined. Specifically, plasma IL-6 levels did not change with three days of cold exposure (4 °C) in wild type mice (Knudsen et al., 2014) or two-hour whole-body cold exposure in humans (Iwen et al., 2011). However, it has been reported that plasma IL-6 might be higher during exercise in cold or wet conditions, although no control trial was performed (Rhind et al., 2001). More recently, it has been demonstrated that plasma IL-6 levels were not different among cyclists during exercise in a cold (0 °C) and control (20 °C) trial (Patterson, Reid, Gray, & Nimmo, 2008). Furthermore, no studies have directly compared the response of plasma IL-6 with exercise in hot, cold, and temperate room temperature environments in a human model.

Therefore, the purpose of this study was to determine the effects of exercise in hot, cold, and temperate environmental conditions on circulating levels of IL-6. The hypotheses of this study were that (1) plasma IL-6 levels will be elevated immediately post-exercise due to the acute-phase response and decrease 3 hr post-exercise because of the short half-life of IL-6 and (2) IL-6 concentration will be elevated in the heat due to an increased stress response and not changed in the cold when compared to a temperate environment.

## Materials and Methods

### Participants

Eleven recreationally trained males served as participants in this study (Table 1). Participants completed a physical activity questionnaire (PAR-Q) and were informed of their rights as a research participant and possible risks associated with the protocol prior to signing written informed consent. All procedures were approved by the University Institutional Review Board (University of Nebraska at Omaha).

Table 1.  
Participant characteristics ( $n = 11$ ).

Characteristic	Mean $\pm$ SD
Age, years	25 $\pm$ 4
Height, cm	178 $\pm$ 5
Weight, kg	79.4 $\pm$ 13.5
Body fat, %	14.7 $\pm$ 3.6
VO <sub>2</sub> peak, l·min <sup>-1</sup>	4.29 $\pm$ 0.86
VO <sub>2</sub> peak, ml·kg <sup>-1</sup> ·min <sup>-1</sup>	54.6 $\pm$ 11.5

### Maximal Aerobic Exercise Capacity

Maximal oxygen consumption (VO<sub>2</sub>peak) and workload associated with VO<sub>2</sub>peak ( $W_{\max}$ ) were measured during the initial visit for each participant by performing a graded exercise protocol on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA, USA). The initial 95 W workload was increased 35 W every 3 min until subjects reached volitional exhaustion.  $W_{\max}$  was determined by taking the sum of the highest stage completed (in watts) and the proportion of time in the last stage multiplied by the 35 W increment. Each participant exercised at 60% of their maximum workload for each trial. Expired gases were collected using a calibrated metabolic cart (ParvoMedics TrueOne Metabolic System, Sandy, UT, USA) and analyzed in 15 s intervals.

### Protocol

Each participant completed three trials, using a randomized, counterbalanced cross-over design. Trials consisted of exercise in a hot (33 °C, 60% humidity), cold (7 °C, 60% humidity), and temperate (20 °C, 60% humidity) environment. All trials were performed in a temperature- and humidity-controlled environmental chamber (Darwin Chambers Company, St Louis, MO, USA) and were separated by at least 4 days but no more than 7 days. Participants were dressed in a gym shirt and shorts and wore the same clothing for each trial. Participants kept an exercise log for 2 days prior and dietary log for 24 hr prior to the initial trial and repeated the recorded exercise and diet before the ensuing trials. Additionally, participants refrained from alcohol, caffeine, and exercise 24 hr prior to each trial. On the day of the trial, subjects arrived after a 12 hr fast and performed 1 hr of cycling at 60% of  $W_{\max}$  in the environmental chamber at the designated temperature. Participants were required to consume 500 ml of water throughout each cycling trial. A 3 hr recovery period in temperate conditions began immediately once cycling was completed. Subjects were asked to remain in a supine position throughout this recovery period.

### Body Composition

Body density was determined using hydrodensitometry and was corrected for estimated residual lung volume and gastrointestinal air volume. Net underwater weights were recording using an electronic load cell-based custom system (Exertech, Dresbach, MN, USA). Body density was then converted to body composition (% fat) using the Siri equation (Siri, 1961).

### Core Temperature and Heart Rate

Core temperature was continuously measured during exercise and recovery. An hour prior to the experimental

trial, each participant ingested a Jonah Core Body Temperature Capsule (JCBC, Hidalgo Limited, Cambridge, UK) followed with 125 ml of water and a fiber bar. The ingested capsule sent a signal to an EQ02 LifeMonitor Sensor Electronics Module (SEM, Hidalgo Limited, USA). This module also measured heart rate using a chest strap sensor and chest skin temperature using an infrared sensor. The infrared thermistor logged skin temperature every 15 s.

#### *Substrate Utilization*

Expired gases were measured during the exercise and recovery periods to determine whole-body carbohydrate and fat utilization. Gases were collected in 5 min intervals at 10, 25, 40, and 55 min of exercise and 25, 85, and 145 min into recovery. The four collections during exercise were averaged to represent the exercise bout and the three collections during recovery were averaged to represent the 3 hr of recovery. Only the last 3 min of the 5 min collection period were averaged to represent the steady-state sample period. Expired  $\text{VO}_2$  and  $\text{VCO}_2$  were used to calculate the respiratory exchange ratio (RER) and were further analyzed to examine carbohydrate and fat oxidation (Jeukendrup & Wallis, 2005).

#### *Measurement of IL-6*

Blood draws were taken at room temperature pre-exercise, immediately post-exercise, and after 3 hr post-exercise for each trial. Blood draws were taken from the antecubital vein into EDTA vacutainer tubes. Hematocrit and hemoglobin levels were measured immediately after collection to correct for any plasma volume shifts known to occur with exercise and heat exposure (Dill & Costill, 1974). If not corrected, an artificial increase in plasma concentration may be observed due to the decreased blood volume associated with sweating. Briefly, 120  $\mu\text{l}$  of whole blood was used to fill two hematocrit tubes (50  $\mu\text{l}$  each) and one hemoglobin micro cuvette (20  $\mu\text{l}$ ). The hematocrit tubes were spun for 5 min (Zipocrit LW Scientific Inc., Lawrenceville, GA, USA) to separate whole blood from plasma. Hemoglobin was measured using a Hemocue HB 201+ Analyzer (Ängelholm, Sweden). The remaining sample was centrifuged at 1000 rpm at 7 °C for 10 min and then stored at -80 °C until analyses were performed. For IL-6 measurement, enzyme-linked immunosorbent assay (ELISA) kits were used according to the manufacturer's protocol (Biosource, MA, USA). Absorbance was read with a spectrophotometric plate reader (Fischer Scientific, Pittsburg, PA, USA) at 450 nm in duplicate to quantify the amount of IL-6 in the blood. According to Biosource, the IL-6 ELISA kits observed no interference with the soluble receptors (sIL-6 and sgp-130). The intra-assay coefficient of variation between duplicate samples was  $5.8 \pm 2.3\%$ .

#### *Statistical Analysis*

Differences in environmental temperatures, core and skin temperature, heart rate,  $\text{VO}_2$ , substrate utilization, and plasma concentration of IL-6 were analyzed throughout each time point among the three trials using a repeated measures two-way ANOVA (time  $\times$  trial). If the *F*-ratio values were significant, a Fisher's protected least significant difference post hoc was performed to determine where significance occurred. A probability of type I error of less than 5% was considered significant ( $p < 0.05$ ). The Statistical Package for Social Sciences software (SPSS 23.0, Chicago, IL, USA) was used to analyze all statistical data. Data are reported as means  $\pm$  SD.

## **Results**

#### *IL-6*

Plasma concentrations of IL-6 were similar ( $p > 0.05$ ) before exercise but increased from pre-exercise to immediately post-exercise and remained elevated 3 hr post-exercise regardless of temperature ( $p = 0.004$  and  $p = 0.017$ , respectively; Figure 1). No statistically significant effect of temperature was observed ( $p = 0.178$ ); however, Cohen's effect size value ( $d = 0.88$ ) suggests a greater response of IL-6 in the hot compared to temperate conditions.

#### *Oxygen Consumption*

During exercise, absolute and relative oxygen consumption were higher in the hot condition and lower in the cold condition when compared to the temperate condition ( $p < 0.001$ ; Table 2). Oxygen consumption was also different between hot and cold conditions ( $p < 0.001$ ; Table 2).

#### *Heart Rate*

Heart rate during exercise was higher in the hot condition when compared to the cold and room temperature conditions ( $p < 0.001$ ; Table 2). There were no differences between the cold and room temperature conditions ( $p = 0.308$ ).

#### *Substrate Utilization*

During exercise, the RER was lower in the hot condition compared to the cold and room temperature conditions ( $p = 0.007$  and  $p = 0.001$ , respectively; Table 2). There was no difference between the cold and room temperature conditions during exercise ( $p = 0.801$ ).

During exercise, carbohydrate utilization was lower in the hot condition ( $p = 0.042$ ; Table 2) when compared to the room temperature condition. There was no difference between

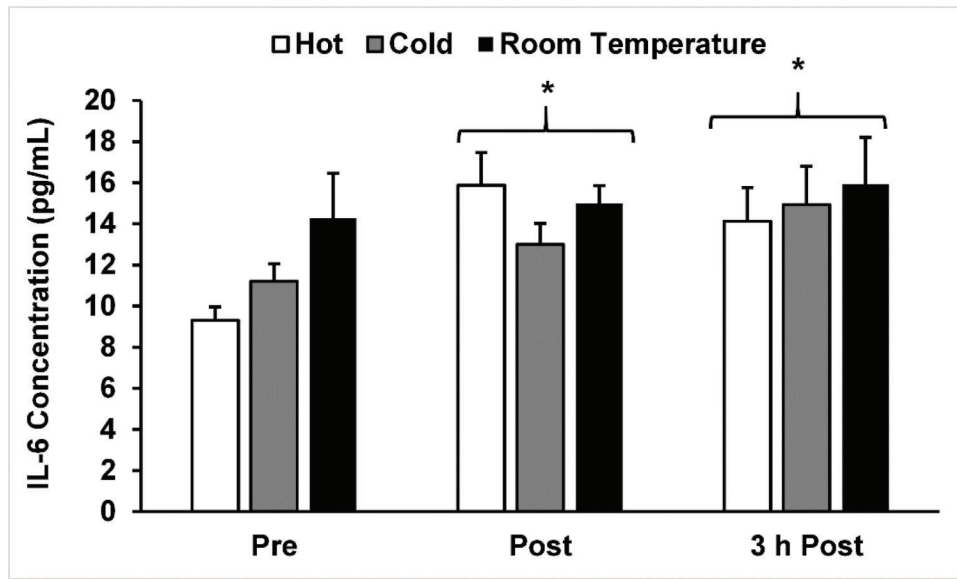


Figure 1. Plasma IL-6 concentrations corrected for exercise- and temperature-induced plasma volume shifts. \* $p < 0.05$  from pre (main effect of time). Hematocrit and hemoglobin levels were measured immediately after collection to correct for any plasma volume shifts known to occur with exercise and heat exposure (Dill & Costill, 1974).

the hot and cold temperature conditions ( $p = 0.123$ ; Table 2) and between cold and room temperature conditions ( $p = 0.535$ ; Table 2).

Fat utilization during exercise was higher in the hot condition when compared to the cold and room temperature conditions ( $p = 0.003$ ,  $p = 0.001$ , respectively; Table 2).

#### Core Temperature

Data from six subjects were used for core temperature analysis (Figure 2) due to technical problems with the ingestible sensor. During exercise, core temperature was similar until 50 min into exercise, at which point core temperature in the hot condition was higher than the cold ( $p < 0.001$ ) and room temperature ( $p < 0.001$ ) conditions. During the first hour of recovery, core temperature in the hot condition ( $37.4 \pm 0.3$  °C) was higher than in the cold ( $37.1 \pm 0.3$  °C,  $p < 0.001$ ) and room temperature ( $37.1 \pm 0.2$  °C,  $p = 0.038$ ) conditions. There was no difference in core temperature throughout the remaining recovery period ( $p > 0.05$ ).

#### Skin Temperature

Skin temperature (Figure 3) during exercise was higher in the hot condition ( $36.6 \pm 0.7$  °C,  $p < 0.001$ ) and lower in the cold condition ( $27.3 \pm 2.3$  °C,  $p < 0.001$ ) at the 10 min mark and thereafter when compared to the room temperature condition ( $32.2 \pm 1.1$  °C). During recovery, there were no differences in skin temperature between the hot, cold, and room temperature conditions ( $34.2 \pm 1.0$  °C,  $33.6 \pm 1.1$  °C,  $34.4 \pm 0.8$  °C, respectively;  $p = 0.101$ ).

Table 2. Metabolic measures.

	Exercise	Recovery
	VO <sub>2</sub> (l min <sup>-1</sup> )	
Hot	3.05 ± 0.48*	0.35 ± 0.06
Cold	2.69 ± 0.38*	0.33 ± 0.06
Room temp.	2.83 ± 0.40*	0.34 ± 0.05
	VO <sub>2</sub> (ml kg <sup>-1</sup> min <sup>-1</sup> )	
Hot	38.8 ± 2.2*	4.5 ± 0.1
Cold	34.4 ± 1.8*	4.3 ± 0.1
Room temp.	35.9 ± 1.8*	4.3 ± 0.2
	Heart rate (bpm)	
Hot	165 ± 11	81 ± 12
Cold	152 ± 9 <sup>†</sup>	74 ± 13
Room temp.	154 ± 13 <sup>†</sup>	76 ± 10
	Respiratory exchange ratio	
Hot	0.89 ± 0.04	0.79 ± 0.07
Cold	0.94 ± 0.04 <sup>†</sup>	0.77 ± 0.07
Room temp.	0.94 ± 0.03 <sup>†</sup>	0.79 ± 0.08
	Carbohydrate utilization (g min <sup>-1</sup> )	
Hot	2.56 ± 0.50	0.13 ± 0.05
Cold	2.91 ± 0.56	0.13 ± 0.05
Room temp.	2.98 ± 0.45 <sup>†</sup>	0.14 ± 0.06
	Fat utilization (g min <sup>-1</sup> )	
Hot	0.56 ± 0.25	0.13 ± 0.05
Cold	0.26 ± 0.21 <sup>†</sup>	0.13 ± 0.05
Room temp.	0.30 ± 0.18 <sup>†</sup>	0.14 ± 0.06

Note. Hot, 33 °C; cold, 7 °C; room temp., 20 °C. Data are mean ± SD.

\* $p < 0.05$  between all conditions (main effect of temperature),

<sup>†</sup> $p < 0.05$  from hot.

#### Discussion

Based on previous research, it was hypothesized that plasma IL-6 would be greater in the heat due to an increased inflammatory response that is typically associated

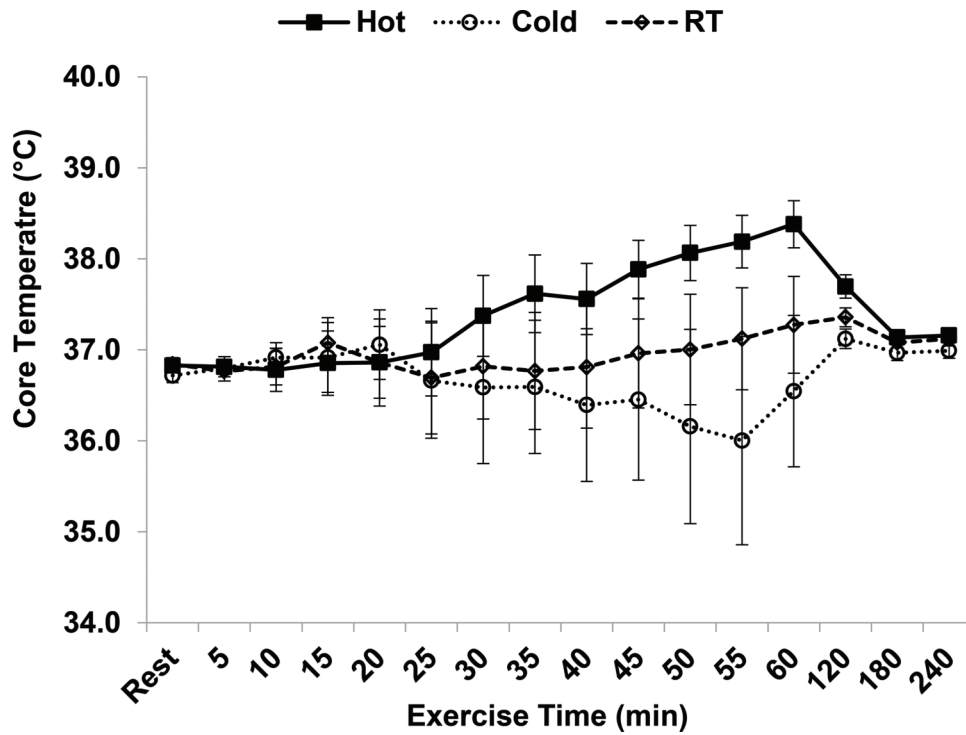


Figure 2. Core temperature during exercise and recovery. \* $p < 0.05$  from room temperature (RT) and cold conditions. Data are mean  $\pm$  SE.

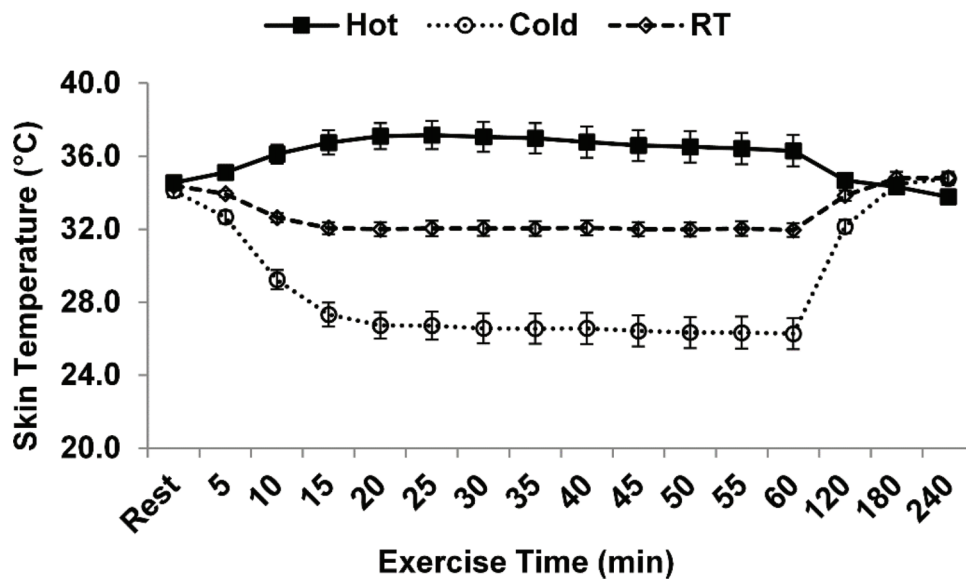


Figure 3. Skin temperature during exercise and recovery. \* $p < 0.05$  between all conditions. Data are mean  $\pm$  SE. RT = room temperature.

with exercising in the heat (Starkie, Hargreaves, Rolland, & Febbraio, 2005). However, the main finding of this study was that exercise in a hot or cold environment did not differentially influence plasma IL-6 concentration compared to exercise in room temperature environment.

Despite the differences in environmental and skin temperatures across conditions, there was a relatively small

difference in core temperature throughout the exercise protocols. This observation of core temperature may be one explanation for the lack of differences in IL-6 between trials. Specifically, core temperature for the first 50 min of the 60 min exercise bout was similar. This finding from the current study is supported by the observation that clamping the rise in core temperature during hyperthermic exercise

diminishes the exercise-related IL-6 response (Rhind et al., 2004). Similarly, our results support other observations that exercise in the cold shows no difference in plasma IL-6 when compared to room temperature environments (Gagnon et al., 2014; Patterson, Reid, Gray, & Nimmo, 2008). Previous work has shown an increase in plasma IL-6 after 8 days of exhaustive exercise in the cold; however, no control condition was performed (Rhind et al., 2001). Furthermore, the observed increase in plasma IL-6 may have been due to the intense exercise regimen performed by the subjects and not due to the effect of temperature. The differences in oxygen and substrate utilization between trials indicate that factors other than exercise intensity may play a role in plasma IL-6 concentration. The findings from the current study agree with other studies that have shown a minor increase in plasma IL-6 concentrations with endurance exercise but no effect of temperature (Cosio-Lima, Desai, Schuler, Keck, & Scheeler, 2011; Lim et al., 2009; Montain, Latzka, & Sawka, 2000; Starkie et al., 2005).

Although temperature did not influence plasma IL-6 levels, exercise elicited a significant increase in plasma IL-6. The increased plasma concentrations following exercise are in agreement with previous investigations (Fischer, 2006; Pedersen, Steensberg, & Schjerling, 2001; Pedersen et al., 2004; Petersen and Pedersen, 2006). Plasma concentrations of IL-6 increase exponentially from exercise and are dependent on fitness level, duration, intensity, and amount of muscle mass recruited (Febbraio & Pedersen, 2002; Pedersen & Febbraio, 2008; Pedersen et al., 2001; Pedersen et al., 2004). Within the current study, a relatively small increase in plasma IL-6 was observed and this could be due to a variety of variables. First, the reasonably fit participants within the current study could explain the relatively small increase due to the known reduction in plasma IL-6 response with training status (Pedersen & Febbraio, 2008). Second, duration of exercise has been shown to be the single most important factor in determining the post-exercise plasma IL-6 response (Fischer, 2006). Only a one- to two-fold increase in IL-6 is typically observed in exercise lasting less than 60 min (Brenner et al., 1999; Fischer, 2006; Lundby & Steensberg, 2004). Therefore, the duration of the trials in the current study may not have been long enough for the exercise-induced response of IL-6 to be of greater magnitude. Third, since high-intensity exercise is associated with shorter duration and vice versa, the response of IL-6 may have been more pronounced if a higher exercise intensity was used (Pedersen & Febbraio, 2008). However, within the current study, the intensity was set at the maximum work rate that allowed the participants to complete the protocol in the hot temperature condition. Lastly, mode of exercise seems to influence the IL-6 response with exercise involving a large muscle mass producing the greatest increase (Ostrowski, Rohde, Zacho, Asp, & Pedersen, 1998). Since the

production of IL-6 during exercise comes mainly from the skeletal muscle itself (Steensberg et al., 2000), it should be noted that exercise involving a limited amount of muscle mass (i.e. cycling) may have been insufficient to elicit a drastic increase in plasma IL-6 compared to other modes of exercise that recruit more muscle mass. Furthermore, the small sample size investigated in the current study may explain the lack of significant findings, despite a large effect size. The noticeably high variation in pre-exercise plasma IL-6 levels may be due to day-to-day variability that has been previously reported in healthy adults (Picotte, Krehlik, & Campbell, 2006).

Interestingly, plasma concentrations of IL-6 remained significantly higher 3 hr post-exercise when compared to resting levels. The prolonged elevation of IL-6 concentrations is typically associated with eccentric exercise that results in a slower decrease of plasma IL-6 during recovery (MacIntyre, Sorichter, Mair, Berg, & McKenzie, 2001; Willoughby, McFarlin, & Bois, 2003). This finding is intriguing due to the minimal eccentric muscle actions associated with cycling and the short half-life of IL-6. One possible explanation may be due to the post-exercise production of IL-6. During recovery, IL-6 production shifts from the muscles during exercise to immune cells (i.e. monocytes, neutrophils, and macrophages), adipose tissue, and/or the brain (Pedersen, 2006). A possible explanation for the increased levels of IL-6 during recovery could be explained by the immune response to inflammation following exercise. The local inflammatory response is accompanied by a systemic response known as the acute phase response in which cytokines initiate an influx of immune cells to aid in tissue repair (Pedersen, 2000). Therefore, the increase in IL-6 production aids in orchestrating this tissue repair and enables inflammatory monocytes that are recruited after skeletal muscle tissue damage to switch into anti-inflammatory macrophages to support myogenesis through phenotype changes (Arnold et al., 2007). Nonetheless, the extended elevation of IL-6 is a unique finding in cycling exercise.

As mentioned earlier, core temperature remained similar across the three conditions for 50 min of the 60 min exercise bout within the current study. Despite this similarity,  $\text{VO}_2$  and heart rate were higher in the hot condition even though the work rate was held constant at 60% of  $W_{\text{max}}$ . From a physiological standpoint, the participants within this study were working harder in the hot condition, which is typically indicated with a higher RER value. However, the RER in the hot condition was lower than in the cold and room temperature conditions. Furthermore, fat oxidation was higher and carbohydrate oxidation was lower in the heat. Thus, a higher concentration of IL-6 may have been expected due to its effects on lipolysis and ability to stimulate the hepatic glucose output (Pedersen & Febbraio, 2008). However, the results from this study indicated no change in plasma IL-6 levels between conditions. Thus, IL-6 did not appear to govern



the alterations in substrate use associated with exercising in hot and cold environments within the current study.

## Conclusions

In conclusion, the plasma IL-6 response with exercise was not altered by temperature. IL-6 increased with exercise and remained elevated following exercise. Furthermore, the present data illustrate that the metabolic roles of IL-6 were not influenced by an acute bout of exercise in different temperatures.

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