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Influence of circulating cytokines on prolactin during slow vs. fast exertional heat stress followed by active or passive recovery

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Wright HE, McLellan TM, Friesen BJ, Casa DJ, Kenny GP.
Influence of circulating cytokines on prolactin during slow vs. fast exertional heat stress followed by active or passive recovery. *J Appl Physiol* 113: 574–583, 2012. First published June 21, 2012; doi:10.1152/japplphysiol.00523.2012.—Prolactin (PRL) has been suggested as an indicator of fatigue during exertional heat stress (EHS), given its strong relationship with body core temperature (T_c); however, the strength of this relationship during different rates of T_c increase and subsequent recovery is unknown. In addition, given the influence that systemic cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)- α , have on the pituitary gland, it would be of interest to determine the relationship between PRL, IL-6, and TNF- α during EHS. The purpose was to examine the PRL, IL-6, and TNF- α heat stress responses during slow and fast heating and subsequent resting or cold water immersion recovery. On 4 days, nine individuals walked at ~45% (slow heating) or ran at ~65% (fast heating) maximal oxygen consumption on a treadmill in the heat (40°C, 30% relative humidity) until rectal temperature (T_{re}) reached 39.5°C (esophageal temperature; fast = 39.41 ± 0.04°C, slow = 39.82 ± 0.09°C). Post-EHS, subjects were either immersed in 2°C water or rested seated until T_{re} returned to 38.0°C. Venous blood, analyzed for PRL, IL-6, and TNF- α , was obtained at rest, during exercise (T_{re} 38.0, 39.0, 39.5°C), the start of recovery (~5 min after 39.5°C), and subsequent recovery (T_{re} 39.0, 38.0°C). IL-6 exhibited myokine properties, given the greater increases with slow heating and lack of increase in TNF- α . A strong temperature-dependent PRL response during slow and fast heating provides additional support for the use of PRL as a peripheral marker of impending fatigue, which is independent of IL-6 and TNF- α cytokine responses.

neuroimmune response; core temperature; peripheral fatigue marker; serotonin and dopamine interplay

DURING EXERTIONAL HEAT STRESS (EHS), the development of central and peripheral fatigue leads to physical and cognitive performance impairments (18, 22), which can increase the risk of injury in recreational and occupational settings. Currently, several mechanisms have been proposed to explain the development of fatigue, or heat intolerance, during exercise in the heat, including the interplay between increased serotonin and decreased dopamine in the brain, or the Central Fatigue Hypothesis (7, 16, 23, 35–36, 63), increased endotoxin leakage at the gut further aggravating hyperthermia (49, 51), the critical core temperature hypothesis (19), and the Central Governor Model of the sensory representation of neural integrative processes (56).

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High core temperatures are believed to alter motor center control in the brain through the interplay of neurotransmitter and hormonal release. The Central Fatigue Hypothesis has been proposed to explain the development of fatigue during EHS (25, 48, 55), given that increased serotonin and decreased dopamine concentrations in the brain have been linked to depressed mood and motivation (3, 29, 33, 55), lethargy, potentially reduced central nervous system drive (66), and altered heat loss responses (i.e., vasodilation) (8).

Direct quantification of the serotonergic and dopaminergic activity within the human brain is not possible. However, changes in circulating prolactin (PRL) concentrations may reflect changes in the release of these neurotransmitters and serve as a peripheral indicator of impending fatigue (5, 29, 32, 65), given that serotonergic and dopaminergic neurons in the brain stem stimulate and inhibit pituitary gland PRL secretion, respectively (4, 29, 62). Previous research has shown that PRL concentrations peak at the point of peak heat strain, during EHS where thermoregulatory strain is intolerable (29). In addition, PRL secretion has been shown to be strongly correlated with perceived exertion (4), mean skin temperature (28), increases in core temperature (i.e., $r \geq 0.55$) during prolonged exercise in the heat (44), and uncompensable heat stress, irrespective of fitness level (65). Although these studies provide support for the use of PRL as an indicator of maximal heat tolerance and impending fatigue, circulating PRL levels at a given core temperature have been shown to be variable among studies, with exercise in trained and untrained individuals to a rectal temperature (T_{re}) of 39.0–39.7°C at exhaustion [~1°C/h, peak PRL (PRL_{peak}) ~2,350–2,500 pmol/l] (65), during active and passive heating to a T_{re} of 38.8°C (~1.6°C/h, PRL_{peak} ~1,000–1,150 pmol/l) (29), and following 45 min of cycling in 30°C with a +1.19°C change in T_{re} (~1.6°C/h, PRL_{peak} ~750 pmol/l) (28), indicating that PRL responses may be influenced by factors in addition to absolute thermal strain. It is unknown whether a faster rate of change in core temperature, associated with greater exercise intensities (50), and/or exposure to higher ambient temperatures (17), together with the concomitant increase in perceived exertion (20), cardiovascular strain, neuroendocrine and immune changes (27), and reduced cognitive performance (22), may help to explain such differences in the PRL responses during EHS. Interestingly, Buckler (6) has shown that the growth hormone response during exercise is directly related to the rate of increase in core temperature. One approach to test whether the rate of change in core temperature affects PRL release is to have participants exercise at different exercise intensities while using core temperature as the independent variable for blood sampling. In addition, examining the relationship between circulating PRL and core temperature,

and changes in PRL, during fast and slow decreases in core temperature (i.e., active and passive cooling, respectively) could be used to further test the premise that PRL can be used as a surrogate marker of the effects of thermal strain on the interplay between brain serotonin and dopamine. Although cold water immersion recovery from EHS results in the quick reestablishment of cardiovascular (i.e., heart rate) and thermal parameters (45, 59), it is unclear whether PRL would exhibit a similar recovery response.

The release of PRL from the pituitary gland is also positively stimulated by cytokines, such as interleukin (IL)-6 and Tumor Necrosis Factor (TNF)- α (61). In response to a single bout of exercise, the immune system exhibits an acute phase response due to the associated local muscle damage, metabolic changes, and hyperthermia (34, 38, 51). Experimental designs where the normal rise in core temperature during exercise has been reduced demonstrate the importance of thermal strain for the release of pro- and anti-inflammatory cytokines, such as IL-6, IL-1ra, and TNF- α , into the circulation (46). Increases in IL-6 and TNF- α during exercise are dependent on the exercise intensity and duration, the muscle mass being utilized, and the endurance capacity of the individual (14, 39, 40, 42). Although PRL secretion is strongly stimulated by increases in core temperature, the strength of the cytokine influence on PRL secretion from the pituitary gland during EHS is unknown. Thus, examining the cytokine and PRL responses simultaneously during different rates of increase in, and recovery of, core temperature will help elucidate both the strength of the influence of cytokines on pituitary PRL secretion and the stimulus for the variability in PRL responses reported in the literature at a given core temperature.

The purpose of this study was, therefore, to examine the influence of different rates of increase (i.e., slow vs. fast heating) and decrease (active vs. passive cooling) in core temperature on PRL and circulating cytokine responses (i.e., IL-6, TNF- α) and, subsequently, the strength of the relationship between cytokine stimuli on the pituitary and potential PRL secretion. Although PRL has been reported to be strongly related to the increase in core temperature, variability of the PRL responses at a given core temperature in the literature may be partially explained by differences in the rate of increase core temperature. Given that cytokine responses are partly dependent on exercise intensity, it was hypothesized that the faster rate of core temperature increase associated with higher exercise intensities would stimulate greater cytokine and PRL responses compared with slow heating. Subsequent to the EHS, it was hypothesized that active, compared with passive, cooling would result in greater reductions in circulating PRL and cytokines, corresponding to the quicker reestablishment of baseline thermal and cardiovascular responses.

EXPERIMENTAL PROCEDURES

Subjects

On receiving approval from the University of Ottawa Health Sciences and Science Research Ethics Board, nine healthy (no history of respiratory, metabolic, or cardiovascular conditions), physically active individuals (7 men, 2 women), aged 20–46 yr, were recruited from the university population and general community. Participants were informed of all experimental procedures, associated risks, and discomforts before providing written, informed consent. Mean \pm SE age, height, mass, body surface area, percent body fatness, and

maximal aerobic power of the participants were as follows: 26.0 \pm 2.5 yr, 174.2 \pm 3.8 cm, 74.6 \pm 4.3 kg, 17.9 \pm 2.8%, and 56.5 \pm 2.2 ml O₂·kg⁻¹·min⁻¹, respectively.

Experimental Design

Testing was conducted in the Human and Environmental Physiology Research Unit at the University of Ottawa. During the preliminary session, participants received an orientation to the instrumentation and experimental protocols, completed the American Heart Association/American College of Sports Medicine Pre-Participation Screening (1) and the Canadian Society for Exercise Physiology Physical Activity Readiness (10) questionnaires to ensure their safety for participation, and performed fitness and body composition assessments. Aerobic fitness was assessed by measuring peak O₂ consumption ($\dot{V}O_{2\text{peak}}$) using a treadmill protocol and open-circuit spiroometry. Participants began running at a self-selected pace, where the treadmill incline was increased 1% each minute to a maximum of 10%, after which speed and incline were increased alternatively each minute by 0.8 km/h and 1% incline, respectively, until the participant could no longer continue. $\dot{V}O_{2\text{peak}}$ was defined as the highest oxygen uptake during the incremental test, taken as the mean of the three highest consecutive 15-s recordings at the end of the test. In addition, hydrostatic weighing was used to measure body density, and percent body fatness was estimated based upon the Siri equation (54).

Following the preliminary session, all participants completed four EHS exposures, which were separated by a minimum of 5 days. Participants were asked to refrain from strenuous exercise (running, swimming, cycling, weight lifting, etc.), alcohol, and the use of over-the-counter medications for 24 h, and caffeine for 12 h, before each EHS session. During the experimental EHS sessions, participants either walked (4.0–4.5 km/h, 2% incline) or jogged (~7.0 km/h, 2% incline) on a treadmill in a hot-dry environment (40°C, 20–30% relative humidity) for a slow or fast rate of T_{re} increase, respectively, while wearing a nonpermeable rain poncho. On reaching a T_{re} of 39.5°C, participants recovered either passively (resting ambient environment, ~29°C) or actively (2°C cold water immersion) until returning to a T_{re} of 38.0°C. Thus the four EHS sessions, which were performed in a randomized order, included the following: 1) slow heating with passive recovery (SP); 2) slow heating with active recovery (SA); 3) fast heating with passive recovery (FP); and 4) fast heating with active recovery (FA).

Physiological Measurements

Core temperature. T_{re} and esophageal temperature (T_{es}) were measured continuously using a pediatric thermocouple probe (Monatherm Nasopharyngeal Temperature Probe, Mallinckrodt Medical, St. Louis, MO). The rectal probe was inserted ~15 cm beyond the anal sphincter. T_{es} was measured by inserting the probe, of ~2 mm in diameter, through the nostril to the level of the heart.

Heart rate. Heart rate was continuously monitored on a receiver using a transmitter (Polar Electro, Oy, Finland) fitted around the chest.

Ratings of thermal comfort and perceived exertion. Ratings of perceived exertion (RPE; scale “6 = no exertion at all” to “20 = maximal exertion”) and thermal comfort (TC; scale “1 = so cold I am helpless” to “13 = so hot I am sick and nauseated”) were recorded at baseline resting (PRE) and at each increment change in T_{re} of 0.5°C. The perceptual strain index (PeSI) was calculated using TC and RPE, according to the following equation:

$$\text{PeSI} = [5 \cdot (\text{TC}_t - 7)/6] + [5 \cdot (\text{RPE}_t - 6)/14]$$

where t represents the T_{re} at which the rating was recorded, the denominators of 6 and 14 represent the number of values on the scale above rest for TC and RPE, respectively, and 6 represents the RPE at rest (60).

Blood sampling and measurements. Venous blood was collected via an indwelling intravenous catheter (Jelco I.V. 18G Catheter,

Smiths Medical International, Rossendale, Lancashire, UK), inserted into an antecubital vein, with a 21-in. extension line. Blood was collected at rest (PRE), at a T_{re} of 38.0°C, 39.0°C, and 39.5°C during EHS, at the beginning of recovery, and at a T_{re} of 39.0°C and 38.0°C during recovery. Blood was drawn into sterile plastic syringes and transferred immediately into serum with no additive, 5.4 mg plasma K₂EDTA, and plasma sodium heparin (NH, 30 USP) BD Vacutainer tubes (BD, Franklin Lakes, NJ). Nonadditive blood sat for 20 min to clot before centrifugation at ~3,300 rpm for 10 min, whereas the EDTA blood was mixed by inversion, used for hematological analyses (Beckman Coulter, Miami, FL) and the measurement of plasma protein concentration (Reichert TS 400 total solids refractometer, Reichert, Depew, NY), and centrifuged immediately with the sodium heparin plasma. Serum and plasma aliquots were transferred into polypropylene Eppendorf tubes, frozen at -20°C, and stored at -70°C until analyzed.

Enzyme immunoassay techniques were used to determine circulating serum PRL (ALPCO Immunoassays, ALPCO Diagnostics, Salem, NH), and serum IL-6 and plasma TNF- α (Quantikine High Sensitivity R&D Systems, Minneapolis, MN) concentrations. The PRL, IL-6, and TNF- α assays' minimum detectable concentrations were 2 ng/ml, 0.016–0.110 pg/ml, and 0.038–0.191 pg/ml, respectively. All samples were analyzed in duplicate following manufacturer instructions, and concentrations were corrected for plasma volume changes, based on the Dill and Costill method using blood hemoglobin and hematocrit changes (11).

Statistical Analyses

A two-way ANOVA with repeated measures of heating (slow vs. fast heating) and temperature (PRE, T_{re} of 38.0, 39.0, and 39.5°C) during exercise, and cooling (Active vs. Passive) and temperature (the start of recovery, and T_{re} of 39.0 and 38.0°C) during recovery was performed on the dependent measures (i.e., heart rate, PeSI, percent plasma volume change, plasma protein, PRL, PRL rate of change relative to T_{re} and T_{es} , IL-6, TNF- α) during the experimental sessions. A one-way ANOVA was used to compare dependent measures at PRE, end of exercise, start of recovery, and end of recovery, the exercise and recovery times and rates of change in T_{re} and T_{es} , and the

PRL response at a given T_{re} in heating vs. cooling. When a significant F ratio was obtained, a Newman-Keuls post hoc analysis was used to isolate differences among treatment means. All ANOVAs were completed using Statistica data analysis software (StatSoft 2007, version 8.0, Tulsa, OK, www.statsoft.com). Results are reported as means \pm SE. Statistically significant differences were identified for $P < 0.05$, and trends were identified for $0.05 < P < 0.1$. An outlier IL-6 response for one participant was identified by calculating the Chauvenet's criterion [$Q = (\text{potential outlier} - \text{maximum value})/\text{range}$], where $n = 8$ for IL-6 based on the data being rejected for a Q greater than the 95% confidence level (for $n = 9$, Q 95% = 0.493).

RESULTS

Physiological Measures

Exercise. By design, the exercise time to reach a T_{re} of 39.5°C was greater for the slow (SP = 76.2 \pm 5.1 and SA = 86.1 \pm 6.8 min) compared with fast (FP = 34.8 \pm 2.6 and FA = 39.8 \pm 4.1 min) ($P < 0.000$) heating, but there was no difference between SP and SA or between FP and FA. These rates of increase correspond to ~1.8 and 4.2°C/h for the slow and fast heating protocols, respectively. There was no difference among the protocols for PRE T_{re} ($P = 0.785$) or T_{es} ($P = 0.986$) or at the end of exercise for T_{re} (SP = 39.50 \pm 0.01, SA = 39.51 \pm 0.00, FP = 39.5 \pm 0.03, and FA = 39.51 \pm 0.00°C, $P = 0.840$). However, T_{es} was significantly higher at the end of exercise following the fast (FP = 39.84 \pm 0.17 and FA = 39.81 \pm 0.11°C) compared with slow (SP = 39.40 \pm 0.08 and SA = 39.41 \pm 0.05°C) ($P = 0.005$) heating. There were no differences between the two slow and the two fast heating conditions for heart rate, percent change in plasma volume, plasma protein concentration, or PeSI (Table 1). No differences were observed for heart rate or plasma protein concentration at PRE ($P = 0.600$ and 0.964, respectively) or the end of exercise ($P = 0.133$ and 0.847, respectively) among the heating protocols.

Table 1. Physiological measures relative to increases in rectal temperature during exercise-induced hyperthermia for the slow and fast heating conditions, followed by active and passive cooling recovery

	Rectal Temperature, °C			
	PRE (36.9)	38.0	39.0	39.5
Heart rate, beats/min				
SP	71 \pm 3	148 \pm 5†	169 \pm 5†	181 \pm 8
FP	71 \pm 3	173 \pm 4†	183 \pm 3†	188 \pm 3
SA	74 \pm 3	152 \pm 4	171 \pm 5	183 \pm 4
FA	74 \pm 1	173 \pm 4	185 \pm 3	191 \pm 3
ΔPV, %				
SP		-7.47 \pm 1.36†	-11.42 \pm 1.21†	-12.53 \pm 1.31
FP		-8.88 \pm 1.26†	-9.31 \pm 1.15†	-9.58 \pm 0.95
SA		-6.10 \pm 1.23	-10.31 \pm 1.38	-11.29 \pm 1.47
FA		-9.24 \pm 1.89	-9.43 \pm 1.80	-9.35 \pm 1.88
PP, g/100 ml				
SP	7.47 \pm 0.15	7.91 \pm 0.19*	8.31 \pm 0.18*	8.43 \pm 0.18*
FP	7.47 \pm 0.20	8.18 \pm 0.14*	8.31 \pm 0.18*	8.31 \pm 0.19*
SA	7.52 \pm 0.16	7.99 \pm 0.23*	8.42 \pm 0.21*	8.55 \pm 0.22*
FA	7.56 \pm 0.13	8.02 \pm 0.18*	8.24 \pm 0.17*	8.32 \pm 0.17*
PeSI				
SP	0.28 \pm 0.14	3.97 \pm 0.37*	6.34 \pm 0.62*	7.65 \pm 0.49*
FP	0.28 \pm 0.14	4.96 \pm 0.41*	6.75 \pm 0.31*	7.43 \pm 0.38*
SA	0.28 \pm 0.14	4.10 \pm 0.66*	6.11 \pm 0.80*	6.87 \pm 0.72*
FA	0.28 \pm 0.14	4.53 \pm 0.50*	5.75 \pm 0.67*	7.57 \pm 0.98*

Values are means \pm SE. PRE, baseline resting temperature; ΔPV, change in plasma volume, PP, plasma protein; PeSI, perceptual strain index; S and F, slow and fast heating conditions, respectively; A and P, active and passive cooling recovery, respectively. *Main effect of temperature, $P < 0.0001$. †Condition \times temperature interaction, $P < 0.005$.

Heart rate increased at a greater rate during the fast compared with slow heating conditions ($P = 0.000$) from PRE to a T_{re} of 39.5°C. Plasma volume exhibited a heating \times temperature interaction ($P = 0.000$), where the percent plasma volume change was significantly greater at a T_{re} of 38.5 and 39.0°C compared with the change at 38.0°C for the slow heating conditions protocols, but the decrease in plasma volume was constant throughout the fast heating protocols. A trend was observed for greater decreases in percent change of plasma volume following the slow compared with fast heating ($P = 0.091$). Plasma protein and PeSI increased significantly throughout exercise, but changes were not different at any given T_{re} among the heating protocols.

Recovery. Cooling recovery time to a T_{re} of 38.0°C tended to be longer following fast compared with slow heating (11.1 ± 2.4 vs. 6.6 ± 0.7 min) ($P = 0.091$), whereas passive cooling recovery time was significantly longer following the fast (76.1 ± 8.0 min) compared with the slow (53.8 ± 5.3 min) ($P = 0.033$) heating protocols. A main effect of condition was observed at the end of recovery for T_{es} ($P = 0.000$) between the two passive (SP = 37.78 ± 0.06 and FP = 37.78 ± 0.09 °C) and two active (SA = 36.27 ± 0.29 and FA = 35.93 ± 0.44 °C) cooling conditions.

Heart rate, plasma volume changes, plasma protein, and PeSI responses during recovery are shown in Table 2. Regardless of the rate of increase in T_{re} during the exercise phase, heart rates at any T_{re} were similar during recovery for all conditions. However, heart rate did significantly decrease as T_{re} decreased. Percent change in plasma volume was not different between the two active nor two passive cooling conditions ($P = 0.978$ and 0.402 , respectively), yet decreased as T_{re} decreased during the passive cooling ($P = 0.000$), but not the active cooling ($P = 0.233$). A temperature \times condition interaction was observed for the four recovery conditions ($P = 0.001$) and the active vs. passive cooling following slow ($P = 0.030$) and fast ($P = 0.012$) heating. Plasma protein concentration was not different between the two active cooling conditions ($P = 0.468$), with decreases in T_{re} during the active cooling ($P = 0.673$), or between the two passive cooling

conditions ($P = 0.593$); however, decreases were observed with decreases in T_{re} during passive cooling ($P = 0.000$).

PRL

PRL exhibited a strong temperature-dependent response throughout exercise from PRE to the end of exercise ($T_{re} = 39.5$ °C, T_{es} fast = 39.8 ± 0.1 °C, T_{es} slow = 39.4 ± 0.0 °C) ($P = 0.000$), with no differences between the fast and slow heating protocols ($P = 0.962$) (Fig. 1, A and C). Regardless of the prior heating protocol, the PRL response during recovery was similar during the two passive and the two active cooling sessions (Fig. 1, B and D). However, PRL values were significantly higher during the active vs. passive cooling protocols at a T_{re} of 39.0 and 38.0°C. The rate of change of PRL relative to T_{es} (pmol/l/°C) was increased during passive recovery following both slow ($P = 0.038$) and fast ($P = 0.000$) heating; however, it was reduced during active recovery following slow and fast heating for both T_{re} ($P = 0.006$ and 0.014 , respectively) and T_{es} ($P = 0.000$ and 0.001 , respectively) (Fig. 2).

Cytokines

IL-6 was not different during EHS between the two slow ($P = 0.328$) or the two fast ($P = 0.907$) heating conditions from PRE to a T_{re} of 39.5°C (Fig. 3, A and C, $n = 8$). A temperature \times condition interaction ($P = 0.024$) was observed for IL-6, where increases were observed at a T_{re} of 38.0, 39.0, and 39.5°C compared with PRE for all conditions, with greater increases at 39.5°C following the slow compared with fast heating conditions. During active and passive recovery, IL-6 tended to be elevated following slow vs. fast heating ($P = 0.076$); however, no effect of temperature was observed ($P = 0.598$) (Fig. 3, B and D). Although no differences were observed between the active and passive recovery conditions ($P = 0.905$) or with decreases in temperature ($P = 0.465$) following slow heating, a temperature \times condition interaction ($P = 0.013$) was observed between the active and passive recovery conditions following fast heating, where IL-6 continued to rise during the passive recovery as T_{re} began to fall.

TNF- α was not different between the two slow ($P = 0.500$) or the two fast ($P = 0.936$) heating conditions from PRE to a T_{re} of 39.5°C (Fig. 4, A and C). An increase in TNF- α was observed at a T_{re} of 39.0 and 39.5°C compared with PRE ($P = 0.038$) during the fast heating conditions only while a trend was observed for a greater TNF- α response at a T_{re} of 39.5°C for the fast compared with slow heating conditions ($P = 0.092$). TNF- α was not different between the active and passive recovery conditions ($P = 0.519$) or with decreases in temperature ($P = 0.349$) (Fig. 4, B and D); however, when comparing the two passive recovery conditions, TNF- α was higher at a T_{re} of 38.0°C compared with the start of recovery following the slow heating ($P = 0.018$). Following slow heating, TNF- α was not different between the active and passive recovery conditions ($P = 0.282$); however, TNF- α was elevated at the end of recovery ($T_{re} = 38.0$ °C) compared with the start of recovery ($P = 0.027$).

DISCUSSION

This study examined the influence of different rates of increase and decrease of core temperature on circulating PRL and cytokine responses, and the influence of circulating cyto-

Table 2. Physiological measures relative to rectal temperature during active and passive cooling recovery following slow and fast heating

	Rectal Temperature, °C		
	Rec (39.7)	39.0	38.0
Heart rate, beats/min			
SP	133 ± 4	$106 \pm 6^*$	$89 \pm 3^*$
FP	137 ± 6	$103 \pm 5^*$	$86 \pm 3^*$
SA	143 ± 8	$101 \pm 9^*$	$94 \pm 5^*$
FA	139 ± 8	$107 \pm 5^*$	$104 \pm 9^*$
$\Delta PV^\dagger, \%$			
SP	-12.13 ± 1.54	$-8.75 \pm 1.95^*$	$-6.45 \pm 2.22^*$
FP	-9.31 ± 1.23	$-6.67 \pm 1.54^*$	$-5.46 \pm 1.64^*$
SA	-11.28 ± 1.64	-9.78 ± 1.78	-8.82 ± 1.97
FA	-9.75 ± 1.46	-10.00 ± 1.62	-10.33 ± 2.20
PP, g/100 ml			
SP	8.70 ± 0.26	$8.41 \pm 0.21^*$	$8.26 \pm 0.23^*$
FP	8.48 ± 0.16	$8.31 \pm 0.15^*$	$8.13 \pm 0.20^*$
SA	8.72 ± 0.23	8.59 ± 0.23	8.59 ± 0.28
FA	8.36 ± 0.16	8.39 ± 0.21	8.46 ± 0.22

Values are means \pm SE. Change in PV is relative to PRE. Rec, start of recovery. *Main effect of temperature, $P < 0.0001$. † Condition \times temperature interaction, $P < 0.005$.

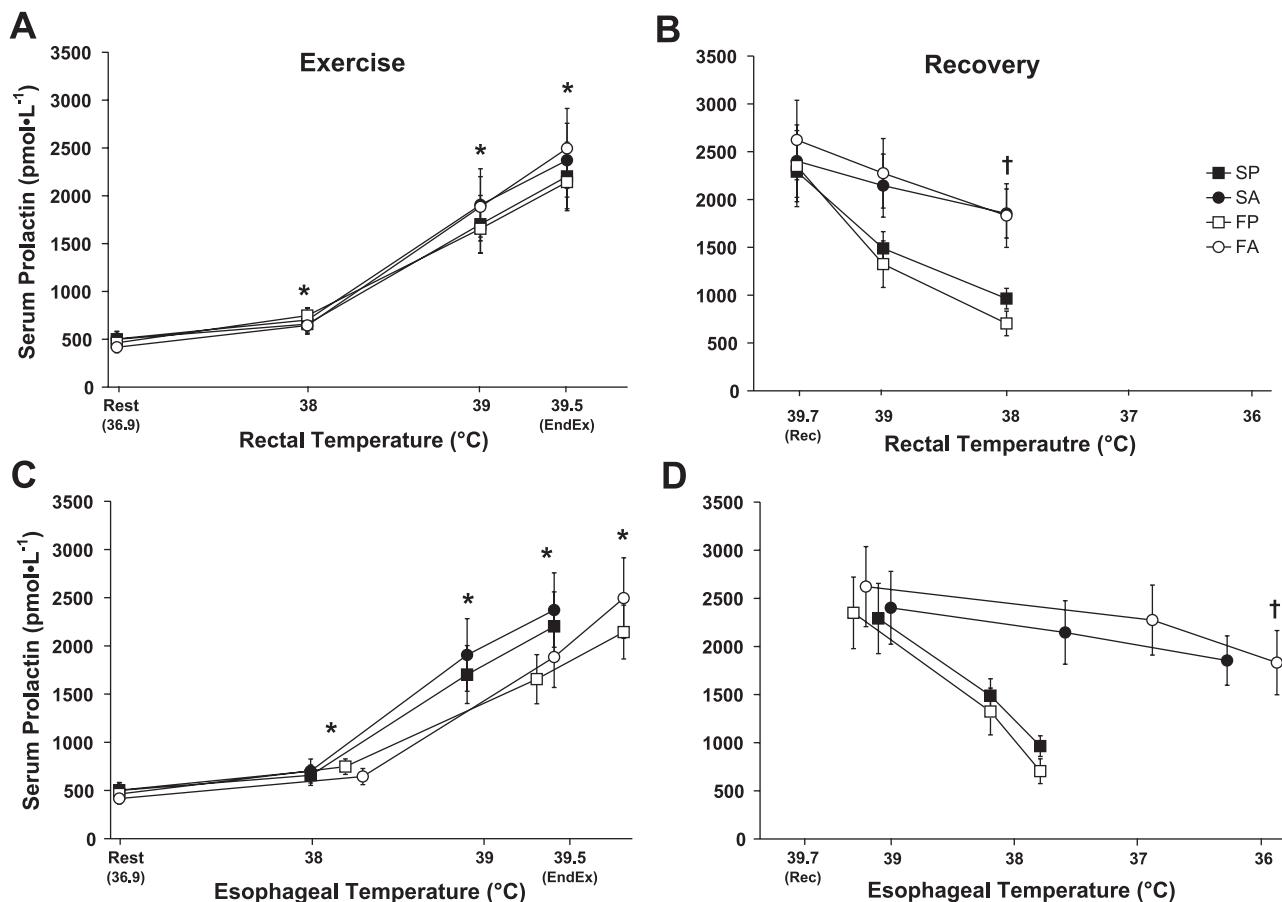


Fig. 1. Circulating serum prolactin (PRL) concentrations during slow (S) vs. fast (F) exertional heat stress (A and C), followed by active (A) vs. passive (P) cooling (B and D), relative to rectal (T_{re} ; A and B) and esophageal temperature (T_{es} ; C and D). Values are means \pm SE. Main effect of temperature ($*P < 0.0001$) and temperature \times condition interaction ($\dagger P < 0.005$) is shown.

kines on PRL responses during EHS and recovery. Furthermore, this study used a slow vs. fast EHS ramping protocol to verify the use of PRL as an indicator of changes within the brain that reflect impending fatigue. Unique to this study was the observation that, regardless of the rate of hyperthermia development, PRL exhibited a strong temperature-dependent response during EHS. However, during recovery, the relationship between PRL and T_{re} was dependent on the rate of cooling. During EHS, increased metabolic demands with long-duration exercise exaggerated the IL-6 response during the slow compared with fast heating protocols, whereas TNF- α responded to the greater exercise intensity during the fast

compared with slow heating. However, the different responses of these circulating cytokines during the heating protocols did not influence the PRL response. In addition, neither recovery method, of active vs. passive cooling, was superior in significantly reducing the circulating cytokines (i.e., IL-6 and TNF- α) following EHS.

PRL and Central Fatigue

Circulating PRL has been implicated as an indicator of central fatigue during heat stress, given that increased values reflect the relationship of increased serotonin and decreased

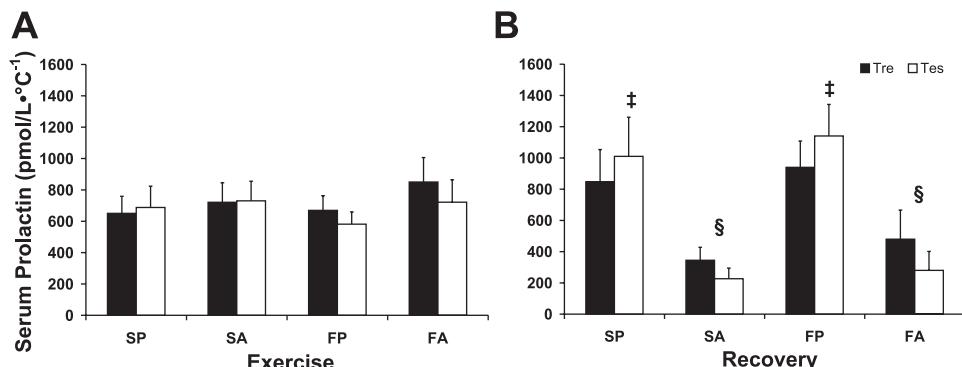


Fig. 2. The rate of change in circulating serum PRL concentrations relative to the change in T_{re} and T_{es} during S vs. F exertional heat stress (A) followed by A vs. P cooling (B). Values are means \pm SE. Significantly different during passive ($\ddagger P < 0.05$) and active ($\ddagger P < 0.05$) recovery compared with exercise.

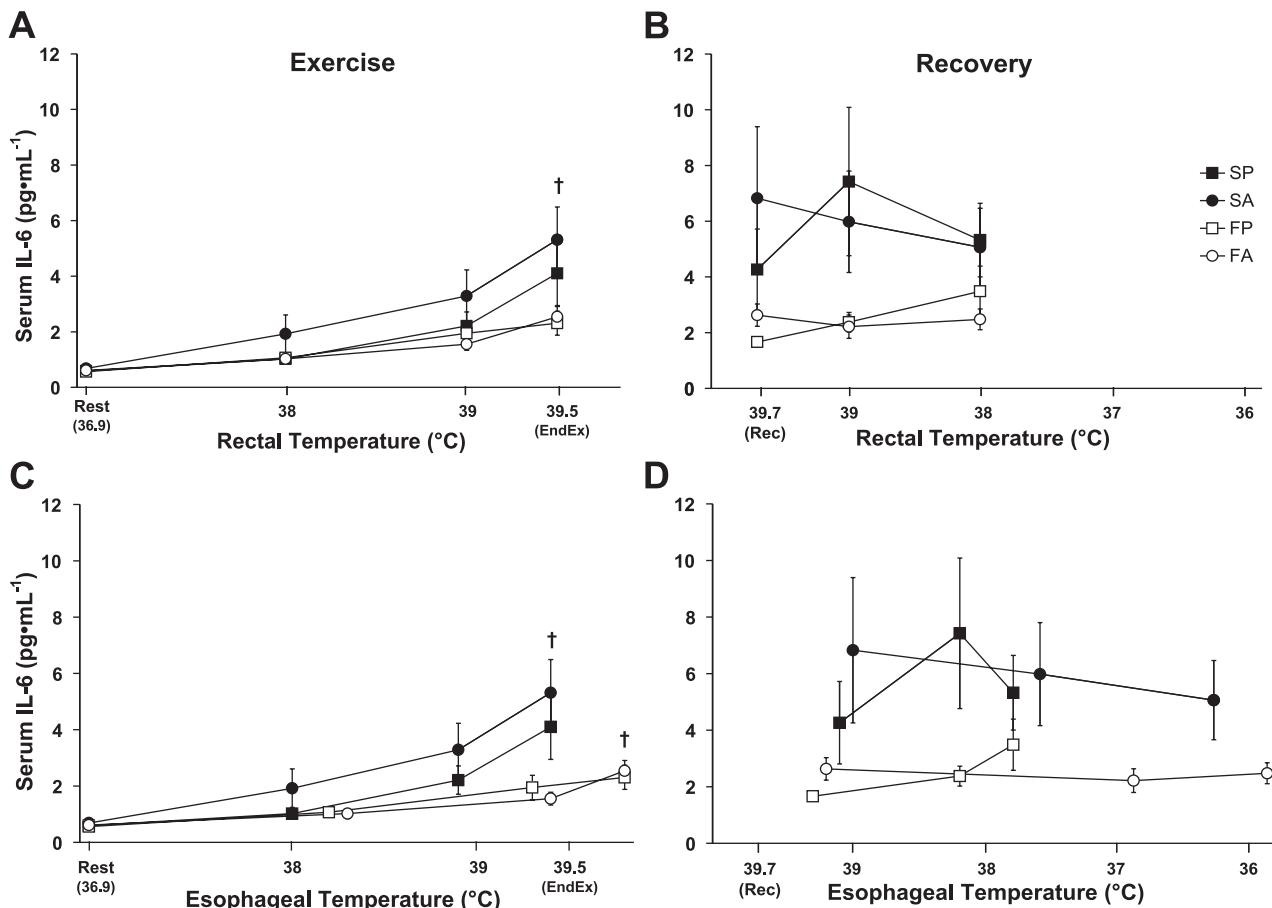


Fig. 3. Circulating serum interleukin (IL)-6 concentrations during S vs. F exertional heat stress (A and C), followed by A vs. P cooling (B and D), relative to T_{re} (A and B) and T_{es} (C and D). Values are means \pm SE. Temperature \times condition interaction ($\dagger P < 0.05$) is shown.

dopamine levels in the brain (4, 29, 62) and are correlated with increases in core temperature (44, 65). The present study showed a strong temperature-dependent PRL response during EHS from PRE ($T_{re} = 36.96 \pm 0.05^\circ\text{C}$) to 39.5°C , which supports the use of PRL as a marker of increased and decreased brain serotonin and dopamine, respectively, also known as the central fatigue hypothesis (31). This temperature-dependent PRL response is consistent with previous studies reporting increases in PRL during EHS in trained and untrained men (65), following 5 h of cycling in trained individuals (57), and in hypohydrated, heat-acclimated men during EHS (7). The greater rate of increase in PRL beyond a T_{re} of 38.0°C is also consistent with our laboratory's earlier work (65) and the other studies reporting increased circulating hormone and immune markers above a T_{re} of 38.0°C (9, 46). Furthermore, this is the first study to report a strong temperature-dependent response for PRL that is independent of different rates of increase in T_{re} [i.e., slow ($\sim 2^\circ\text{C}/\text{h}$) vs. fast ($\sim 4^\circ\text{C}/\text{h}$) hyperthermia development] associated with greater exercise intensities (Fig. 5). Although it was hypothesized that different rates of increase in core temperature would contribute to the variable PRL responses at a given core temperature reported in the literature, the present study refutes this possibility, given that the PRL response was independent of the rates of increase in T_{re} . This is further supported by Fig. 2, which shows no differences in the rate of change in PRL relative to T_{re} and T_{es} .

(pmol/l/ $^\circ\text{C}$) between the slow and fast heating and our laboratory's previous work, which showed similar PRL responses between trained and untrained individuals at a slower rate of T_{re} increase of $\sim 1^\circ\text{C}/\text{h}$, with very similar PRL values at a T_{re} of 39.5°C for the trained group ($2,353 \pm 220$ pmol/l) (65) compared with the present study with individuals of similar fitness levels ($2,303 \pm 360$ pmol/l). Thus, during EHS, the present study provides further support for the use of PRL as a peripheral marker of impending fatigue at high core temperatures.

Despite the present study and previous research showing a strong relationship between PRL and core temperature (44, 65), the divergent PRL response at a given core temperature between the two recovery methods in the present study indicates that thermal strain alone cannot solely explain the PRL responses and may provide some explanation for the variable PRL responses reported in the literature. Regardless of the rate of increase in core temperature during EHS, the manipulation of the rate of change in core temperature during recovery resulted in PRL concentrations decreasing at a slower rate during the active compared with passive cooling recovery. Since it is believed that T_{es} better reflects brain temperature compared with T_{re} (53), representing PRL responses relative to T_{es} may be more indicative of the serotonergic and dopaminergic changes within the brain and thus circulating PRL concentrations (Figs. 1B and 2B). However, PRL responses in

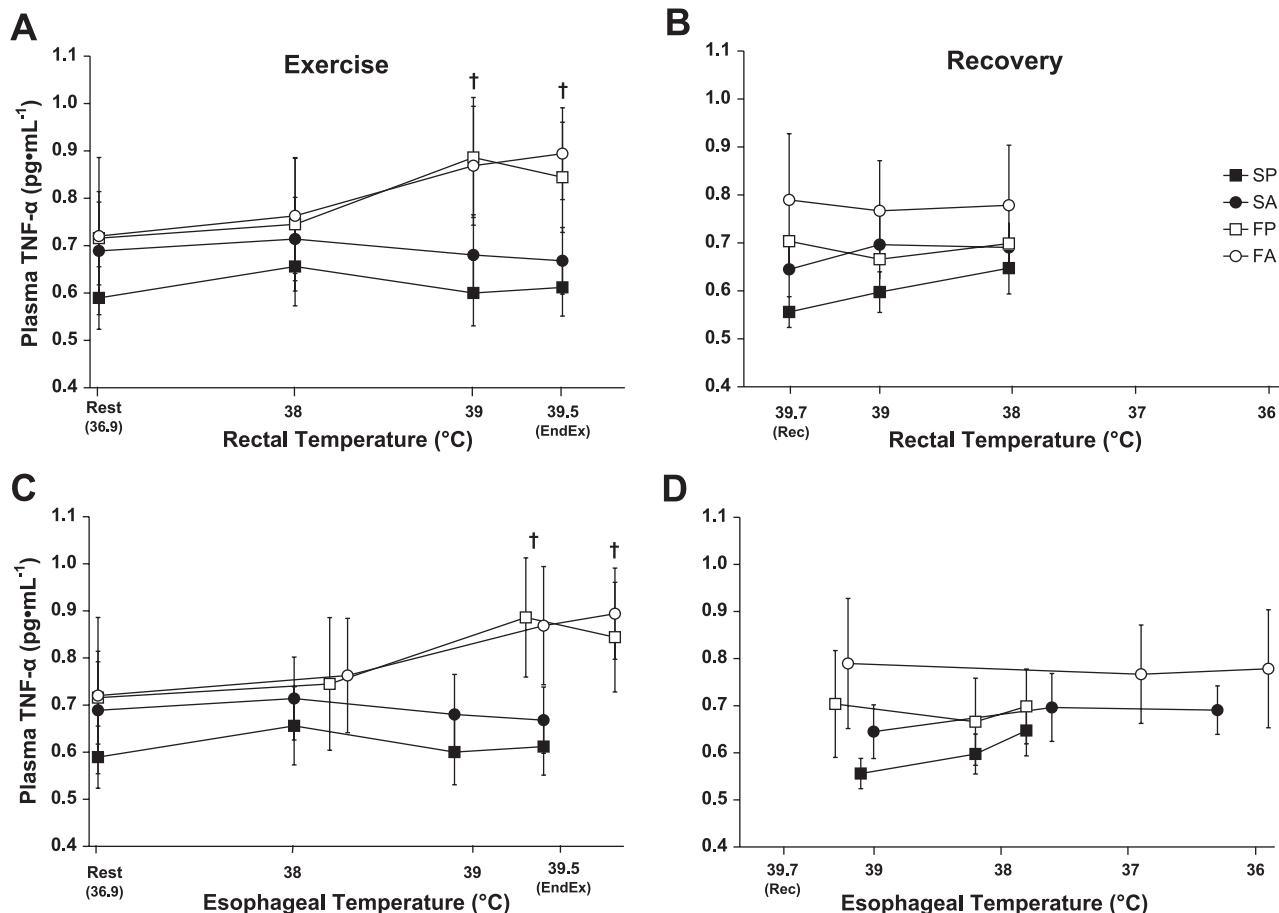


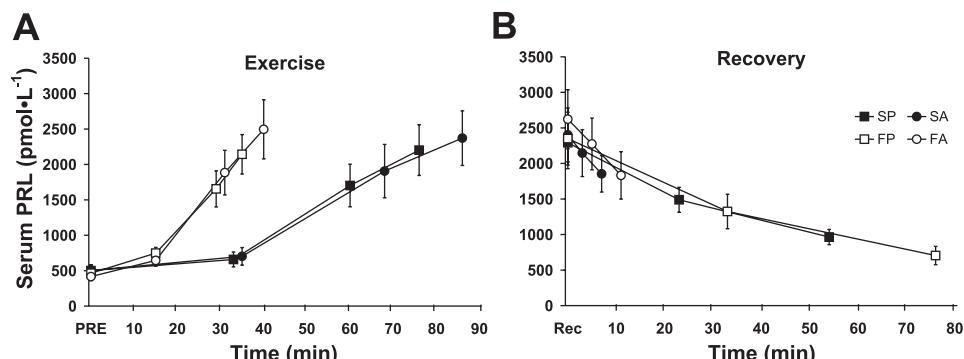
Fig. 4. Circulating plasma tumor necrosis factor (TNF)- α concentrations during S vs. F exertional heat stress (A and C), followed by A vs. P cooling (B and D), relative to T_{re} (A and B) and T_{es} (C and D). Values are means \pm SE. Temperature \times condition interaction ($\dagger P < 0.05$) is shown.

the present study were more strongly correlated with T_{re} than T_{es} during slow ($r = 0.67$ and 0.61 , respectively) and fast ($r = 0.72$ and 0.64 , respectively) heating, and during passive ($r = 0.58$ and 0.48 , respectively) but not active recovery ($r = 0.24$ and 0.31 , respectively). Furthermore, a greater divergence in PRL response is observed during recovery when represented relative to T_{es} . Despite similar PRL concentrations at the start of recovery in all conditions, PRL concentrations at a given T_{re} during passive recovery reflected EHS concentrations better than the PRL responses observed during active recovery. For instance, at the end of recovery, at a T_{re} of 38.0°C , PRL values were not different than PRL values at a T_{re} of 38.0°C during

EHS ($P = 0.120$), although they were still different at 39°C ($P = 0.045$) and were better correlated with T_{re} and T_{es} during passive ($r = 0.58$ and 0.48 , respectively) but not active ($r = 0.24$ and 0.31 , respectively) recovery. Although active cooling reestablishes heart rate and thermal parameters (i.e., T_{re} , T_{es}) more quickly than passive cooling (45, 59), the present study shows that circulating PRL concentrations lag behind the changes in T_{re} during active cooling, indicating that factors other than this measure of thermal strain are contributing to this response.

The divergent PRL response during the active cooling recovery may be attributed to the likely reduced clearance capacity with the decreased peripheral blood flow from cold

Fig. 5. Circulating serum PRL concentrations during S vs. F exertional heat stress (A), followed by A vs. P cooling (B), relative to time. Values are means \pm SE.



water vasoconstriction and/or a reduced inhibition of PRL secretion by dopamine in the brain. Circulating PRL concentrations are predominantly affected by inhibitory and stimulatory actions of dopamine and thyrotropin-releasing hormone, respectively; however, clearance by the kidneys also influences concentrations (i.e., reduced renal function reduces PRL clearance) (12, 52). During the active recovery, not only is cold water diuresis, an increase in urine production due to peripheral vasoconstriction, likely reduced in the present study due to the extent of dehydration from the EHS, but reduced renal function can also occur during cold water immersion (30). Together, the reduced cold water diuresis and renal function may have resulted in a reduced clearance of PRL from circulation, leading to the sustained elevation in PRL during active recovery. Furthermore, PRL secretion is inhibited by dopamine in the brain (2, 62), and thus the reduced peripheral blood circulation may be indicative of reduced circulation within the brain, blunting the rate of dopamine-induced inhibition of PRL, and delaying the decrease in PRL at a given core temperature during active cooling recovery. In addition, although chronic elevations in PRL are indicative of disease (i.e., hypothyroidism, kidney disease, tumors), PRL secretion may have actually continued during the cooling recovery, given that the cold stress may have further stimulated PRL release (15, 26). The divergence between the active and passive recovery may have been further exacerbated by an increased clearance during passive recovery. Given that the rate of change in PRL relative to the change in T_{re} ($\text{pmol/l}/^{\circ}\text{C}$) is greater during passive recovery compared with slow and fast heating (Fig. 2), and that blood is redistributed away from the gut splanchnic area with increasing core temperatures with hyperthermia (49, 51), clearance of PRL during exercise may have been progressively decreasing and potentially reversed during passive recovery, such that clearance was increased. In addition, circulating norepinephrine levels have been shown to be strongly related to core temperature (64) and could be used as an indicator of sympathetic response and the extent of splanchnic bed vasoconstriction in the present study. For instance, heart rates at a T_{re} of 39.0°C during exercise were ~ 170 – 180 and ~ 105 beats/min during passive recovery, with similar differences at a T_{re} of 38.0°C (Tables 1 and 2), suggesting that lower norepinephrine levels, as reflected by the lower heart rates, may be promoting stronger renal blood flow and increased clearance of PRL during passive recovery.

PRL-Cytokine Interplay

Although the immune system acute phase response observed during exercise and heat stress has been well documented (43, 51, 58), the role of the immune system in the development of fatigue is not fully understood. Given the importance of thermal strain on the release of pro- and anti-inflammatory cytokines (i.e., IL-6, TNF- α) into circulation during exercise (46), and that PRL secretion is not only strongly stimulated by increases in core temperature, but also positively stimulated by such cytokines, this study was unique in examining the relationship and the extent of the influence of cytokines on the PRL secretion from the pituitary during different rates of increase in, and recovery of, T_{re} . During EHS, the present study showed divergent increases in cytokine responses between the slow and fast heating conditions, whereby IL-6 was elevated at higher

core temperatures of 39.5°C (i.e., end of exercise) for the slow compared with the fast heating, yet TNF- α showed a trend for the reverse (i.e., elevated for fast compared with slow heating). Although cytokine (i.e., IL-6, TNF- α) secretion is partially dependent on exercise intensity (40, 42), the elevated IL-6 responses at higher core temperatures during the slow heating, or lower intensity exercise, are consistent with an increased IL-6 secretion observed when muscle glycogen is low following long-duration exercise (13, 41). The addition of heat stress to exercise also accelerates the utilization of muscle glycogen, which stimulates the release of IL-6 to a greater extent (24). Thus it appears that IL-6 may be driven by metabolic demands, acting as a myokine to mobilize substrates and possibly enhance lipolysis (37). The trend for a reversed response for TNF- α between the slow and fast heating protocols is likely due to the fact that intense exercise is a strong stimulant for TNF- α secretion (41, 43), as would be the case during the fast rather than during the slow heating. In addition, given that IL-6 exhibits anti-inflammatory properties by inhibiting the production of the pro-inflammatory cytokine TNF- α (43), the elevated IL-6 during the slow heating protocol may have contributed to the lack of increase in TNF- α during these sessions.

Neither active nor passive cooling influenced the cytokine responses, although there were trends for elevated IL-6 concentrations throughout both recovery methods following the slow compared with the fast heating and an increase in TNF- α at 38.0°C compared with the start of passive recovery following slow heating. The lack of change in circulating IL-6 with active or passive cooling is consistent with Halson et al. (21), who also observed no effect of cold water immersion on circulating IL-6 concentrations and other neuroendocrine and immune (i.e., cortisol, C-reactive protein) markers following cycling in the heat ($\sim 34^{\circ}\text{C}$), despite subjective reports of enhanced recovery following cold water immersion compared with passive recovery. Similarly, although no formal assessment was conducted, most participants in the present study verbally commented that they felt an enhanced recovery following active compared with passive cooling recovery.

Given that IL-6 and TNF- α are partially dependent on exercise intensity, it was hypothesized that these cytokines would exhibit a greater increase during the fast compared with slow heating conditions (i.e., higher and lower exercise intensities, respectively). Furthermore, although speculative, IL-6 and TNF- α are believed to positively stimulate the secretion of PRL from the pituitary gland (61) and thus may enhance the secretion of PRL during the fast heating conditions. However, contrary to our hypothesis, PRL remained tightly correlated to T_{re} and similar during both fast and slow heating, despite the observed changes in IL-6 and TNF- α . Given that IL-6 has been reported to be positively correlated with increased sensations of fatigue (47), the elevated IL-6 concentrations during the slow heating may show evidence for the role of IL-6 as a myokine, with the sustained IL-6 elevation during both cooling protocols following slow heating potentially reflecting a greater level of fatigue following prolonged EHS and thus a delayed recovery (47). Thus it appears that PRL was influenced by continued thermal stimulation (i.e., cold stress) and/or reduced clearance from circulation rather than IL-6 and/or TNF- α .

This was the first study to systematically examine the effects of different rates of increase, and recovery, of core temperature

on PRL as a peripheral marker of central fatigue and the influence of circulating cytokines on this relationship. PRL exhibited a strong temperature-dependent response during exercise, despite a twofold variation in the rate of increase in thermal strain and during subsequent passive cooling recovery, which required ~60 min to return T_{re} to 38.0°C. However, during active cooling recovery, which rapidly decreased core temperature, much higher PRL values were observed at any given core temperature compared with passive cooling. IL-6 exhibited myokine and anti-inflammatory properties during exercise, as shown with the increases in IL-6 and lack of increase in TNF- α during the slow heating, while being elevated during recovery following slow heating as an indicator of low muscle glycogen and increased fatigue. Thus it appears that IL-6 and TNF- α acted independently of PRL during EHS and recovery, in that these cytokines did not influence the release of PRL from the pituitary gland. Rather, the predominant stimulus affecting the interplay between serotonin and dopamine control of PRL release appears to be the level of thermal strain.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

H.E.W., T.M.M., D.J.C., and G.P.K. conception and design of research; H.E.W. and B.J.F. performed experiments; H.E.W. analyzed data; H.E.W., T.M.M., and G.P.K. interpreted results of experiments; H.E.W. prepared figures; H.E.W. drafted manuscript; H.E.W., T.M.M., B.J.F., D.J.C., and G.P.K. edited and revised manuscript; H.E.W., T.M.M., B.J.F., D.J.C., and G.P.K. approved final version of manuscript.

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