

EXERCISE, HEAT STRESS AND THE INTERLEUKIN-6 RESPONSE: SUPPORT FOR TEMPERATURE-MEDIATED NEUROENDOCRINE REGULATORY MECHANISMS

Toby Mündel^{1,2 (A-F)}, Jaime P. Cox^{2(B)} and David A. Jones^{2,3(A, E)}

¹ Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

² School of Sport and Exercise Sciences, The University of Birmingham, Birmingham, UK

³ Institute for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University, Manchester, UK

Abstract

Aim of study: The purpose of the present investigation was to determine a) whether or not the addition of heat stress augments exercise-induced circulating interleukin-6 (IL-6), and b) if this was accompanied by an associated neuroendocrine response.

Materials and methods: Eight trained, non-heat acclimated males (26 ± 8 years, $\dot{V}O_{2\max}$: 60 ± 7 ml·kg⁻¹·min⁻¹) each performed two 60-min cycling trials at $70 \pm 2\%$ maximal oxygen uptake at either 20°C (CON) or 35°C (HOT). Samples of venous blood were taken (rest, 30min, 60 min, +30 min) for determination of IL-6, prolactin and growth hormone. Indirect calorimetry was used to estimate whole-body substrate oxidation at 20 and 40 min during exercise, and measures of oesophageal (core) temperature and heart rate recorded.

Results: All eight subjects reported exercise as more difficult during HOT than CON, as evidenced by significantly higher ratings of perceived exertion ($P < 0.01$). The rise in core temperature was greater during HOT than CON ($P < 0.05$), increasing by $\sim 1.8^\circ\text{C}$ and $\sim 1.2^\circ\text{C}$, respectively. Plasma concentrations of IL-6 increased approximately twice as much in the HOT condition than CON ($P < 0.05$). Concentrations of plasma growth hormone were significantly higher during HOT than CON ($P < 0.05$) as were concentrations of plasma prolactin ($P < 0.01$), whilst estimated total carbohydrate oxidation was similar between trials ($P > 0.05$).

Conclusions: The present results confirm the additive effect of heat stress to the IL-6 response during exercise. It is unlikely that this response is related to fuel selection, as whole-body carbohydrate oxidation was similar between conditions. Rather, the greater rise in core temperature during HOT was accompanied by significantly higher levels of growth hormone and prolactin, supporting a role for temperature-mediated neuroendocrine regulation of exercise-induced IL-6.

Key words: Exercise, core temperature, interleukin-6, prolactin, growth hormone

Introduction

Physical exertion can present a serious challenge to the homeostatic mechanisms of the body, and the changes occurring during strenuous or prolonged exercise have been described as a 'stress response' [1]. High ambient temperatures exacerbate this response and have a detrimental effect on endurance performance [2]. When compared with temperate conditions, exercise in the heat is associated with a progressive hyperthermia which is thought to be the primary stimulus for more pronounced concentrations of circulating stress hormones. In particular, the addition of heat stress to exercise is a potent stimulus of both the sympathetic nervous system and the hypothalamic-pituitary-adrenocortical axis [3]. Concentrations of the catecholamines and the anterior pituitary hormones prolactin and growth hormone have been shown to increase exponentially with core temperature (T_{core}), as long as this increase is at least 0.6°C or $> 38^\circ\text{C}$ [4]. In general, these hormones con-

tribute towards the maintenance of cardiovascular and glucose homeostasis, however, more recently the focus has turned to the additional impact of heat exposure on exercise-related immunological changes such as leukocytosis and cytokinaemia [5].

The acute-phase response to exercise typically involves the release of several inflammatory cytokines into the circulation, with by far the most immediate and dramatic increase being that of interleukin (IL)-6 [6]. The additional effects of heat stress during exercise on IL-6 are unclear, since the heat may [7, 8, 9] or may not [10, 11] augment circulating concentrations. Possible reasons for this discrepancy may be the different modes (running *versus* cycling), intensities ($45\text{--}75\%$ $\dot{V}O_{2\max}$) and duration (40-90 min) of exercise used, or ambient temperatures whilst exercising ($28\text{--}40^\circ\text{C}$). Therefore the primary purpose of the present study was to clarify *whether or not* the addition of heat stress to prolonged exercise augments the IL-6 response.

The mechanisms underlying the IL-6 response are a complex interplay of neuroendocrine, metabolic and thermal changes associated with exercise [12, 13]. However, Rhind *et al.* [8] were able to demonstrate that the elevation of T_{core} during exercise is a critical determinant of cytokine release. In their study, subjects cycled for 40 minutes whilst immersed in water at 39°C and 18°C, thereby manipulating the rise in T_{core} to ~1.9°C and ~0.5°C by the end of exercise, respectively. Immersion in cold water significantly blunted the neuroendocrine response to exercise and abolished the increases in plasma IL-6 concentrations, when compared to exercise immersion in warm water. The authors concluded that exercise-induced increases in T_{core} mediate the neuroendocrine response, which subsequently contributes to increase circulating IL-6 [8]. Therefore, a secondary function of the present study was to determine whether any exercise-induced increase in IL-6 was associated with an appropriate neuroendocrine response.

Our experimental hypothesis was that when compared to a temperate climate, exercise in the heat would further elevate T_{core} , amplify the neuroendocrine response, as evidenced by concentrations of growth hormone and prolactin, and augment circulating IL-6.

Methods

Participants

Eight trained, non-heat-acclimated males volunteered their written informed consent to participate in the study. The study was performed according to the Declaration of Helsinki and was approved by the Local Ethics Committee. Their physical characteristics were (mean $\pm$ SD): age = 26 ± 8 years; body mass = 79 ± 10 kg; height = 180 ± 4 cm; maximal O_2 consumption ($\dot{V}O_{2max}$) = 60 ± 7 ml·kg⁻¹·min⁻¹; maximal aerobic power (W_{max}): 339 ± 34 W; maximal heart rate = 186 ± 9 beats·min⁻¹. All participants were familiar with laboratory cycle ergometry, with most having participated in at least one of our previous studies [14, 15, 16] and had completed a general health questionnaire to rule out any obvious contra-indications for exercise. All participants were asked to abstain from vigorous exercise, alcohol and caffeine for at least 24 h and arrived at the laboratory by 09:00 h after an overnight fast, having consumed 500 ml of water upon waking.

Experimental Procedure

Participants completed a preliminary visit to the laboratory in which $\dot{V}O_{2max}$ was determined using a standard progressive exercise test to exhaustion [see 17]. The participants then completed two counterbalanced sessions, each comprising a 60 min cycle trial at either 20°C (CON) or 35°C (HOT). Participants were advised to consume a diet high in carbohydrates in

the 24-h period prior to each visit. To minimise differences in muscle glycogen content between visits, subjects were asked to record their diet in the 24-h period before the first visit and instructed to follow the same diet before the second visit. Shortly after arriving at the laboratory, a cannula (20-G, Venflon) was inserted into an antecubital vein and kept patent with saline (Baxter, UK) during the test. The participant then rested seated in thermo-neutral conditions for 40 min before emptying his bladder. A thermistor (Ellab, UK) to measure oesophageal temperature was then inserted through the nasal passage to a depth of one-fourth the subject's standing height. A resting blood sample was taken after which a bolus of water (8 ml·kgbw⁻¹) was given to ensure adequate hydration during the exercise protocol. The participant was transferred to an environmental chamber and began to exercise at a constant work rate equal to $70 \pm 2\%$ $\dot{V}O_{2max}$ (220 ± 8 W) for 60 min at an ambient temperature of 20°C (CON) or 35°C (HOT); relative humidity was $33 \pm 2\%$. Subjects wore shorts and shoes and a fan set at ~ 0.5 m·s⁻¹ and in the same position for all trials, was used to circulate air in the chamber. Following the exercise, participants recovered sitting in a thermo-neutral environment and were allowed water *ad libitum* while waiting for the final blood sample to be taken (+30 min recovery).

Cardio-Respiratory Measures, Body Temperature and Perceived Exertion

During the exercise trials, standard Douglas bags (Cranlea, UK) were used to collect expired air for three min at the 20 and 40 min time points and were analysed with a gas analyser (Servomex, UK) and gas meter (Harvard, UK). Standard temperature pressure dry (STPD) values for expiratory minute ventilation ($\dot{V}_E$), rate of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were determined. Volume was calibrated using a 3 l calibration syringe, and the gas analysers were calibrated using a 5.03% CO_2 – 94.97% N_2 gas mixture. These values were then used to estimate the rates of fat and carbohydrate (CHO) oxidation [18]. Ambient temperature was measured during each exercise protocol and relative humidity calculated from the wet and dry bulb thermometer differential. The oesophageal thermistor was connected to a Squirrel data logger (Grant Instruments, UK) and values recorded every 10 min. Heart rate was recorded every 10 min by telemetry (Polar Accurex Plus, Polar Electro Oy, Finland) and ratings of perceived exertion were taken every 10 min during exercise using the 15-point Borg scale [19].

Blood Collection and Analyses

Venous blood samples were taken at rest, after 30 and 60 min of exercise and 30 min after cessation of

exercise (+30 min recovery). For each sample, the initial 2ml drawn was discarded and then blood was collected into a 4.5 ml, EDTA-containing tube (Becton Dickinson, UK). The tube was placed on melting ice until centrifuged (2300 g at 4°C for 10 min) and the plasma aliquotted and stored at -70°C until analysed. Prolactin and growth hormone concentrations were measured using a radioimmunoassay (Skybio Ltd, UK); average inter- and intra-assay coefficients of variations were 2.7% and 5.9%, respectively. Concentrations of IL-6 were determined using a high-sensitivity sandwich ELISA (DiaClone Research, France). Plates were read on a Labsystems Original Multiskan MS at 450 nm. The manufacturers report sensitivity of less than 0.8 pg·ml⁻¹, with intra-assay and inter-assay variation of 5.5% and 1.4%, respectively.

Data and Statistical Analyses

Data were tested for approximation to a normal distribution. Data were analysed using a two-way (time × condition) analysis of variance (ANOVA) with repeated measures for time. Values from ANOVA were assessed for sphericity and, if necessary, corrected using the Huynh-Feldt method. Following a significant main effect, pair-wise comparisons were made using Tukey's honestly significant difference (HSD) *post hoc* procedure. Where appropriate, Student's paired *t*-tests were performed. Eta-squared (η^2) is reported as a measure of effect size. Pearson product moment correlation coefficients were used to identify relationships between the main variables (IL-6 and T_{core} , prolactin and growth hormone). A 5% significance level was adopted throughout. Data are reported as means ± SE and analyses performed on $n = 8$, unless otherwise stated.

Results

Core Temperature and Perceived Exertion

All eight subjects successfully completed 60 min of cycling in both conditions and reported exercise in the CON condition relatively easy (end values 12.8 ± 0.6). In contrast, exercise in the HOT condition was more demanding and several of the subjects had difficulty completing the full 60 min (end values 14.8 ± 0.8). This is clearly depicted in Fig. 1, where during CON values for perceived exertion remained relatively stable and did not increase over time. In the HOT condition, however, values were increased after 30 min of exercise and were significantly higher than CON from 40 min onwards ($P = 0.003$, $\eta^2 = 0.742$).

There were no differences in T_{core} between conditions before the start of exercise ($P = 0.654$). A main effect of time was observed ($P = 0.013$, $\eta^2 = 0.996$), such that T_{core} had increased significantly by the 30-min time point in both conditions. A main effect of condition was found during exercise ($P = 0.02$, $\eta^2 = 0.560$), with

T_{core} rising higher in the HOT condition than in CON. Values at 60 min had reached 37.73 ± 0.06 and 38.27 ± 0.17 °C for CON and HOT, respectively.

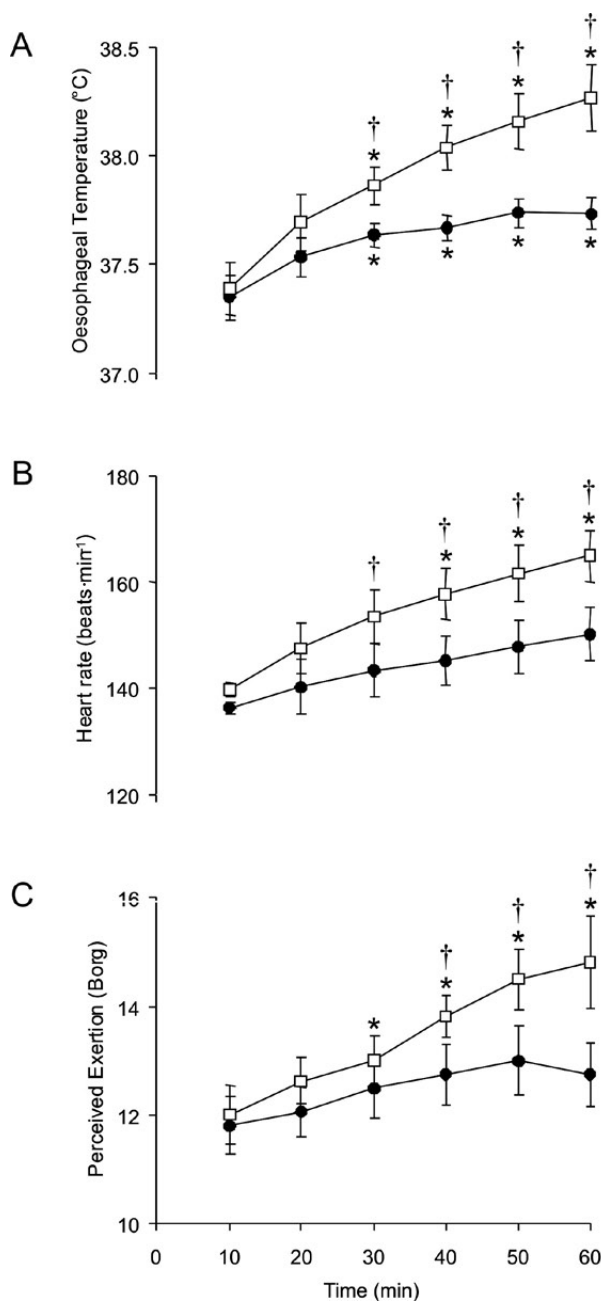


Figure 1. Mean (SE) oesophageal temperature (A), heart rate (B) and ratings of perceived exertion (C) during exercise in CON (●) and HOT (□) conditions.

* Significantly different from 10 minute value within a trial. † Significantly different to CON at that time point.

Cardio-Respiratory Measures and Fuel Oxidation

There were no differences in resting values of heart rate between conditions ($P = 0.516$). After an initial increase from rest, there were no significant increases in heart rate during CON. In contrast, heart rate had increased significantly by 40 min during HOT and values were significantly higher than CON from 30 min onwards ($P < 0.001$, $\eta^2 = 0.907$). A significant ef-

fect of time was found for ventilation ($P = 0.05$, $\eta^2 = 0.472$), such that ventilation increased during HOT but not during CON (Table 1). Rates of O_2 consumption and CO_2 production are presented in Table 1. There were no significant main effects of time, condition or time $\times$ condition interactions for either variable (all $P > 0.05$). No significant differences were found in estimated total CHO (HOT, 119 ± 22 g; CON 112 ± 20 g; $P = 0.196$) or fat oxidation (HOT, 57 ± 10 g; CON 60 ± 11 g; $P = 0.660$) between conditions.

Table 1. Ventilation ($\dot{V}_E$), rates of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) during exercise. Values are mean $\pm$ SE.

	Condition	Time (min)	
		20	40
$\dot{V}_E$ (l $\cdot$ min $^{-1}$)	CON	70 ± 5	73 ± 4
	HOT	70 ± 6	$79 \pm 4^*$
$\dot{V}O_2$ (l $\cdot$ min $^{-1}$)	CON	3.46 ± 0.25	3.65 ± 0.23
	HOT	3.51 ± 0.12	3.62 ± 0.11
$\dot{V}CO_2$ (l $\cdot$ min $^{-1}$)	CON	2.91 ± 0.21	3.02 ± 0.16
	HOT	2.95 ± 0.15	3.05 ± 0.11

* Significantly different to 20-min value.

Plasma IL-6, Growth Hormone and Prolactin

The responses of IL-6, growth hormone and prolactin to exercise are shown in Fig. 2, with resting concentrations of each similar in the two conditions (all $P > 0.05$). A main effect of time was observed for IL-6 ($P < 0.001$, $\eta^2 = 0.859$) such that concentrations had increased by 30 (HOT) and 60 min (CON), with concentrations significantly higher during HOT from 30 min onwards ($P = 0.024$, $\eta^2 = 0.539$). Peak values reached 2.2 ± 0.3 and 3.7 ± 0.6 pg $\cdot$ ml $^{-1}$ for CON and HOT, respectively.

A main effect of time was observed for plasma growth hormone ($P < 0.001$, $\eta^2 = 0.827$), such that concentrations had increased significantly by the 30-min time point in both conditions. A main effect of condition was found ($P = 0.025$, $\eta^2 = 0.667$), with levels rising higher in the HOT condition than in CON. Peak values reached 60 ± 11 and 95 ± 12 mU $\cdot$ l $^{-1}$ for CON and HOT, respectively.

A main effect of time was observed for plasma prolactin ($P = 0.004$, $\eta^2 = 0.566$), such that concentrations increased during HOT but not CON. A main effect of condition was found ($P = 0.008$, $\eta^2 = 0.659$), with concentrations significantly higher during HOT at 30 and 60 min than in CON. Peak values reached 316 ± 50 and 728 ± 145 mU $\cdot$ l $^{-1}$ for CON and HOT, respectively.

Correlations

The analyses on all common data points (0, 30, 60 min) revealed significant (all $P < 0.05$) correlations between IL-6 and core temperature of $R = 0.44$, IL-6 and prolactin of $R = 0.51$ and IL-6 and growth hormone of $R = 0.40$.

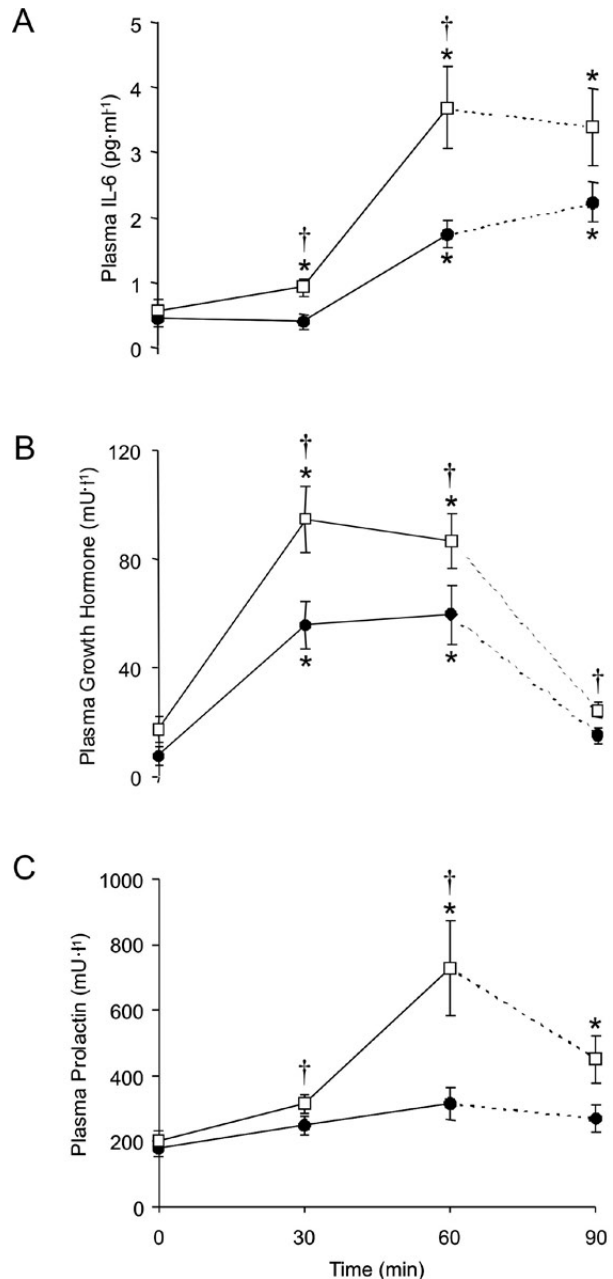


Figure 2. Mean (SE) plasma interleukin-6 (A), growth hormone (B) and prolactin (C) at baseline, during exercise and +30 minute recovery in CON (●) and HOT (□) conditions. * Significantly different from baseline value within a trial. † Significantly different to CON at that time point.

Discussion

Systemic IL-6 concentrations increase as a consequence of prolonged exercise [6], yet confusion exists over the plasma IL-6 response to prolonged exercise with additional heat stress, since this may or may not augment circulating concentrations [11, 9]. This discrepancy in the literature may be due to differences in protocols used (mode, intensity and duration of

exercise or heat load imposed). Therefore, in an attempt to clarify this we have used the regimes most commonly employed in exercise-stress investigations with or without an imposed heat load i.e. ~1h of cycle ergometry at ~70% $\dot{V}O_{2\max}$, carried out at 18 - 21°C or > 30°C [2, 20]. Under these conditions, our results confirm that the addition of heat stress to prolonged exercise augments circulating IL-6.

Our observation of approximately double the increase in circulating IL-6 during HOT compared to CON is similar to that of Starkie *et al.* [9], who cycled subjects for 90 minutes (70% $\dot{V}O_{2\text{peak}}$) at 15°C and 35°C. However, this is in contrast to the findings reported by Niess *et al.* [11] who used a 60-minute running protocol (90% anaerobic threshold) with ambient temperatures of 18°C and 28°C, where IL-6 increased similarly in both trials. Running would possibly elicit a greater local/muscle inflammatory response than cycling due to its weight-bearing nature, and this might explain the differences between studies. In support of this, it has been shown that eccentric compared to concentric exercise induces a more pronounced increase in IL-6 [21]. Moreover, the concentrations of IL-6 observed following exercise by Niess *et al.* [11] at 18°C are higher than those observed at the same time-point by ourselves and Starkie *et al.* [9] at 35°C.

Exercise duration appears to be an important factor influencing the IL-6 response, with a logarithmic relationship having previously been demonstrated [22]. It would be expected that a greater exercise duration than used in the present study would induce a greater IL-6 response in both environmental conditions, possibly by further depleting muscle glycogen stores. Starkie *et al.* [9] cycled subjects for 90 minutes at the same intensity and under similar environmental conditions as the current study. However, our results are very similar, in terms of concentrations of cytokine and the time course of appearance, to those reported by Starkie *et al.* [9]. Furthermore, whilst an exercise duration > 90 minutes might be expected when using trained subjects at this intensity (~70% $\dot{V}O_{2\max}$) in temperate conditions, it is often difficult to maintain constant-load exercise of this intensity in the heat for 90 minutes and is most likely limited by factors other than endogenous CHO stores, i.e. a high body temperature [2, 20].

The source or consequence of this additional IL-6 release due to heat stress has yet to be resolved. The likely consequences of this increased IL-6 are beyond the scope of the present study and discussion. Nevertheless, a regulated cytokine response – including IL-6 as both pro- and anti-inflammatory – is important for proper immune function and host defence, and exercise with additional heat stress has the potential to cause greater immune disturbances [e.g. 11]. Starkie and colleagues [9] examined plasma and intracel-

lular cytokine responses to 90 min of cycling at 70% $\dot{V}O_{2\text{peak}}$ with (35°C) and without (15°C) heat stress. Despite observing significantly higher plasma concentrations of IL-6 during exercise in the heat when compared to the control condition, no such difference was observed for monocyte cytokine production, suggesting that IL-6 was being produced by tissues and organs other than blood mononuclear cells [9]. It is unlikely that the liver contributes to the augmented IL-6 as it has been shown that it clears rather than produces IL-6 during exercise [23]. However, IL-6 is produced and released from skeletal muscle [24] and this is exacerbated with low intramuscular glycogen [25]. Febbraio and colleagues [26, 27, 28] have demonstrated that muscle glycogen use is augmented during exercise in the heat, and that this effect is most likely due to an increased muscle temperature. Therefore, Febbraio and colleagues [9] raised the possibility that higher IL-6 during exercise in the heat may be a signal to the brain that muscle glycogen is being depleted at a faster rate. However, there have been other reports indicating that glycogen use is unaffected by heat stress [29, 30] and in the present study, estimated total CHO oxidation was similar between conditions (HOT, 119 ± 22 g; CON 112 ± 20 g) suggesting that metabolism of muscle glycogen was not affected by the ambient temperature and, by inference, that the increased IL-6 concentrations in HOT were not due to greater glycogen depletion.

Indirect calorimetry only provides data about whole-body CHO oxidation and it is possible that increased muscle CHO oxidation in HOT could have been exactly balanced by a decreased consumption in some other tissue. However, since the consumption of CHO by muscle far exceeds that of other sources, it would be difficult to find another tissue that would balance the muscle utilisation. The present protocol could have included further sampling of gas in addition to the samples at 20 and 40 minutes during the exercise protocol; more frequent sampling may have allowed a more thorough conclusion to be drawn, however, as there were no differences in CHO oxidation by the 40-minute time-point (Table 1) yet concentrations of IL-6 were significantly higher during HOT by the 30-minute time-point (Fig. 2), it seems unlikely that fuel selection was an important part of the IL-6 response during the present study. However, although we believe this to be the first study to concurrently investigate IL-6 release and substrate use during exercise heat stress, without measures of muscle glycogen we cannot rule out a role of increased glycogen depletion contributing towards this greater IL-6.

Evidence exists to support neuroendocrine-immune interaction during exercise [1]. There are numerous factors known to modify the hormonal responses to exercise, but the combination of high

metabolic and environmental heat during prolonged exercise is a particularly potent stimulus for the release of several hormones, with studies having found a positive correlation between the rise in T_{core} and hormone release [4, 31]. To examine this further, Cross *et al.* [32] designed a protocol whereby during 40 minutes of cycling ($65\% \dot{V}O_{2max}$), T_{core} was either clamped ($\Delta T_{core} < 0.5^{\circ}C$) by immersion in cold ($23^{\circ}C$) water or allowed to rise substantially ($\Delta T_{core} \sim 2.0^{\circ}C$) by immersion in warm water ($38^{\circ}C$). The results demonstrate a significantly blunted neuroendocrine response during exercise with a thermal clamp. Results from the present study appear to support this, with concentrations of growth hormone and prolactin significantly higher during HOT at times when T_{core} had risen further than CON (Figs. 1 and 2). Using a similar thermal-clamp protocol as described above, Rhind *et al.* [8] demonstrated that exercise-hyperthermia augmented the circulating IL-6 and neuroendocrine response, whilst T_{core} clamping abolished these responses. This led the authors to suggest that exercise-associated elevations in T_{core} are important in triggering the hormone-cytokine axis [8]. The present data are in agreement with this suggestion, with significant associations between IL-6 and T_{core} , prolactin and growth hormone. Figure 2 clearly shows a similar time-course of increase between IL-6, growth hormone and prolactin and significant differences between trials when T_{core} was elevated during HOT.

Exercise in the heat also pushes the cardiovascular system further towards its maximum when compared to more temperate conditions. To maintain thermal homeostasis, a greater proportion of cardiac output is directed towards the skin to improve heat loss due to an elevated T_{core} – this can be seen in Fig. 1, where the cardiac drift during HOT follows the increase in T_{core} . This increases competition for cardiac output between the skin and active muscle, and is balanced by increased renal and splanchnic vasoconstriction [33]. A reduced splanchnic blood flow may produce hypoxia and oxidative and nitrosative stress to the intestinal wall, resulting in cellular necrosis, increased epithelial permeability and tight-junction opening [34]. Leakage of endotoxin can activate tissue macrophages and circulating monocytes to secrete inflammatory cytokines, such as IL-6 [34]. Although this has been demonstrated in animal models [35, 36], further substantiation is needed in humans before being recognized as a plausible explanation.

In conclusion, we have confirmed previous observations that exercise with additional heat stress augments the increase in circulating IL-6. It is unlikely that this is related to an increased rate of glycogen depletion as whole-body CHO oxidation was similar between conditions. Rather, the greater rise in T_{core} ($\sim 0.6^{\circ}C$) during exercise in the heat was accompanied

by higher concentrations of growth hormone and prolactin, supporting a role for temperature-mediated neuroendocrine regulation of exercise-induced IL-6. Whether this rise is pro- or anti-inflammatory, and the possible consequences of this increased IL-6 during exercise heat stress remain unresolved and to be investigated.

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Address for correspondence:

Toby Mündel, PhD

Institute of Food, Nutrition and Human Health

Massey University

Private Bag 11 222

Palmerston North

New Zealand

Tel +64 6 350 5799 ext 7763

Fax +64 6 350 5657

Email T.Mundel@massey.ac.nz

David A Jones: D.A.Jones@Bham.ac.uk