LIMITING FACTORS IN SELF-PACED EXERCISE TOLERANCE - THE ROLE OF NEUROINFLAMMATION AND CENTRAL SENSITISATION

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DECLARATION OF AUTHORSHIP

I hereby declare that this submission is my own work and to the best of my knowledge and belief, understand that it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

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Signed: ________________________________

Date: 9/11/2016 ________________________________

Nicole Vargas
B. Sci (Ex Sci), B. Ex. Sci. (Hons)
PhD Student
ACKNOWLEDGEMENTS

If someone told me five years ago that I would be living in Australia, about to complete a
PhD and learning about life, I would have never believed it. Alas, life throws us curve balls
when we least expect them. I’m sure most would agree that the time spent doing a PhD is
not confined to research and study. Indeed, I had no idea what was in store for me when I
jumped the big pond. I can say, however, that what has come of it highly exceeded every
one of my expectations. I have met new friends, laughed, cried, travelled, and even thought
about moving home at times. I encountered a very difficult time in learning to manage the
stress of PhD where I lost my hair, I tore my ACL, suffered anxiety and the onset of
depression. I lost myself and I found myself. I managed to push through, however, I am not
a one woman team, and therefore, have countless others to acknowledge in this short
section.

To Mom, Dad, Mike, Nanda and Marty, you guys have been there for me, sending me gifts
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encouraging me when I needed it the most. I couldn’t ask for a better support team even
though I only get to see you once or twice each year. Luckily, phone calls, skype and
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when it felt most unbearable. To my Grandma and Grandpa, leaving you behind to move
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of what I have accomplished and only wish you were still with us so I could give you a hug
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hope you know how much I appreciate everything you have done for me!

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many a late night studying.

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made me think twice and question more. You guys have all shaped me in some way and I
am so lucky have friends like you. To my American counterpart, Dawn – well, you know
everything you are to me. Thank you dearly for being one of those Americans with me!
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to find my way here at CSU, and thank you also for the bright smiles and friendly chats throughout the years.

Obviously, to all of my fellow PhD colleagues, well, we know the ins-and-outs of the last several years. We’ve cupped elbows, hugged, celebrated, panicked, and cried together. And for that I am thankful. I feel very lucky to have had such a great group coming through and the friendships and professional relationships I have developed with each and every one of you will carry on into the future. Thank you especially for your guidance throughout the PhD journey, and in life. I know our PhD bond will keep us together for years to come.

Finally, to my supervisor, Frank, I’m not sure where I would be without you. Your calm nature, curious mind, and understanding personality proved to be a great match as a supervisor. Our conversations outside the realm of PhD have helped shaped my world views, but your guidance and encouragement throughout the PhD process has truly helped shape me as a researcher and thinker. I can’t tell you how great it has been working with you for the last few years and I look forward to future collaborations.

And to Rob, who knew when I asked you 6 years ago, what research was all about, that I would end up in Australia too. I have always admired your passion, willingness to ruffle some feathers, and dedication to your work. Thank you for being someone open for a conversation and willing to answer questions.

An individual is a reflection of those who they share their life with and I have to admit that my reflection looks pretty darn good, thanks to all of you! I look forward to the next chapter post-PhD in my life and in each of yours as well. And with that, one quote comes to mind that sums up how important each of you are to me. How lucky am I?

‘You will never be completely at home again, because part of your heart will always be elsewhere. That is the price you pay for the richness of living and loving people in more than one place’

- Mick and Wout
**PUBLICATIONS**


**CONFERENCE PRESENTATIONS**


Vargas, N., & Marino, F. The effects of carbohydrate swill or ingestion on interleukin-6 and its soluble receptors during a 30km time trial performance. *ESSA Meeting*. April 2016. Oral Presentation; Young investigator award finalist.
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<td>5-hydroxy tryptamine</td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>ASIC</td>
<td>Acid sensing ion channels</td>
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<td>APSS</td>
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<td>Central Nervous System</td>
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<td>ES</td>
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<td>Deoxyhaemoglobin</td>
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**HS**<sub>12</sub>  Heat Stress, RPE 12
**HS**<sub>16</sub>  Heat Stress, RPE 16
**HR**  Heart Rate
**HPA-axis**  Hypothalamic pituitary adrenal-axis
**IBU**  Ibuprofen
**IL**  Interleukin
**kΩ**  Kiloohm
**La<sup>-</sup>**  Lactate
**LPS**  Lipopolysaccharide
**LT**  Lactate Threshold
**MC**  Motor Cortex
**NBW**  Near-Infrared Spectroscopy
**NSAID**  Non-steroidal anti-inflammatory
**NT**  Thermoneutral
**NT<sub>12</sub>**  Normothermic, RPE 12
**NT<sub>16</sub>**  Normothermic, RPE 16
**PaCO<sub>2</sub>**  Partial Pressure of Carbon Dioxide
**PC**  Parietal Cortex
**PFC**  Prefrontal Cortex
**PGE**  Prostaglandin
**PLAC**  Placebo
**PO**  Power output
**PGE**  Prostaglandin
**PSD**  Power spectral density
**PVH**  Paraventricular Hypothalamus
**PVN**  Paraventricular Nucleus
**rh**  Recombinant human
**Rh**  Relative Humidity
**RM**  Repeated Measures
**RPE**  Rating of Perceived Exertion
**sIL-6R**  Soluble IL-6 Receptor
**sgp130**  Soluble glycoprotein 130
**SBC**  Selective Brain Cooling
**SEM**  Standard error of the mean
**SS**  Snapshot
**X**
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<td>$\theta$</td>
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<td>$T_c$</td>
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<td></td>
</tr>
<tr>
<td>$T_{sk}$</td>
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<td>TNF</td>
<td>Tumour necrosis factor</td>
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<td>USG</td>
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Previously, IL-6 has been implicated in sickness behaviours during illness, disease, and in perceptions of fatigue in exercise. IL-6 is an immune biomarker first implicated in the inflammatory response and works in various ways and among numerous systems throughout the body. It is a known pro- and anti-inflammatory cytokine, a humoral regulator of blood glucose homeostasis during exercise, and has been reported to be a temperature sensing protein in the muscles and the core. Of most importance in the present thesis, it is widely implicated in neuroimmunology of acute and chronic disease and exercise, and effectively provides bi-directional brain communication between the periphery and central regions. Notably, nociceptive fibres are sensitised by IL-6 in the periphery, and it is possible that their signals stimulate subcortical regions in the thalamus, which can interact with higher cortical regions, thereby modulating cortical activity and, potentially, overall exercise tolerance, pacing and fatigue. Additionally, the role it plays as a temperature sensing protein and in blood glucose regulation during exercise may further modulate performance decrements and fatigue in heat stress or during high intensity exercise, as a mechanism of protection.

In chronic pain and fatigue models, cortical activity is altered due to chronic sensitisation of the central nervous system (CNS), ultimately leading to altered excitatory and/or inhibitory pathways, and lowering the threshold for pain and fatigue. While central sensitisation is primarily considered a chronic state, the initial acute effects of sensitising neuronal fibres are likely to cause changes in cortical activity as well. It is well established that peripheral responses inform the brain during exercise and therefore, changes in cortical activity at any given time could be likened to the cumulative effect of peripheral responses from afferent feedback and central responses to efferent drive. In a given exercise task, changes in cortical activity have been recorded using electroencephalography (EEG). These changes are likely due to continuous informational processing and integration of systemic responses throughout the task.
Considering the evidence supporting central regulation during exercise, few investigations have studied the overall state of the brain and cortical activity via changes in EEG, particularly in self-paced exercise protocols. Likewise, the notion of acute central sensitisation through afferent feedback, and the effect it may have on perceptions of effort, fatigue and subsequent behavioural modification during exercise has yet to be elucidated. Despite the multifaceted roles of IL-6 within the body and recent literature that highlights its implications in fatigue during exercise, little has also been determined of the complex interactions between IL-6, its signalling receptors, and other physiological systems during prolonged, self-paced exercise of different intensities and environmental loads, and their effects on perception of effort and regulation of power output or performance.
Abstract

Hence, the studies in the present thesis aimed to: (a) elucidate the interactions of heat stress and exercise intensity on cortical activity measured using EEG during self-paced, internally regulated exercise; (b) identify the interactions of heat stress and exercise intensity on the release of IL-6 and other systemic responses during the same self-paced, internally regulated exercise task; (c) modulate IL-6 with a non-steroidal anti-inflammatory drug (NSAID) during heat stress to understand its interaction with heat, cortical changes and other systemic responses; and (d) modulate the release of IL-6 using a carbohydrate mouth rinse solution in an attempt to see if blunting the response through a neural mechanism would alter performance or fatigue.

The first study evaluated changes in alpha (α) and beta (β) waves and the α/β ratio during exercise across prefrontal (PFC), frontal (FC), motor (MC) and parietal (PC) cortices using electroencephalography (EEG). The protocol employed was clamped at a rating of perceived exertion (RPE) of either ‘hard – very hard’ (RPE 16) or ‘light – somewhat light’ (RPE 12) from the Borg 6-20 RPE scale. Seven active males completed 4 separate 60 min cycling trials in thermoneutral (NORM) or heat stress (HOT) environments set at a rating of perceived exertion that was hard (HIGH), or moderate (LOW). PO revealed that HIGH was greater than LOW intensity in the respective HOT (p=0.002) and NORM conditions (p<0.001) while NORM HIGH was greater than HOT HIGH (p=0.01). Over the duration of the protocol, α waves in the PFC were greater for HOT HIGH than HOT LOW (p<0.001). FC (p<0.001), MC (p<0.001) and PC (p=0.01) also showed increases in α waves in HIGH compared to LOW intensity exercise. PFC, MC and PC all had an increase in β waves in HIGH compared to the respective LOW intensity protocol (p<0.01), while FC had a main effect for both HIGH compared to both LOW intensity protocols (p<0.001) and HOT was also greater than NORM (p=0.001). Across all conditions, α/β ratio decreased compared to baseline (p<0.05). PFC showed a greater change in
α/β ratio in high compared to low intensity (p=0.01). In FC (p=0.01) and MC (p=0.04), HOT HIGH, NORM HIGH and Hot LOW all had greater changes than NORM LOW, while in PC, HOT had greater changes than NORM (p=0.001). The data demonstrates a significant reduction in PO during high intensity in the heat, accompanied by an exacerbated α and β wave activity in high intensity exercise and heat stress. These findings do not support conventional decreased arousal owing to increased α/β ratio, but may be indicative of central sensitisation to intense exercise and heat stress from increased afferent sensory information and a concomitant increase in efferent drive in an effort to maintain the designated RPE.

The second study presented in the thesis is data that were collected simultaneously during the first study and evaluated the magnitude of change (Δ) of IL-6, its soluble receptors, soluble interleukin receptor (sIL-6R) and soluble glycoprotein 130 (sgp130), and other peripheral and central responses during the same cycling protocol as the first study in heated or normothermic environments at high or low intensities. Behaviour modifications owing to perceptual fatigue were quantified by Δ in PO between the first (0-30min) and second (30-60min) half of the protocol. Peripheral measures included cardiovascular (HR), metabolic (lactate (La⁻) and blood glucose (BG)), and skin temperature (Tsk). Central measures included total haemoglobin (HbTot) and the difference in haemoglobin concentration (HbDiff) at the PFC, and neuromuscular drive (EMG). IL-6 (p=0.002) and sIL-6R (p=0.002) increased in HIGH intensity during the second half of the protocol, while sp130 only revealed a main effect showing an increase across all conditions during the protocol (p=0.01). PO decreased significantly in HOT compared to NORM in the second half of the protocol (p=0.005). HR was greater for HIGH (p<0.001), while Tsk was only greater in HOT HIGHT compared to NORM HIGHT (p=0.02). La⁻ increased in the first half in high compared to low intensity (p<0.001), while BG increased in HOT compared to NORM (p=0.02). HbTot (p=0.004) was attenuated in the first and EMG in the second half of the protocol in HOT HIGH (p=0.02). The data suggests down regulated HbTot and EMG activity in HOT conditions, in conjunction with exacerbated IL-6 and La⁻ signalling.
Abstract

in HIGH intensity exercise, which may contribute to perception of effort and regulation of PO during an internally regulated exercise task.

In the third study, the IL-6 response to exertional HS was manipulated using a non-selective cyclooxygenase (COX) inhibitor, Ibuprofen (IBU). IBU has been shown to alter perceptual and thermal loads during exercise and exacerbate the response of IL-6 in an attempt to contest the release of lipopolysaccharides (LPS) from increased intestinal wall permeability in exertional HS. This study aimed to evaluate whether the ingestion of 800mg IBU or a placebo (PLAC) prior to exertional HS exacerbates the response of IL-6 and its soluble receptors, and if they are associated with changes in PO. A further aim was to identify Δ in peripheral measures of HR, $T_{sk}$ and core temperature ($T_c$) and central measures of $Hb_{Tot}$, $Hb_{Diff}$, EMG and cortical activity via measures of $\alpha$, $\beta$ and the $\alpha/\beta$ ratio in the PFC, FC, MC and PC regions. Eight active males performed two separate 60 min clamped RPE 16 cycling tasks in HS. ΔIL-6 was greater in the second half for both conditions ($p=0.004$), but ES suggest an exacerbated response in IBU in the second half ($d=1.5$). There were no changes in ΔsIL-6R or Δsgp130 over the duration of the protocol ($p>0.05$). ΔPO was not different between IBU or PLAC, and did not decrease significantly over 60 min ($p>0.05$). $T_c$ was not different, although a moderate ES indicated a slightly attenuated $T_c$ in the IBU ($d=0.5$). Δ$Hb_{Tot}$ decreased in the second half in both conditions ($p<0.001$), while there were no significant changes in Δ$Hb_{Diff}$, although ES revealed a large increase in the first half ($d=2.4$) and a large decrease in the second half ($d=0.9$). There were only increases in Δ$\alpha/\beta$ ratio in both conditions in the PFC ($p=0.02$), FC ($p=0.01$) and MC ($p=0.01$), where a large ES revealed a greater increase for PLAC in MC compared to IBU ($d=0.8$). The data suggests that IL-6 may signal central areas, but the opposing effect of an attenuated $T_c$ may counteract this and hence reduce cortical activity overall. In contrast, PLAC may have greater cortical activity due to increased afferent signalling. As none of the responses resulted in changes in performance, there are likely other mechanisms that influenced regulation of power output, hence suggesting integration of numerous physiological systems in the self-paced exercise task.
Finally, it is known that during exercise, ingesting carbohydrates (CHO) can attenuate the IL-6 response during exercise. It has also been shown that rinsing CHO in the oral cavity can have performance benefits, although mechanisms remain focused on central areas. The purpose of this study was to determine whether or not rinsing or ingesting CHO improves performance during a 30km cycling time trial (TT) and if it is related to the attenuated release of IL-6 and its soluble receptors. A further aim was to determine Δ in central measures of Hb$_{Tot}$ and Hb$_{Diff}$ in the PFC and in cortical activity via α and β waves and the α/β ratio in the PFC, FC, MC and PC regions. Eight healthy participants completed three separate 30km TT in a randomised crossover design. Participants either ingested water (WATER) or a CHO (CARB) solution, or rinsed the same CHO solution (RINSE). ΔIL-6 increased significantly in WATER compared to CARB from 10-20 and 20-30km (p=0.01). ΔsIL-6R increased in WATER compared to CARB from 10-20km (p=0.01) and Δsgp130 increased in RINSE compared to WATER from 0-10km (p=0.04), but WATER increased greater than both CARB trials in 10-20km (p=0.04). There were no differences in TT completion (p>0.05). There was a main effect for time for ΔPO (p<0.001), showing a decrease after the first 10km in all trials. Hb$_{Tot}$ and Hb$_{Diff}$ only showed an increase in the first 10km for all trials (p=0.01) and decreased thereafter. Only α activity in the PFC was greater in CARB compared to RINSE between 0-10km (p=0.05), but was less than rinse at 10-20km (p=0.04). The data suggests that ingesting CHO attenuates the IL-6 response, with a similar trend when rinsing, which also appears to reduce the receptor response. However, ingesting or rinsing CHO does not increase performance in a 30km TT.

Overall, results from the studies revealed a likely effect of high intensity exercise and heat stress on central areas which may become sensitised and modulate performance. However, there was no definitive evidence for a direct association between IL-6 and regulation of PO or performance, despite an increase in IL-6 in the high intensity clamped RPE trials, exacerbated by heat stress, and in the water ingestion TT. In contrast, they do provide an overall representation of dynamic physiological responses and behavioural modifications that occur in order to perform a self-paced perceptual or performance based exercise task. Future research should aim
to distinguish how the culmination of physiological responses is accurately reflected in cortical activity and changes in brain waves. Furthermore, the changes in cortical activity should be elucidated further and the relationship with the development of fatigue should be studied as an easy diagnostic tool for acute exercise and/or chronic fatigue, potentially as a consequence of altered central sensitisation.
1. INTRODUCTION
OVERVIEW

Like most things in life, our capacity to exercise exists on a continuum, constantly modified at any given moment by internal and external factors that contribute to the sensory information we receive and process. Processing of the stimuli occurs within the brain and remains elusive except in that it provides us with an intangible experience—a feeling, giving rise to a perception that subsequently modulates our behaviour. In psychological and social-behavioural fields, this is termed the ‘dynamic behaviour model’, where behaviour at any given moment is characterised by perception and action, possessing a ping-pong like relationship to one another (Warren, 2006). When applied to an exercise paradigm, it is termed the complex integrated systems theory (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005). Both paradigms amalgamate with one evolutionary goal in mind: protection of the individual systems and overall organism from danger.

In the context of this thesis, control of perception begins with the most miniscule of stimuli from a) environmental factors, b) stress to the physiological systems whereby homeostasis is shifted, and/or c) previous experience and knowledge of a given task. Glass (2001) describes this as the synchronisation of rhythmic processes in physiology. When under stress, one or more of these systems can be shifted in a non-linear dynamic, providing sensory feedback and modulating perceptions and actions performed by the individual (Glass, L., 2001; St Gibson, A. et al., 2006). Consequently, our behaviour is modified based on the desire to maintain or change the level of perception at any point in time.

Exertional stress follows the same dynamic behaviour pattern where changes in the cardiovascular, metabolic, inflammatory, neuroendocrine and CNS are dependent on fitness level, intensity, duration and environment, and culminate in a perceptual level of effort at any given moment. This perception of effort is commonly identified as a rating of perceived exertion (RPE) where the intensity of exercise can be between ‘very, very light’ (RPE 6) to ‘very, very hard’ (RPE 20) (Borg, G.A., 1982). Dynamic behaviour of the aforementioned physiological systems has been studied.
individually, providing conventional science with knowledge about overall regulation and individual roles of numerous biomarkers within each system. These biomarkers and chemical messengers enable integration within and between physiological systems during rest and periods of homeostatic imbalance.

INTEGRATION OF PHYSIOLOGICAL SYSTEMS DURING EXERCISE

In a healthy state at rest, our bodies remain under homeostatic balance where physiological systems are tightly regulated by omnipresent mechano-, chemo- and thermo- receptors that sense changes and lead to necessary modifications (Glass, L., 2001). Controlled by the autonomic nervous system, modifications occur without our awareness, except in the event that they require a change in behaviour. In this event, sensory signals are received that provide a perceptual feeling or sensation in order to prompt the necessary action (Warren, W.H., 2006). For example, the sensation of hunger prompts us to seek food. Fatigue prompts us to rest. Underpinning these perceptions are numerous physiological changes that rely on feedback loops such as those signalling low blood glucose levels. In turn, we consume food that is high in energy to replenish blood glucose. Importantly, the regulation and integration of these systems is not limited to a single pathway or biomarker.

As we begin exercise, a host of physiological changes occur within central and peripheral areas, none which exceeds another in importance. Exercise initiation begins with a thought, followed by a decision that propagates a neuronal signal from the motor cortex to perform the desired muscular action (Gandevia, S.C., Allen, G.M., Butler, J.E., & Taylor, J.L., 1996; Kayser, B., 2003). For example, in beginning to run, the first step alone is a complicated task involving integration of both central and peripheral regions. As we continue to run, integration becomes increasingly complex. To begin, there is an initial increase in efferent sympathetic drive based on a self-selected intensity and the demand to meet oxygen (O_2) requirements
and carbon dioxide (CO$_2$) release for energy production. Changes in partial pressure concentrations of O$_2$ and CO$_2$ are sensed within the baroreceptor system and signal the medulla oblongata (Robinson, B.F., Epstein, S.E., Beiser, G.D., & Braunwald, E., 1966). The hypothalamus receives these signals, along with changes in peripheral resistance and blood pressure control, and adjusts the HR accordingly (Perini, R. & Veicsteinas, A., 2003; Robinson, B.F., Epstein, S.E., Beiser, G.D., & Braunwald, E., 1966).

Delivery of O$_2$ to the muscles is used for mitochondrial respiration, in conjunction with the breakdown of muscular glycogen, or up-take of blood glucose into the cell to generate adenosine tri-phosphate (ATP). Biochemical breakdown during glycolysis results in metabolic by products in the form of lactate (La$^-$) and other molecules (Robergs, R.A., Ghiasvand, F., & Parker, D., 2004). La$^-$ is initially shuttled through and used to make additional ATP, however, in the event that the accumulation of La$^-$ increases to levels beyond that which can be used, it appears in the circulating blood, and can signal nociceptive nerve fibres, travelling within the CNS (Light, A.R. et al., 2008).

Additionally, the augmented sympathetic drive and initial muscular contraction simultaneously increases muscular haemodynamics (Rådegran, G. & Saltin, B., 1998) while decreasing cerebral perfusion (Ogoh, S. & Ainslie, P.N., 2009; Toronov, V. et al., 2000). Blood flow within the peripheral vasculature is primarily regulated through arterial pressure (Ogoh, S. & Ainslie, P.N., 2009). During steady state exercise, Hb$_{Tot}$ remains stable while the level of intensity designates the corresponding oxyhaemoglobin (HbO$_2$) and deoxyhaemoglobin (HHb) concentrations. When the intensity reaches a level near or above the lactate threshold (LT), and is maintained, arterial CO$_2$ (PaCO$_2$) concentration increases, which reflects decreased oxygenation or HbO$_2$, and increased deoxygenation or HHb (Ekkekakis, P., 2001). Although cerebral haemodynamics bears a linear relationship to cardiac output, they are not as sensitive to sympathetic drive as is the peripheral vasculature (Ogoh, S. & Ainslie, P.N., 2009). Cerebral regions often react through vasoconstriction and cerebral vascular resistance. Hence, an overall increase in cardiac output results in increased
regional cerebral blood flow (CBF) during steady state exercise, only to a lesser extent than skeletal muscle blood flow (Ekkekakis, P., 2001).

As the internal rate of heat storage increases due to biochemical breakdown, increased peripheral haemodynamics facilitates capillary perfusion as a thermoregulatory mechanism for heat dissipation (Schlader, Z.J., Prange, H.D., Mickleborough, T.D., & Stager, J.M., 2009). In the event that exercise intensity is greater than the release, or the environment does not favour release of heat, there is an increase in core temperature ($T_c$) within the body (Marino, F.E., 2004; Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011). Increased $T_c$ is largely implicated in performance decrements in fixed intensity exercise protocols (González-Alonso, J. et al., 1999; Nielsen, B., Hyldig, T., Bidstrup, F., González-Alonso, J., & Christoffersen, G.R.J., 2001; Parkin, J., Carey, M., Zhao, S., & Febbraio, M., 1999), however self-paced exercise allows individuals to manipulate the intensity in order to complete the task, thus minimising changes in $T_c$ (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011; Tucker, R. et al., 2006). Still, it is believed that temperature signals processed by the brain modulate power output in favour of preventing cellular or whole damage to the organism (Marino, F.E., 2004; Schlader, Z.J., Prange, H.D., Mickleborough, T.D., & Stager, J.M., 2009; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006).

Continued increases in $T_c$, combined with diffusion of peripheral blood flow away from the visceral organs, compromise the permeability of the intestinal wall and releases endotoxins into the circulation, especially during heat stress (Lambert, P., 2008; Starkie, R.L., Hargreaves, M., Rolland, J., & Febbraio, M.A., 2005; Zuhl, M. et al., 2014). The release of endotoxins stimulates an inflammatory response, which is initially mediated through the acute-phase response involving a host of anti-inflammatory cytokines such as IL-6 (Rhind, S.G. et al., 2004; Starkie, R.L., Hargreaves, M., Rolland, J., & Febbraio, M.A., 2005). In conjunction with the inflammatory response, IL-6 is implicated in blood glucose regulation during exercise (Febbraio, M.A., Hiscock, N., Sacchetti, M., Fischer, C.P., & Pedersen, B.K., 2004; Steensberg, A., van Hall, G., et al., 2001) and in thermoregulatory processes
both in the gut (Jiang, Q. et al., 1999; Kozak, W. et al., 1998; Leon, L.R., 2002) and muscle (Welc, S.S. et al., 2012).

Fatigue in a self-paced exercise task may arise from afferent signals due to internal peripheral changes, combined with external environmental, visual and motivational factors, which equate to a subjective perception of effort and subsequent decline in power output (PO). The signals find their way through the CNS, to the thalamus, and propagate to higher levels within the sub cortical and cortical areas of the brain (St Gibson, A. et al., 2006). Cortical activity within the brain is measured in frequency waves and have been associated with information flow (Mima, T., Matsuoka, T., & Hallett, M., 2001) and overall brain state (Nielsen, B. et al., 2001), where the beta (β) and gamma (γ) frequencies represent efferent drive, alpha (α) and theta (θ) frequencies represent afferent input (Mima, T., Matsuoka, T., & Hallett, M., 2001), and an increase in α and concomitant decrease in β reflects a shift in overall volitional fatigue and increased RPE (Nielsen, B. et al., 2001). The effect of sensory information on nerve fibres within the CNS can alter cortical activity through changing excitatory and inhibitory pathways, in an event termed central sensitisation.

The integration of the physiological systems during exercise can be further complicated when taking into account environmental, nutritional, fitness level and motivational factors. Nonetheless, it is clear that exercise capacity, tolerance and effort are truly a cumulative product of regulation within and between all physiological systems. Furthermore, the systems themselves react in an anticipatory or reactionary manner dependent upon previous experience, current mental and/or physiological state, and knowledge of the desired task at hand. While all systems are imperative in exercise performance, specific molecules may enhance our understanding of why exercise tolerance is reduced at times. IL-6 is a signalling molecule that is implicated in the aforementioned glucose, inflammatory and temperature regulation, and potentially in exercise tolerance and fatigue due to its ability to communicate between the central and peripheral systems in numerous ways and sensitise the CNS. For these reasons, it is postulated
that IL-6 may contribute to the complex integrated systems theory of fatigue.

**INTERLEUKIN-6 AS A CONTRIBUTING FACTOR TO CENTRAL SENSITISATION AND FATIGUE**

IL-6 is a prominent molecule released in immune, heat, exercise and inflammatory stress alike. Due to the copious release of local and systemic IL-6, it is also implicated in both transient and chronic fatigue during illness (Maier, S.F. & Watkins, L.R., 1998; Watkins, L.R., Maier, S.F., & Goehler, L.E., 1995). High levels of circulating plasma IL-6 have been correlated with sickness behaviours such as malaise and loss of appetite and depression (Alesci, S. et al., 2005; Harden, L.M., Plessis, I.d., Poole, S., & Laburn, H.P., 2008). The psychoneuroimmunology theory places IL-6 at the base of these behaviour modifications as it has been shown to stimulate nerve cells within the periphery, especially the vagus (Goehler, L.E. et al., 2000) and other sensory (Brenn, D., Richter, F., & Schaible, H.-G., 2007; Hoheisel, U., Unger, T., & Mense, S., 2005) nerve fibres. Likewise, IL-6 can propagate signals in the brain through receptors at the circumventricular organs and can cross the blood brain barrier (Watkins, L.R., Maier, S.F., & Goehler, L.E., 1995).

There is growing evidence that IL-6, along with other inflammatory cytokines, can stimulate neurons in the dorsal root ganglion and ultimately affect the CNS (Coderre, T.J. & Melzack, R., 1991; Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008; Yunus, M.B., 2007). Indeed, it has been shown that peripheral sensory nerves possess the gp130 receptor which enables IL-6 signalling (Andratsch, M. et al., 2009). The signalling of nociceptive fibres can alter neuronal excitation and inhibition, which can further modify the threshold for pain tolerance (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008) and fatigue (Nijs, J. et al., 2012) through central sensitisation (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008; Nijs, J. et al., 2012; Yunus, M.B., 2007). Central sensitisation is actually a chronic adaptation and is well known in chronic pain and fatigue.
paradigms, but less so in exercise, although inhibitory pathways have been implicated in down regulation of efferent drive through central fatigue (Taylor, J.L. & Gandevia, S., 2011).

Central sensitisation to afferent signalling is believed to have a cumulative effect on perceptions of pain and fatigue (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008), which may therefore contribute to the perception of fatigue and down regulation of the CNS during exercise. Consequently, considering the aforementioned evidence in terms of the increase in circulating IL-6 and the potential for IL-6 to signal through nociceptive fibres throughout the periphery, it may be an important regulatory, or at least, contributor to transient feelings of fatigue during prolonged exercise.

**SUMMARY**

The complex integrated theory of fatigue posits that all physiological systems work together and culminate in a perceptual sensation that subsequently modifies exercise behaviour at any given time (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005). Additionally, there is a motivational factor that plays a role in perception of effort which cannot be discounted (Smirmaul, B.P.C., Dantas, J.L., Nakamura, F.Y., & Pereira, G., 2013). It can be presumed that changes in cortical activity reflect cumulative information processing from all sensory stimuli - the contribution of peripheral metabolic and inflammatory by products and environmental, motivational and anticipatory factors alike. Furthermore, central sensitisation caused by afferent signalling is likely to be reflected in measures of cortical activity. IL-6, specifically, has been implicated in both transient and chronic fatigue in illness and disease (Maier, S.F. & Watkins, L.R., 1998; Watkins, L.R., Maier, S.F., & Goehler, L.E., 1995), and is released during exercise in an intensity, time and environmentally dependent manner. Likewise, IL-6 is implicated in the regulation of numerous physiological processes and as a signalling molecule from the periphery to the CNS. While IL-6 is only a single signalling molecule itself, it is likely to be a potent contributor to overall perception, exercise tolerance and capacity regulated through the complex integrated theory of fatigue. Hence,
determining the association between IL-6, exercise tolerance, perception of effort and regulation of power output during endurance exercise in different environments and intensities may help further elucidate the contributions of IL-6 towards acute exercise fatigue.

**STATEMENT OF THE PROBLEM**

The complexities of the human body cannot be dispelled, especially in relation to exercise and integration of external and internal factors that help to regulate intensity during self-paced exercise at any given time. While IL-6 has been implicated in hepatic glycogen breakdown, blood glucose regulation, inflammatory processes and temperature signalling for many years, only recently has it been identified as a potential marker for fatigue (Robson-Ansley, P. et al., 2009; Robson-Ansley, P.J., Milander, L.d., Collins, M., & Noakes, T.D., 2004). Hence, the association between IL-6 and performance is an important aspect of exercise tolerance and regulation that warrants further investigation. Furthermore, central sensitisation is a model used to explain chronic pain and fatigue, but may be analogous to acute exercise fatigue and can potentially be measured through altered cortical activity. Cortical activity and associated IL-6 release has yet to be evaluated during self-paced exercise of different perceptually based intensities and environments, or during a self-paced time trial (TT) protocol. Likewise, there is no previous literature that provides an overall indication of the magnitude of change of specific peripheral and central physiological systems during self-paced exercise, and their association with regulation of power output and perception of effort and performance.

**RESEARCH AIMS AND HYPOTHESES**

The aims of this thesis, therefore, were to evaluate the changes in cortical activity and magnitude of change in peripheral and central physiological systems during self-paced exercise that was either regulated by perception of effort or based on performance during a TT protocol. To do so, we initially measured cortical activity during a prolonged cycling protocol that clamped the RPE at different intensities under heat stress and in
thermoneutral conditions. We evaluated PO during the protocol to identify whether there is evidence of central sensitisation and decreased PO. Next, we evaluated the magnitude of change in IL-6, its soluble receptors, PO, and other peripheral and central measures between the first and second half of each protocol as an indicator of behaviour modification employed in order to maintain the designated RPE in the presence of proposed afferent stimuli. Consequently, we then aimed to determine whether manipulating the IL-6 response using a nonsteroidal anti-inflammatory drug (NSAID) during a high intensity clamped RPE protocol in heat stress would effect the magnitude change in cortical activity, IL-6, its soluble receptors, other peripheral and central measures between the first and second half of the protocol, and subsequent regulation of power output. Finally, we aimed to determine whether manipulating the IL-6 response during a 30 km cycling TT through either ingesting or rinsing a carbohydrate solution would have performance benefits, while also evaluating the magnitude of change in cortical activity and within peripheral and central systems between every 10km completed of the TT.

**STUDY 1 – CORTICAL ACTIVITY DURING SELF-PACED EXERCISE IN THERMONEUTRAL AND HEATED ENVIRONMENTS – EVIDENCE FOR ACUTE CENTRAL SENSITISATION**

Research Aims:

This study aimed to evaluate changes in:

1. α waves,
2. β waves and
3. The α/β ratio as an indicator of overall cortical activity and central sensitisation

Within the prefrontal (PFC), frontal (FC), motor (MC) and parietal (PC) cortices and the associated PO during a clamped RPE protocol of low or high intensity exercise in thermoneutral or heated environments in:

Hypotheses:

It was hypothesised that
1. There would be an increase in α wave and a concomitant decrease in β wave activity as PO decreased and,
2. α / β ratio would increase over the duration of the exercise protocol, especially as power output decreased and that,
3. Overall activity in EEG bandwidths would be intensity and environmentally dependent, with the greatest changes seen in the heated high intensity condition, with comparable changes between thermoneutral high intensity and heated low intensity, and fewest changes in the thermoneutral low intensity condition.

**STUDY 2 – EFFECTS OF IL-6 AND OTHER PERIPHERAL AND CENTRAL CHANGES ON REGULATION OF POWER OUTPUT DURING INTERNALLY REGULATED, SELF-PACED EXERCISE**

Research Aims:
This study aimed to evaluate the magnitude of change in:
1. The release of IL-6 and its soluble receptors, sIL-6R and sgp130;
2. The regulation of power output as an indicator of behavioural modification in an effort to maintain the desired perception of effort;
3. Peripheral cardiovascular (HR), metabolic (La⁻ and BG) and thermoregulatory (Tsk) changes; and
4. Central changes in HbTot, HbDiff, and EMG
Between the first and second half of a clamped RPE protocol of low or high intensities during self-paced exercise in thermoneutral or heat stress environments on:

Hypotheses:
It was hypothesised that
1. The greatest changes in IL-6 and its receptors would occur in the heated high intensity condition and in the second half of the protocol;
2. The greatest change in PO would occur in the heated high intensity condition and in the second half of the protocol;
3. The high intensity protocols would result in greater physiological changes in the peripheral and central systems compared to the low intensity protocols;
4. And the heat stress conditions would exacerbate responses relative to the respective thermoneutral condition.

**STUDY 3 – THE EFFECTS OF IBUPROFEN INGESTION ON THE IL-6 RESPONSE AND REGULATION OF POWER OUTPUT DURING INTERNALLY REGULATED SELF-PACED EXERCISE IN THE HEAT**

Research Aims:
This study aimed to examine the effect of Ibuprofen during a clamped RPE protocol of high intensity in heat stress on:
1. The release of IL-6 and its soluble receptors, sIL-6R and sgp130;
2. Cortical activity and central sensitisation via α waves, β waves, and the α/β ratio;
3. The regulation of power output as an indicator of behavioural modification in an effort to maintain the desired perception of effort;
4. Peripheral changes in HR, Tc and Ts;
5. Central changes in HbTot, HbDiff, and EMG

Hypotheses:
It was hypothesised that Ibuprofen ingestion prior to exercise in heat stress would
1. Exacerbate the release of IL-6 and sIL-6R;
2. Increase α wave activity and increase the α/β ratio;
3. Cause a reduction in PO;
4. Attenuate the HR and Tc response; and
5. Increase EMG activity while maintaining HbTot and HbDiff.

**STUDY 4 – THE EFFECTS OF CHO INGESTION AND RINSE ON THE IL-6 RESPONSE AND PERFORMANCE DURING A 30KM CYCLING TIME TRIAL**

Research Aims:
The final study aimed to identify potential mechanisms that might underpin previous literature which reports a performance benefit from simply rinsing
CHO within the oral cavity. More specifically, it aimed to determine the magnitude of change of:

1. The release of IL-6 and its soluble receptors, sIL-6R and sgp130;
2. Cortical activity and central sensitisation via α waves, β waves, and the α/β ratio;
3. Overall TT performance;
4. Central changes in Hb$_{Tot}$, Hb$_{Diff}$, and EMG

Within each 10km distance completed during a self-paced 30km TT performance while ingesting or rinsing a CHO solution.

Hypotheses:

It was hypothesised that

1. IL-6 and sIL-6R would be attenuated when CHO was ingested and rinsed.
2. Performance would be better in the CHO ingestion and rinsing conditions;
3. α wave activity would be reduced in the CHO trials compared to the water trial and β activity would increase in CHO. α/β ratio would be increased in the water ingestion trial.
4. There would be increased cerebral haemodynamics during both carbohydrate conditions compared to the water condition.
2. REVIEW OF LITERATURE
Part of the research presented within this chapter has been published within the following articles:

- Vargas NT & Marino F.  Heat stress, gastrointestinal permeability and Interleukin-6 – Implications for exercise. *Temperature* (Accepted for publication March, 2016).

**INTRODUCTION**

Advances in neuroimaging have facilitated an immense mapping of the brain’s sensory pathways, promoting numerous models of sensory integration, especially in response to pain and temperature (Coghill, R. et al., 1994; Johnson, A.K. & Gross, P.M., 1993; Liang, M., Mouraux, A., & Iannetti, G.D., 2011; Ploner, M., Schmitz, F., Freund, H.-J., & Schnitzler, A., 1999). Fatigue, however, remains largely an anomaly. There is certainly no scarcity of literature in several disciplines such as psychology, pathophysiology, immunology and exercise physiology (Maier, S.F. & Watkins, L.R., 1998; Olofsson, P.S., Rosas-Ballina, M., Levine, Y.A., & Tracey, K.J., 2012; St Gibson, A. et al., 2006) which examines the phenomenon and mechanisms of fatigue. Drawing on evidence from these disciplines can help improve current models and broaden our knowledge and understanding of physiological processes that influence fatigue in exercise research. While a definition of fatigue is largely dependent on the circumstance, for the purpose of this thesis, fatigue is defined in two different ways. The first definition of fatigue is either a chronic or transient, overall sensation of tiredness or exhaustion in times of sickness. The second definition is a transient sensation of tiredness or exhaustion that is felt during acute bouts of exercise and can be quantified through changes in the regulation of power output and performance. Although the study of fatigue is encompassed in numerous theories, the complex integrated systems theory and the neuroinflammatory model of acute fatigue during exercise underpin the present thesis.
The purpose of this review of literature is to firstly, discuss the history of the study of fatigue and mechanisms of acute fatigue during exercise, highlighting numerous theories that have been developed through the years. However, beyond these definitions and the historical backdrop, it is essential that we move to a more complete understanding of potential mechanisms of fatigue distinct from the most popular theories. Therefore, the second aspect of this literature review highlights the differences between peripheral IL-6 release during disease and exercise in thermoneutral and heat stress conditions and the biochemistry of cytokine receptor signalling, using evidence that receptors located ubiquitously on tissues within the body can send signals back to the brain, hence highlighting the neuroinflammatory model of acute exercise fatigue. The implications of manipulating the IL-6 response on fatigue, power output and performance will further be discussed, followed by responses of other physiological systems which integrate together to inform the brain of what is happening in the periphery at any given moment. Finally, the culmination and processing of all peripheral sensory signals in the brain and the overall cortical activity and central sensitisation, which may indicate perceptions of fatigue and subsequent behaviour modification, will be considered (St Gibson, A. et al., 2006).

**History of the Study of Fatigue**

Centuries ago, Giovanni Borelli and Niels Stensen identified that direct nerve stimulation could induce a muscular contraction and that overall neural function may contribute to muscular fatigue (Giulio, C.D., Daniele, F., & Tipton, C.M., 2006). The fatigue curve was characterised shortly after by Angelo Mosso where both voluntary and involuntary muscle contractions of the index finger were recorded using an ergograph (Giulio, C.D., Daniele, F., & Tipton, C.M., 2006). For this, Mosso was credited with the knowledge that peripheral fatigue is distinguishable from that originating in central regions (Giulio, C.D., Daniele, F., & Tipton, C.M., 2006; Marino, F.E., Gard, M., & Drinkwater, E.J., 2011). Like all good science, the study of fatigue has followed a Kuhn and Popper dynamic in which scientists drill down to find the specific contributing pathways or physiological
mechanisms contributing to fatigue, followed by new overriding theory for further investigation (Lakatos, I., 1968). Although Mosso pioneered the idea of the brain as the overriding factor that drives exercise, studying fatigue has come full circle since then. During the 1900s, the chief focus of studying fatigue was on changes in the peripheral system due to advancing methods for testing biochemical contributions (Robergs, R.A., Ghiasvand, F., & Parker, D., 2004). Towards the end of the 20th and into the 21st century, however, we have witnessed the focus shift again to central regions (Blomstrand, E., HassmÉN, P., Ek, S., Ekbom, B., & Newsholme, E.A., 1997; Hassmén, P., Blomstrand, E., Ekbom, B., & Newsholme, E.A., 1994; Negro, M., Giardina, S., Marzani, B., & Marzatico, F., 2008) and eventually to the integration of the whole body (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005; Marino, F.E., 2004; Noakes, T.D., Peltonen, J.E., & Rusko, H.K., 2001; Noakes, T.D., St Clair Gibson, A., & Lambert, E.V., 2004; St Gibson, A. et al., 2006).

MODELS AND THEORIES OF ACUTE EXERCISE FATIGUE

Classical models of fatigue focused on cardiovascular, neurotransmission and energy supply and demand, suggesting that fatigue is caused by decreased blood flow from exercise stress on the heart (Pandolf, K.B., Cafarelli, E., Noble, B.J., & Metz, K.F., 1972), failure of neurotransmission and muscle excitation at the neuromuscular junction (Bigland-Ritchie, B. & Woods, J.J., 1984), and imbalances between ATP supply and demand, especially due to glycogen depletion (Bergström, J., Hermansen, L., Hultman, E., & Saltin, B., 1967; Hill, A.V. & Lupton, H., 1923). Consequently, and owing to the dependency of these systems on one another, an accumulation of metabolites during biochemical processes of regenerating ATP was assumed to play a large role in the development of fatigue (Bangsbo, J., Madsen, K., Kiens, B., & Richter, E.A., 1996; Cady, E.B., Jones, D.A., Lynn, J., & Newham, D.J., 1989; Noakes, T.D., St Clair Gibson, A., & Lambert, E.V., 2004; Vøllestad, N.K. & Sejersted, O.M., 1988).
It was standard, and remains common, to study prolonged exercise responses within individual physiological systems using a fixed intensity or externally regulated exercise protocol based on a certain percentage of an individuals’ maximal oxygen consumption (VO_{2max}). As such, much of what we know about the physiological response to exercise is skewed based on the requirement that an individual maintain a certain exercise intensity and ultimately reach volitional exhaustion. In a true Khunian fashion, responses of individual physiological systems, including the cardiovascular (Cornelissen, V.A., Verheyden, B., Aubert, A.E., & Fagard, R.H., 2009), thermoregulatory (González-Alonso, J. et al., 1999; Nielsen, B. et al., 1993), inflammatory (Mendham, A., Donges, C., Liberts, E., & Duffield, R., 2011; Ostrowski, K., Schjerling, P., & Pedersen, K.B., 2000), metabolic (Romijn, J., Coyle, E., Sidossis, L., Rosenblatt, J., & Wolfe, R., 2000; Romijn, J.A. et al., 1993) endocrine (Felsing, N.E., Brasel, J.A., & Cooper, D.M., 1992), muscular (Bigland-Ritchie, B. & Woods, J.J., 1984), and even whole brain (Nybo, L., Moller, K., Volianitis, S., Nielsen, B., & Secher, N.H., 2002; Roelands, B. & Meeusen, R., 2012; Wilson, W.M. & Maughan, R.J., 1992) have been investigated based solely on a designated percentage of lactate threshold (LT) or VO_{2max}. Although scrutinised due to its lack of transferability to field based scenarios, the targeted research was driven by some important theories and provided new ideas that have advanced our knowledge accordingly.

**THE CARDIOVASCULAR/ANAEROBIC LIMITING THEORY**

In an early study, Astrand and Saltin (2003) attempted to record a greater VO_{2max} in individuals using different protocols of increasing work rate and hence, decreasing the time to reach VO_{2max}. In this study, despite the altered work rate, VO_{2max} still peaked at nearly the same place for each participant, and was repeatable, thus suggesting a ‘ceiling’ effect (Åstrand, P.-O., 2003). The cardiovascular/anaerobic model therefore suggests that there is a point whereby energy demands become too great for the cardiorespiratory system to supply enough oxygen, thus shifting towards a reliance on glycolytic pathways and increasing La\(^{-}\) production and accumulation (Bassett, D.R. & Howley, E.T., 2000). Bassett and Howley (2000) identify several limiting
factors to VO$_{2\text{max}}$ including the pulmonary diffusing capacity, maximal cardiac output, the oxygen carrying capacity within the blood and skeletal muscle oxygen uptake.

At rest, the arterial oxygen concentration is around 200 mL O$_2$·L$^{-1}$, however, during heavy exercise, it decreases to only ~20-30 mL O$_2$·L$^{-1}$ as it is utilised by muscles for energy (Bassett, D.R. & Howley, E.T., 2000). Hence, the combination of pulmonary efficiency, or how much oxygen can be breathed in and carbon dioxide off-loaded, the maximal cardiac output, and skeletal utilisation of oxygen, were believed to be limiting factors in exercise capacity, with 75-80% of limitation coming from the maximal cardiac output alone (Bassett, D.R. & Howley, E.T., 2000). The link between oxygen delivery and maximal oxygen uptake is further supported through blood doping studies which show an increase of up to 10% VO$_{2\text{max}}$ when 900-1,350 mL blood is reinfused, thereby increasing haemoglobin (Hb)-oxygen binding affinity and associated oxygen delivery to the working muscles (Gledhill, N., 1985). Moreover, this effect is not present in the event that Hb is not altered, thereby resulting in minimal increases in VO$_{2\text{max}}$ when plasma volume is increased without parallel increases in Hb in untrained individuals (Warburton, D.E.R., Gledhill, N., & Quinney, H.A., 2000). The evidence clearly shows the physiological dependency on the cardiovascular system, however, further studies revealed more contributing factors to VO$_{2\text{max}}$.

**Theories of Thermoregulation and Fatigue**

In addition to physiological limits of the cardiovascular, pulmonary, skeletal and central systems during exercise, performance decrements and exacerbated changes within each of the aforementioned systems during exertional heat stress provided early evidence that fatigue ensues when T$_c$ or oesophageal temperature (T$_{es}$) increases to approximately ~40°C (Fuller, A., Carter, R.N., & Mitchell, D., 1998; González-Alonso, J. et al., 1999; Nielsen, B. et al., 2001; Walters, T.J., Ryan, K.L., Tate, L.M., & Mason, P.A., 2000). This critical core temperature limiting theory was shown in several studies exposing participants to heat stress between 35-40°C and
required them to exercise at fixed intensity (~60% VO$_{2\text{max}}$) until exhaustion. One study found a correlation between initial T$_{es}$ starting temperature (36, 37 or 38°C) and performance time (63, 46 and 28 min, respectively), while the end temperature reached an identical level in all trials (~40.1°C) (González-Alonso, J. et al., 1999). This evidence sparked considerable interest in the limiting core temperature theory, but more recent research as suggested that core temperature alone does not account for the primary reduction in power output and performance (Ely, B.R. et al., 2009; Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011; Tucker, R., Rauch, L., Harley, Y., & Noakes, T., 2004).

Indeed, a study by Ely et al. (2009) presented evidence against the limiting core temperature theory in their study which revealed that when heat dissipation is not constrained to narrow core-to-skin gradients (i.e. When there is ample ability to release heat from the body, such as during cooler weather) and in conditions which produced high muscle temperatures, trained runners were able to surpass the 40°C threshold without compromising performance. Likewise, other research has identified a relative exercise intensity theory in which high skin temperatures, or external thermal sensory information, is a greater contributor to limiting aerobic exercise performance, coupled with a reduction in cardiovascular reserve (Sawka, M.N., Leon, L.R., Montain, S.J., & Sonna, L.A., 2011), especially when the core-to-skin temperature gradient is narrow (Sawka, M.N., Cheuvront, S.N., & Kenefick, R.W., 2012). Most notably with these more recent studies, is the shift from a fixed intensity exercise protocol to self-paced types of protocols which enable the complex integration of the physiological systems to be studied in greater detail. While each of these theories are largely based on peripheral limitations, the alternative fatigue theory features the central areas with limitations due to changes in neurotransmitter concentrations, such as serotonin (Meeusen, R., Watson, P., Hasegawa, H., Roelands, B., & Piacentini, M.F., 2012).

**THE CENTRAL FATIGUE HYPOTHESIS**
The central fatigue hypothesis was developed in an effort to identify how changes in serotonin concentrations in the brain can contribute to fatigue during prolonged exercise (Newsholme, E.A. & Blomstrand, E., 2006; Roelands, B. & Meeusen, R., 2012). In this hypothesis, neuronal activity is dependent on the production of serotonin, or 5-hydroxytryptamine (5-HT), which is synthesised by neurons themselves (Meeusen, R. et al., 2012). However, synthesis is, in turn, dependent on the uptake of tryptophan (Trp) and the availability of branched chain amino acids (BCAAs) to produce Trp (Davis, J.M., Alderson, N.L., & Welsh, R.S., 2000; Newsholme, E.A. & Blomstrand, E., 2006). An increase in 5-HT levels in presynaptic neurons leads to increased 5-HT release and ultimately to greater electrical activity in the post synaptic neuron (Newsholme, E.A. & Blomstrand, E., 2006). The serotonin fatigue hypothesis posits that fatigue ensues with performance decrements when 5-HT is not synthesised quick enough to compensate for increased energy demands (Davis, J.M., Alderson, N.L., & Welsh, R.S., 2000). Hence, supplementing with BCAAs is a common method of researching whether or not increases in the Trp pre-cursor, may increase synthesis of 5-HT, thereby increasing serotonin and performance.

Evidence supporting the central fatigue hypothesis, and of other neurotransmitters including epinephrine, norepinephrine and noradrenaline is variable, with some suggesting nutritional supplementation of BCAAs increases serotonin synthesis, reduces mental fatigue and the associated rating of perceived exertion (RPE), and increases cognitive ability, thereby defending the brain against fatigue (Blomstrand, E. et al., 1997; Hassmén, P., Blomstrand, E., Ekblom, B., & Newsholme, E.A., 1994). Still, others show no performance benefits when supplementing with BCAAs (Negro, M., Giardina, S., Marzani, B., & Marzatico, F., 2008; van Hall, G., Raaymakers, J.S., Saris, W.H., & Wagenmakers, A.J., 1995). Despite the variable findings, it is generally accepted that there are contributions from altered concentrations of neurotransmitters that are likely to contribute to motor control, power output and overall feelings of fatigue.

Further to the implications that reduced serotonin may have on fatigue; there is also interplay between it and dopamine. Similar to serotonin, dopamine
concentrations at end exercise are found to decrease due to fatigue (in rats) (Roelands, B. & Meeusen, R., 2012). The ratio between serotonin and dopamine, therefore, has previously been identified as a factor that may influence central fatigue. Dopamine supplementation has been shown to influence performance in heated (Watson, P. et al., 2005) but not temperate conditions (Meeusen, R., Roeykens, J., Magnus, L., Keizer, H., & De Meirleir, K., 1997; Piacentini, M.F., Meeusen, R., Buyse, L., De Schutter, G., & De Meirleir, K., 2004).

A final neurotransmitter hypothesised to play a role in the central fatigue hypothesis is noradrenaline due to its role in arousal, consciousness and reward (Roelands, B. & Meeusen, R., 2012). Again, research is largely inconclusive. It has been shown that the combination of a serotonin/noradrenaline reuptake inhibitor does not alter performance (Piacentini, M.F., Meeusen, R., Buyse, L., De Schutter, G., & De Meirleir, K., 2002), while a noradrenaline reuptake inhibitor alone revealed a slight, yet not significant decrease in time to complete a 90 min time trial (Piacentini, M.F., Meeusen, R., Buyse, L., De Schutter, G., Kempenaers, F., et al., 2002).

These aforementioned models helped to understand the changes within and between each physiological system and provided a foundation for basic understanding of central and peripheral contributions, yet they left out the most important aspect of exercise tolerance and regulation – the overall perception of effort by the individual and the ability to modulate behaviour based on these feelings. The polarised methods of researching physiological processes within the body reiterate the relationship between Kuhn and Popper ideologies whereby a recent Popperian type paradigm shift towards self-paced exercise highlighted the notion of central regulation of exercise intensity and effort, thereby furthering our understanding of the perceptual basis of exercise tolerance, pacing and fatigue.

**A new era of central regulation, dynamic behaviour and the complex integrated systems theory**
The shift in paradigms to self-paced exercise consequently changed the focus to the intervening entity capable of manipulating energy consumption and pacing strategies to prevent end state catastrophe due to the cascade of metabolic and neuromuscular events (Noakes, T.D., St Clair Gibson, A., & Lambert, E.V., 2004). Instead, the teleo-anticipatory and central governor theories identified the brain as the regulator of exercise and fatigue (St Clair, G.A., Lambert, M.I., & Noakes, T.D., 2001; Ulmer, H.-V., 1996), suggesting that there is a feedback mechanism from somatosensory or other afferent feedback during exercise to help calculate and regulate the amount of energy needed, relative to some anticipated end point of exercise and based on the current state of the body at any given point in time (Marino, F.E., 2004; Noakes, T.D., St Clair Gibson, A., & Lambert, E.V., 2004; Ulmer, H.-V., 1996). Furthermore, both theories highlighted the ability to mediate efferent drive based on metabolic reserve, metabolic rates of energy consumed and time required for completing the required exercise task (Marino, F.E., 2004; Noakes, T.D., Peltonen, J.E., & Rusko, H.K., 2001; Ulmer, H.-V., 1996). These theories have been further elucidated by Edwards and Polman (2013) in their review on pacing and awareness, highlighting the differences between consciousness and awareness in exercise and pacing, whereby there is increased awareness of a particular exercise situation due to sensory stimuli ‘bombarding’ the brain with negative cues and, in high intensity exercise, becoming increasingly severe to the point that they stimulate awareness.

These models of fatigue and pacing involving central regulation have since eliminated the ‘brainless’ theories of fatigue and initiated an inquiry into the perceptual and behavioural aspects of exercise tolerance. The dynamic behaviour theory posits that perception of fatigue or other danger is an integral aspect of human life that allows us to adapt to present situations based on stimuli from external and internal factors (Allport, F.H., 1955; Warren, W.H., 2006). Dynamic behaviour involves distal stimuli or the event to be perceived, such as the exercise protocol; and proximal stimuli, analogous to somatosensory responses to the exercise protocol caused by both external stimuli from the environment and internal stimuli from changes within the physiological systems (Allport, F.H., 1955). In dynamic
behaviour models, proximal stimuli produce afferent signalling to the brain and an array of responses from the central and autonomic nervous systems culminate in efferent drive that is continually modulated by the afferent feedback (Glass, L., 2001; Lambert, E., St Clair Gibson, A., & Noakes, T., 2005; Robson-Ansley, P.J., Milander, L.d., Collins, M., & Noakes, T.D., 2004; St Clair Gibson, A. & Noakes, T.D., 2004). This theory has underpinned that of the complex integrated systems of fatigue during exercise, which posits that physiological systems work together to maintain homeostasis and eliminate catastrophe through anticipatory regulation initially, followed by alterations in effort based on afferent feedback from internal, external and motivational factors (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005).

**FATIGUE DURING HEAT STRESS AND CENTRAL SENSITISATION**

Reductions in performance in heat stress have also been associated with complex integration of physiological systems involving perturbed cardiovascular (González-Alonso, J., 2012), metabolic (Parkin, J.M., Carey, M.F., Zhao, S., & Febbraio, M.A., 1999), perceived exertion (Nybo, L. & Nielsen, B., 2001) and motivational (Nielsen, B. et al., 1993) responses and is accompanied by fatigue that is exacerbated due to the thermal load.

Heat stress has been shown to alter cortical EEG activity in passive hyperthermia alone with a generalised increase in fast cortical activity and a dispersed, concomitant increase in slow cortical activity that predominates within the posterior head region (Dubois, M. et al., 1980). Central sensitisation is seen primarily in disease and chronic pain, whereby there is exacerbated afferent feedback, or reduced inhibition of sensory feedback, thereby causing a sensitising effect that alters the overall modulation of neural output (Zambreanu, L., Wise, R.G., Brooks, J.C., Iannetti, G.D., & Tracey, I., 2005). Though yet to be discerned, changes in cortical EEG activity could identify central sensitisation based on altered brain wave patterns.
It is clear that down-regulation of the central nervous system (CNS) plays a critical role in fatigue during exertional heat stress, as high core temperatures have shown to be associated with reduced central drive (Nybo, L & Nielsen, B, 2001; Sabiosky, J., Marino, F., Kay, D., & Cannon, J., 2003). Furthermore, it has been shown that when core temperature is manipulated during passive heat stress, voluntary activation is reduced and systematically returns to resting values in an orderly fashion (Morrison, S., Sleivert, G.G., & Cheung, S.S., 2004). These studies collectively show that exercise induced hyperthermia invokes a strong central component which reduces the capability of the brain to continue to drive motor output at a level that sustains exercise.

Research has primarily focused on the role of the CNS and the relationship between rising core temperature and neuronal activity (Nielsen, B. et al., 2001), cerebral blood flow (Rasmussen, P. et al., 2010) and energy turnover (Nybo, L., Moller, K., et al., 2002; Trangmar, S.J. et al., 2014) as mechanisms for reduced performance or increased fatigue in the heat. Most of these studies, however, use fixed intensity exercise protocols to evaluate the changes. In self-paced exercise, it has been shown that CNS down regulation may be attributed to initial increased thermal cues (heat), leading the participant to choose a lesser intensity initially than they would if the conditions were cooler (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011), and hence, reducing neuromuscular drive and power output in an anticipatory manner. In this study, participants wore a full body liquid-perfused suit that was perfused with hot water initially, hence increasing thermal cues, followed by cool water half way through the protocol. Total work (due to a lesser initial power output) was reduced when the water was first hot and then cold, compared to when cool water was perfused initially, followed by hot (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011). The author’s attribute the reduction in total work to the reduction in initial power output in the hot to cold condition where, even when the water turned cool, they couldn’t make up for the difference in power output compared to the cool to hot trial (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011). Likewise, it has been shown that thermal sensations alone, or hot skin
temperature, contribute to reductions in aerobic performance in euhydrated states, while hypohydration exacerbates the effects (Sawka, M.N., Cheuvront, S.N., & Kenefick, R.W., 2012). While these examples don’t necessarily reflect fatigue, they do provide further understanding of how we manage signalling from the environment and the behavioural changes that are made in order to regulate performance.

In addition to altered CNS regulation, it has also been known for some time that peripheral responses to strenuous exercise in the heat are exacerbated. For instance, muscle glycogen depletion is accelerated by heat stress, though exhaustion can occur long before depleted energy stores cause fatigue (Cheuvront, S.N., Kenefick, R.W., Montain, S.J., & Sawka, M.N., 2010). Cardiovascular demands are also altered as blood flow is directed to the skin ultimately increasing cutaneous venous volume while simultaneously increasing heart rate for more blood delivery to the muscles, with a concomitant reduction in plasma volume, thus reducing cardiac filling and stroke volume and overall cardiac output (Cheuvront, S.N., Kenefick, R.W., Montain, S.J., & Sawka, M.N., 2010; Sawka, M.N., Cheuvront, S.N., & Kenefick, R.W., 2012).

Additionally, heat stress promotes alterations in gastrointestinal (GI) permeability (Brock-Utne, J. et al., 1988; Øktedalen, O., Lunde, O.C., Opstad, P.K., Aabakken, L., & Kvernebo, K., 1992), leading to increased leakage of lipopolysaccharides (LPS, endotoxin) from the intestinal lumen to the internal environment. A number of factors could be responsible for the increased gut leakage including, but not limited to, reduced intestinal blood flow (Rowell, L.B., 1974), tissue hypoxia (Hall, D.M., Baumgardner, K.R., Oberley, T.D., & Gisolfi, C.V., 1999), dehydration (Lambert, G. et al., 2008) and nonsteroidal anti-inflammatory drugs (Lambert, G.P., Boylan, M., Laventure, J.P., Bull, A., & Lanspa, S., 2007). A consequence of the increased gut permeability is the promotion of inflammatory events. Endotoxin release from the gut mucosa triggers a response involving pro-inflammatory and anti-inflammatory cytokines including tumor necrosis factor alpha (TNF-a), and the interleukins (IL)-1b and IL-6 (Pedersen, B.K., 2000), whereby afferent CNS signaling may be increased, contributing,
potentially to central sensitisation, and overall, to the complex systemic integration of fatigue in heat stress. The following sections provide an extended review on systemic peripheral responses to exercise in thermoneutral and heat stress conditions, hence providing a greater understanding of how altered mechanisms may contribute to exercise regulation and fatigue.

**PERIPHERAL RESPONSES TO EXERCISE**

Physiological responses to exercise have, for many years, been informed by fixed intensity protocols. While this has been beneficial to understanding responses when they are externally regulated, studying the power output and pacing strategies in closed-loop, internally regulated protocols have become increasingly popular. Hence, the below literature aims to discuss research that employs a self-paced protocol. Unfortunately, the literature in the area is quite scattered amongst the different types of protocol - internally regulated (clamped RPE) (Edwards, A. & Lander, P., 2012; Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006) or time trial, performance based (Marino, F.E. et al., 2001; Sherman, W.M., Peden, M.C., & Wright, D.A., 1991; Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000); and interventions (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004; Mauger, A.R., Jones, A.M., & Williams, C.A., 2010; Robson-Ansley, P.J., Milander, L.d., Collins, M., & Noakes, T.D., 2004). Moreover, they don’t all report findings on the same variables. Consequently, the following review focuses on self-paced exercise responses where possible, but includes responses of fixed intensity as well. To review some of the self-paced literature more systematically, please refer to table 2-1 which aims to highlight the discrepancy in protocols/variables measured and the respective results from the literature. Furthermore, it highlights the lack of investigation into cerebral responses during self-paced exercise.

**CARDIOVASCULAR AND THERMOREGULATORY RESPONSES IN TEMPERATE CONDITIONS**
When the level of exercise surpasses initial reductions in parasympathetic drive, heart rate is increased initially through efferent sympathetic drive and subsequent changes in cardiac output, vascular resistance and arterial pressure (Victor, R.G., Seals, D.R., & Mark, A.L., 1987). Heart rate increases in a linear fashion with increasing exercise intensity to meet energy demands through increased blood perfusion to the working muscles which imposes a greater cardiac output (Périard, J.D., Cramer, M.N., Chapman, P.G., Caillaud, C., & Thompson, M.W., 2011). In trained athletes, an increase in stroke volume due to left ventricular hypertrophy and an increase in cardiac contractility and blood plasma volume aides in meeting the demands of exercise through a greater cardiac output.

In addition to the heart rate, stroke volume and cardiac output responses, baroreceptors and metaboreceptors help to regulate the blood pressure response during exercise (González-Alonso, J., Crandall, C.G., & Johnson, J.M., 2008; Rowell, L.B., O'Leary, D.S., & Kellogg, D.L., 2011). Specifically, the baroreflex and metaboreflex can cause muscular vasoconstriction in order to maintain blood pressure within range. This, however, is believed to limit muscle blood flow by reducing cardiac output when pressure becomes too high (González-Alonso, J., Crandall, C.G., & Johnson, J.M., 2008). Indeed, it has been shown that while cardiac output and muscular blood perfusion increase linearly during incremental exercise to exhaustion, there is a reduction in the rate of both oxygen delivery from the blood when exercise intensity increases above 50% VO$_{2\text{max}}$, and a plateau in cardiac output at intensities greater than 90% VO$_{2\text{max}}$ (Mortensen, S.P. et al., 2005). Hence, the baro- and metaboreflexes closely monitor the blood pressure in an effort to minimise changes and in effect, provide a ceiling effect for cardiac output when intensity becomes too high, thereby limiting muscular blood flow and contributes to eventual cessation of exercise.

Heart rate responses have been shown to be similar between an internally regulated self-paced protocol and a work-load matched, externally regulated protocol (Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009). However, the physiological responses during a an internally regulated, clamped RPE
protocol are seemingly modality dependent with motorised treadmill running producing a higher mean intensity, heart rate and lactate concentration than the same protocol performed in a rowing test at the same intensity (Edwards, A. & Lander, P., 2012). This is likely a consequence of the size of the muscles being used in running and rowing, respectively, and the additional metabolic and associated blood volume demands which would increase heart rate accordingly.

Exercise in thermoneutral or cool environments does not stress the thermoregulatory system to the same extent as heat stress, although core temperature does increase in thermoneutral or cool environments in response to exertion at high intensities due to increased metabolic activity. Nevertheless, when a large temperature gradient exists between deep muscle and subcutaneous or skin tissue, as in thermoneutral conditions, the removal of heat is sufficient and performance is not often impaired (Schlader, Z.J., Prange, H.D., Mickleborough, T.D., & Stager, J.M., 2009). Interestingly, however, core temperature and associated RPE, has been shown to be significantly reduced during self-paced internally vs. externally regulated exercise, although there is no difference in mean skin temperature, hence suggesting a greater heat loss and physiological demand from externally paced protocols than when individuals were allowed total control over the pacing (Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009). Importantly, these findings oppose the theory that core temperature is directly responsible for reductions in power output (González-Alonso, J. et al., 1999; Nybo, L & Nielsen, B, 2001; Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000) and hence, supports the integration of numerous physiological systems contributing to power output modifications.

CARDIOVASCULAR AND THERMOREGULATORY RESPONSES TO EXERTIONAL HEAT STRESS

Despite minimal changes in cardiovascular responses between internal and external protocols, the addition of thermal stress has been shown to have a large effect on power output and cardiovascular strain (Hargreaves, M., 2008). Increased heart rate in heat stress is caused by greater cardiovascular
strain owing to a reduced plasma volume, and therefore reduced stroke volume, mean arterial pressure and cardiac output (Périard, J.D. et al., 2011). Additionally, thermal strain is regulated through thermoreceptors and is accompanied by increased blood perfusion to the skin and away from the splanchnic region to reduce internal temperature and overall thermal sensation (González-Alonso, J., Crandall, C.G., & Johnson, J.M., 2008). Importantly, the increased perfusion to the skin limits the muscular blood flow too. Limitations in muscular blood flow therefore increase the demands for cardiac output and exacerbate the heart rate response, especially as exercise duration is prolonged.

The aforementioned changes in cardiac function are further exacerbated by a reduction in total blood volume or mean arterial pressure from sweating, although they may not directly affect exercise performance to the same extent that increases in skin temperature do (Ely, B.R. et al., 2009; Périard, J.D. et al., 2011; Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000). Nevertheless, the reduction in total blood volume results in a reduced stroke volume and hence a greater cardiac output for the same intensity of exercise as would otherwise be performed in thermoneutral conditions. These effects eventually lead to the known cardiac drift that occurs during heat stress in which heart rate continues to increase while cardiac output is maintained or reduced (Coyle, E. & Gonzalez-Alonso, J., 2001).

Some literature using fixed intensity protocols report significantly increased heart rate in heat stress due to the loss of fluid from thermoregulatory processes compromising plasma volume (González-Alonso, J., Mora-Rodríguez, R., Below, P.R., & Coyle, E.F., 1997), while others report only non-significant increases, if any decreases, that don’t alter performance or time to fatigue (Kay, D. & Marino, F.E., 2003; Marino, F.E., Kay, D., & Serwach, N., 2004), despite an increase in RPE (Rasmussen, P. et al., 2010).

Heart rate responses during self-paced exercise in heat stress, however, are increased significantly compared to thermoneutral conditions using a clamped RPE protocol that is rated between ‘hard to very hard’ (Tucker, R.,
Marle, T., Lambert, E., & Noakes, T., 2006), with similar findings in a 30 min cycling time trial (Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000). Both studies suggest a marked increase in thermal stress and, consequently, an increase in heart rate and decrease in power output (Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006). This may highlight numerous contributions from heat stress to the overall rating of perceived exertion and performance.

The integrated systemic responses are further highlighted in a study comparing 20km cycling TT performance in heat stress compared to a cool environment (Tucker, R., Rauch, L., Harley, Y., & Noakes, T., 2004) where heart rate was not significantly increased in heat stress, however, there was a significant reduction in power output in the heat stress condition (Tucker, R., Rauch, L., Harley, Y., & Noakes, T., 2004). This highlights the dependency between the anticipatory response, information processing, and the regulation of power output and ability to modulate performance based on physiological responses. The integration of cardiovascular and thermoregulatory systems have also been demonstrated by cooling the skin temperature to 5-6°C below baseline, which resulted in an increase in performance despite a non-significant rise in heart rate (Kay, D., Taaffe, D.R., & Marino, F.E., 1999). This therefore presents an opposite side of the cardiovascular-thermoregulatory performance spectrum in that reducing thermal loads can benefit performance, whilst minimising cardiovascular strain.

The understanding of thermoregulatory responses, and especially that of skin and core temperature, has improved immensely since the original proposal that an increase in core temperature to ~40°C reduces performance, exercise tolerance and induces fatigue (Nielsen, B. et al., 1993). When exercising in the heat, core temperature increases due to a combination of increased metabolic activity, reduced efficiency for eliminating internal heat by the blood to the skin, and, especially in humid conditions, limited loss of heat from sweat (González-Alonso, J., 2012). It has been suggested that two avenues of heat exchange in the body include intercellular conductive
heat transfer and vascular convective heat transfer, where, in cool conditions, a large temperature gradient between deep muscle and skin allow efficient heat removal (González-Alonso, J., 2012). Notably, core temperature is reported to be similar between thermoneutral and heat stress environments during self-paced exercise, accompanied by significantly greater skin temperature, and reduced power output heat stress (Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000; Tucker, R., Rauch, L., Harley, Y., & Noakes, T., 2004).

Several studies have reported that core temperature alone may not be directly responsible for reductions in power output in exertional heat stress. For instance, Ely et al. (2009) report that average running velocity at core temperatures > 40˚C were not different to those < 40˚C. Other studies have found that performance is reduced long before the rate of heat storage reaches a proposed threshold (Kay, D., Taaffe, D.R., & Marino, F.E., 1999; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006; Tucker, R., Rauch, L., Harley, Y., & Noakes, T., 2004) and hence, even thermal sensations alone are believed to have a large impact.

Schlader et al. (2011) found $T_{sk}$ to be an important regulator of determining exercise intensity in the initial stages of a self-paced protocol, while subsequent changes in $T_{sk}$ over the duration of the protocol were found to be less implicated in pacing. Participants were required to perform a self-paced protocol in which they commenced in a cool environment and finished in a heated environment, or vice-versa. Interestingly, the thermoregulatory response in terms of whole body sweat rate were similar between both protocols, while plasma osmolality was increased when exercising from cold to hot, but stayed the same when exercise commenced in hot and went to cold (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011). The authors conclude that thermal sensations and afferent input are responsible for the selected pace and power output regulation in the beginning 5 min of the protocol, but a culmination of signals is responsible for modulating power output throughout the duration of the protocol (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011).
In fixed intensity exercise, reductions in plasma volume and hence other measures of cardiovascular strain have been shown to have a direct effect on power output (González-Alonso, J., Mora-Rodríguez, R., Below, P.R., & Coyle, E.F., 1997; Périaud, J.D. et al., 2011). However, hydration studies revealed that even replacing 100% of the fluid lost due to sweating during both fixed intensity and self-paced exercise, does not improve cardiac strain, hence eliminating the proposed performance benefits (Kay, D. & Marino, F.E., 2003; Marino, F.E., Kay, D., & Serwach, N., 2004). The literature discussed throughout the previous two sections highlights the importance of the cardiovascular and thermoregulatory systems during exercise and the regulatory processes that are involved. Furthermore, they attempt to elucidate the effects of thermal strain on cardiac function and discuss how the literature varies in terms of exercise protocols used. Nevertheless, although it is a central component to exercise and delivery of oxygen for energy requirements, there are numerous factors in conjunction with the cardiac system that must be acknowledged and discussed in order to better understand complex integration and feelings of fatigue during exercise.

**METABOLIC RESPONSES – BLOOD GLUCOSE AND LACTATE**

Pacing strategies allow preservation or distribution of energy as required to maintain the desired intensity for the duration of the exercise task. It is generally established that during an all-out TT competition, an initial high energy output, followed by a plateau or reduction in power output and a burst of anaerobic capacity at the end is desirable for optimal racing (Stone, M.R., Thomas, K., Wilkinson, M., St Clair Gibson, A., & Thompson, K.G., 2011). Furthermore, it has been suggested that there should be minimal, if any, residual anaerobic energy left at the end of the TT (Foster, C. et al., 2004). Muscle glycogen breakdown and carbohydrate oxidation is known to provide the majority of fuel during high intensity exercise, in addition to, although to a lesser extent, fatty acid oxidation (Romijn, J. et al., 2000; Romijn, J.A. et al., 1993). The overall glycogen content and blood glucose levels are therefore believed to inform the brain of available energy stores in an anticipatory manner (Rauch, H., Gibson, A.S.C., Lambert, E., & Noakes, T., 2005).
Ample amounts of blood glucose and glycogen storage prior to performing endurance exercise tasks can help to sustain exercise intensity for long durations. Specifically, endurance trained individuals will be able to train at higher intensities primarily using oxidation of their fat stores and delaying the use of carbohydrates as fuel. As intensity and/or duration increases, however, the use of carbohydrates as energy is inevitable (Romijn, J.A. et al., 1993). Hence, it has been shown that carbohydrates can have a potentially limiting role in endurance exercise, especially in the event that carbohydrate supplementation is not performed throughout the exercise bout. For instance, a review of the literature by Coyle et al. (1992) reveals that carbohydrate supplementation can delay fatigue for 30-60 min during endurance exercise by providing additional blood glucose for energy. Likewise, one study determined a strong trend towards an increase in 64km time trial performance when carbohydrates were supplemented regardless of glycaemic index (Earnest, C.P. et al., 2004), while large fluid replacement of carbohydrate solution has been shown to improve performance in 1hr cycling at 50% VO2max by 6.3% (Below, P.R., Mora-Rodríguez, R., González-Alonso, J., & Coyle, E.F., 1995).

As carbohydrates are increasingly used as fuel for exercise at intensities above the lactate threshold, metabolic by-products accumulate within the blood. Several studies have revealed an apparent sensitivity to the build-up of metabolic by products, especially lactate (Foster, C. et al., 2004; Karlsson, J. & Saltin, B., 1970) associated with a reduced power output, increase in RPE and increase in time to exhaustion (Karlsson, J. & Saltin, B., 1970; Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009). Interestingly, self-paced exercise has an apparent attenuating effect on lactate accumulation, owing to constant behavioural modifications in an effort to defend homeostasis compared to fixed intensity, externally regulated exercise (Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009). Mechanisms for this response may be due to a reduction in physiological strain that accompanies internally regulated self-paced protocols. Namely, being able to alter pacing strategies so that at times, the dominant fuel for exercise is fats and at other time, carbohydrates, results improved lactate shuttling hence maintaining blood pH, while attenuating the core
temperature, thereby enabling a constant metabolic challenge that can be maintained throughout the duration of the exercise task (Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009).

**METABOLIC RESPONSES TO EXERTIONAL HEAT STRESS**

Muscle metabolism in heat stress is known to shift substrate use and enhance glycolysis, potentially due to a reduction in muscle blood flow (Fink, W.J., Costill, D.L., & Van Handel, P.J., 1975). Enhanced glycolysis has been related to increases in epinephrine released during exertional heat stress along with increased muscle temperature which causes an increase in allosteric activation of phosphofructokinase and phosphorylase, resulting in an increase in glycolytic flux (Febbraio, M.A., Snow, R.J., Stathis, C.G., Hargreaves, M., & Carey, M.F., 1994). It has since been shown that carbohydrate supplementation (glucose + fructose) during the heat enhances exogenous carbohydrate oxidation rates compared to glucose alone, and reduces endogenous carbohydrate oxidation rates compared to supplementing with water alone (Jentjens, R.L.P.G. et al., 2006). However, another study found that while carbohydrate supplementation increases time to fatigue during both low (60%) and high (73%) fixed intensity exercise protocols (Carter, J., Jeukendrup, A.E., Mundel, T., & Jones, D.A., 2003), there was no clear metabolic effect, but rather, the author’s associated the performance benefits to an increased tolerance to rising core temperatures (Carter, J., Jeukendrup, A.E., Mundel, T., & Jones, D.A., 2003). Similar findings have been found in other fixed intensity, carbohydrate supplementation studies where exogenous carbohydrate increased endurance exercise capacity compared to water (Carter, J., Jeukendrup, A.E., & Jones, D.A., 2005) and where availability of carbohydrates resulted in a correlation between power output and neuromuscular drive compared to the placebo condition (Abbiss, C.R. et al., 2008). Unfortunately the role that carbohydrate supplementation plays during self-paced exercise in heat stress remains somewhat elusive. Nevertheless, an increase rate of glycolysis results in an additional accumulation of lactate that occurs in exertional heat stress.
The accumulation of lactate in exertional heat stress, and the effects that self-paced exercise has on lactate accumulation is somewhat counterintuitive. For instance, in a study that required individuals to complete 30 min submaximal (70% peak treadmill speed), followed by an 8km self-paced time trial, lactate accumulation post submaximal bouts of exercise were not different between cool and heated conditions, however, post the performance run, lactate was significantly greater in the cool compared to the heated condition (Marino, F.E. et al., 2001). Similar findings have been shown in self-paced cycling time trials where lactate was not different between heat stress and cool environments (Abbiss, C.R. et al., 2008; Duffield, R., Green, R., Castle, P., & Maxwell, N., 2010).

Duffield et al. (2010), required participants to perform a 40km cycling TT in 33°C heat stress where they were either pre-cooled or not. Although time trial performance was better in the pre-cooled condition than the heat stress condition, there was no difference in lactate accumulation between the two (Duffield, R., Green, R., Castle, P., & Maxwell, N., 2010). Similarly, Abbiss et al. (2008), showed no difference in lactate accumulation between hot and temperate conditions in a 16.1km time trial, although lactate concentration was greater and performance increased in when supplementing with carbohydrates compared to a placebo. This finding further highlights the potential for increased carbohydrate energy to improve performance through increased glycolysis, hence resulting in an increased lactate accumulation (Abbiss, C.R. et al., 2008). The multifaceted response of lactate during heat stress and temperate conditions, therefore suggest complex integration between energy fuel and metabolic processes, combined with thermal sensations and during prolonged exercise in the heat.

CENTRAL RESPONSES TO EXERCISE

CEREBRAL HEMODYNAMICS

Activity and oxygenation patterns within the brain can be measured through near infrared spectroscopy (NIRS) (Ekkekakis, P., 2001; Ogoh, S. & Ainslie, P.N., 2009). Furthermore, cerebral blood flow (CBF) can be
quantified using the Kety-Schmidt technique and an injected tracer that calculates the global (g) CBF based on blood gases and haemoglobin concentration [Hb] at any given time (Kety, S.S. & Schmidt, C.F., 1948). Measures of cerebral autoregulation can also be inferred through measuring the mean arterial pressure (MAP) and PaCO\(_2\) from the brachial artery, along with oxygen saturation from the jugular vein (Ogoh, S. et al., 2005). Collectively, these techniques have provided a good overall indication of cerebral changes that occur, however, NIRS is arguably the most common tool used to assess changes during exercise due to its good temporal resolution, low cost and minimal artefact compared to other techniques (Ekkekakis, P., 2001). Still, there are many limitations involved with the interpretation of signals due to the relatively low spatial resolution and ability to only measure the superficial tissues (Ekkekakis, P., 2001). Additionally, interpretation of NIRS can be difficult.

Although at times misinterpreted, regional (r) CBF is not coupled with regional cerebral metabolic rate (rCMRO\(_2\)), and hence, limits the ability for NIRS to be interpreted in terms of oxygen demand and metabolism (Ekkekakis, P., 2001; Ide, K., Horn, A., & Secher, N.H., 1999). Instead, it is recommended that NIRS is reported using three measures (Ekkekakis, P., 2001): (a) the change (∆) in deoxyhaemoglobin concentration ([HHb]), as this is believed to be a reliable measure of tissue deoxygenation and neuronal activation (Billaut, F., Davis, J.M., Smith, K.J., Marino, F.E., & Noakes, T.D., 2010; Garcin, M., Mille-Hamard, L., Billat, V., Humbert, L., & Lhermitte, M., 2005; Subudhi, A.W., Dimmen, A.C., & Roach, R.C., 2007), (b) the ∆ oxyhaemoglobin concentration ([HbO\(_2\)]), as this reflects cerebral activation (Ekkekakis, P., 2001) and (c) the ∆ haemoglobin difference (∆[Hb\(_{Diff}\) = [HbO\(_2\)] – [HHb]) as a measure of cerebral oxygenation (Coxy) as it correlates well with CBF.

Several studies have helped to understand changes in CBF and Coxy using both NIRS and a variety of the aforementioned techniques during exercise. Incremental exercise has been shown to reduce Coxy when exercise intensity reaches the respiratory compensation ratio (Bhambhani, Y., Malik, R., & Mookerjee, S., 2007) and between 75-100% peak power output
(Subudhi, A.W., Dimmen, A.C., & Roach, R.C., 2007), likely due to the decreased blood pH and associated reductions in the PaCO$_2$, which has a direct effect on CBF and hence, C oxy (Ogoh, S. et al., 2005). During prolonged fixed intensity exercise at 30% peak PO, all measures of HbO$_2$, HHb and Hb$_{Tot}$ (measured through NIRS) have been shown to increase from baseline, and are exacerbated, yet remain stable at 60% peak PO (Ide, K., Horn, A., & Secher, N.H., 1999). These findings were accompanied by an increase in C oxy, yet a decrease for arterial-to-internal jugular venous oxygen demand and glucose uptake at 30%, and increase at 60% peak power, respectively. This, along with the changes in arterial-venous lactate concentration, support the notion that during exercise, CBF increases greater than demand, and that lactate metabolism is responsible for some energy supply, thereby reducing carbohydrate uptake (Ide, K., Horn, A., & Secher, N.H., 1999).

Similar findings have been reported in terms of CBF using the Kety-Schmidt technique at 50% VO$_{2\text{max}}$, where gCBF increased in the first 15 min, but stabilised for the remainder of the protocol in thermoneutral conditions (Nybo, L., Moller, K., et al., 2002; Rasmussen, P. et al., 2010). However, contrary to thermoneutral conditions, gCBF is attenuated in hyperthermia (Nybo, L., Moller, K., et al., 2002; Rasmussen, P. et al., 2010), again due to a decreased PaCO$_2$, owing either to hyperventilation or decreased cardiac output during heat stress (Nybo, L & Nielsen, B, 2001). This was also elegantly shown during self-paced exercise in a study by Periard & Racinas (Periard, J.D. & Racinais, S., 2015) where they revealed that during a 60 min time trial in heat stress and thermoneutral conditions, the blood volume in the median cerebral artery decreased greater in heat stress compared to thermoneutral conditions after a small initial rise in both conditions in the first 10 minutes. The authors relate the exacerbated drop in heat stress to a hyperventilation-induced decline in expired CO$_2$ (Periard, J.D. & Racinais, S., 2015).

Additionally, the cerebral metabolic rate of oxygen metabolism is increased in thermoneutral exercise and exacerbated in heat stress (Rasmussen, P. et al., 2010). During thermoneutral exercise, the cerebral mitochondrial
oxygen tension is maintained during moderate intensity and decreases when intensity becomes strenuous, hence not being able to meet the oxygen demands for CMRO$_2$ due to a reduction in CBF (Rasmussen, P. et al., 2010). In heat stress, a reduction in cerebral mitochondrial oxygen tension is further exacerbated, which again limits the ability to meet CMRO$_2$ demand due to an attenuated CBF (Rasmussen, P. et al., 2010). It is therefore suggested that CBF is an important aspect in maintaining exercise tolerance and capacity, especially in heat stress (Rasmussen, P. et al., 2010), and may be downregulated as a preventative mechanism.

Only one study to date has reported on cerebral haemodynamics during self-paced exercise using NIRS (Billaut, F. et al., 2010). Results showed an increase in total [Hb] in the prefrontal cortex PFC for the first half of a 5 km TT and a plateau until the end. $C_{Oxy}$ increased and remained constant up until 4.5km, where it decreased for the final 0.5 km, owing to a decrease in [HbO$_2$], and a concomitant increase in [HHb] (Billaut, F. et al., 2010). Importantly, this study highlights the ability for $C_{Oxy}$ to be maintained during high intensity, self-paced exercise, while the common end-spurt in a self-paced performance protocol is reflected in the cerebral haemodynamics (Billaut, F. et al., 2010).

**CORTICAL ACTIVITY**

Exercise induced cerebral changes are also evident in the level of cortical activity at any given time. Cortical activity is largely influenced by concentrations of excitatory and inhibitory neurotransmitters, glutamate/acetylcholine (Glut/ACh) and gamma-aminobutyric acid-γ (GABA), respectively. Levels of excitation and inhibition in the brain are optimally maintained during resting states, however, during exercise, the amount of Glut/ACh and GABA is changed relative to the conditions, ultimately modulating cortical activity. While not a direct reflection of the excitatory and inhibitory state based on neurotransmitters, there are several non-invasive techniques used to measure cortical activity including functional MRI, positron emissions tomography (PET), transcranial magnetic stimulation (TMS), and electroencephalography (EEG). In TMS
studies, it has been documented that cortico-motor excitability is affected by both endurance and localized, repetitive muscle movement exercise due to an increased firing of central motor units from cortical areas of the brain when movement is initiated (Benwell, N.M. et al., 2005; Ross, E.Z., Middleton, N., Shave, R., George, K., & Nowicky, A., 2007; Sacco, P., Thickbroom, G.W., Byrnes, M.L., & Mastaglia, F.L., 2000). Similarly, cortical activity measured through EEG has shown relative changes in low frequency alpha (α) and theta (θ) and high frequency beta (β) and gamma (ϒ) waves during exercise (Bailey, S.P., Hall, E.E., Folger, S.E., & Miller, P.C., 2008; Nybo, L & Nielsen, B, 2001; Robertson, C.V. & Marino, F.E., 2015).

TMS-EEG theory posits that higher frequency waves (β and ϒ) are likely to be cortical in origin (Fitzgerald, P.B., Maller, J.J., Hoy, K., Farzan, F., & Daskalakis, Z.J., 2009) and provide information flow from the cortex to the muscle in an EEG-EMG direction (Mima, T., Matsuoka, T., & Hallett, M., 2001). Furthermore, long interval cortical inhibition stimuli induced through TMS to the motor cortex has shown to be regulated by GABA neurotransmission, and reduces higher frequency EEG waves (Farzan, F. et al., 2009; Fitzgerald, P.B. et al., 2009). Collectively, these findings suggest that GABA induces cortical inhibition, and is an important regulator of higher frequency brain waves. Contrary to high frequency waves, low frequency waves may be related to states of relaxation or rest (Brownsberger, J., Edwards, A., Crowther, R., & Cottrell, D., 2013) and are thought to stem from sub-cortical areas in the brain, stimulated by sensory information and possessing a non-directional flow (Mima, T., Matsuoka, T., & Hallett, M., 2001; Mima, T., Steger, J., Schulman, A.E., Gerloff, C., & Hallett, M., 2000). Unfortunately, the exact physiological underpinnings in EEG waves are not well understood and this makes interpretation of EEG changes in response to exercise difficult. Furthermore, methodology related to EEG data collection and analyses is not standardised and hence the complex nature of numerous cortical sites and frequencies of brain waves render the comparison between studies problematic.
While EEG provides a non-invasive measure of cortical activity, signals that are measured come from cerebral sources, or populations of neurons surrounding the nearest electrode, but at times can be from artefact such as movement, eye-blinking, muscular contraction, respiration, electrocardiographic activity, among others (Thompson, T., Steffert, T., Ros, T., Leach, J., & Gruzelier, J., 2008). For this reason, decontamination of the EEG signals is required and ensuring there is enough good data epochs (or seconds) available for analysis is necessary (Thompson, T. et al., 2008). Several methods of processing EEG data have been used and largely depend on the number of electrodes and robustness of the experimental design. The majority of literature using EEG in conjunction with exercise tasks relies primarily on basic digital filtering to isolate specific frequencies within alpha, beta and sometimes even theta band waves (Thompson, T. et al., 2008), followed by fast Fourier transformation into power spectral densities in individual frequency bands (Bailey, S.P., Hall, E.E., Folger, S.E., & Miller, P.C., 2008; Nybo, L & Nielsen, B, 2001; Robertson, C.V. & Marino, F.E., 2015). Recently, more sophisticated software has become available for performing the decontamination, checking the quality of EEG data and transforming the data in PSD spectra using ‘in house’ proprietary algorithms which make the systems efficient and understandable for amateur EEG users (Johnson, R.R. et al., 2011).

Studies that actually measure EEG activity during exercise are typically either incremental (Bailey, S.P., Hall, E.E., Folger, S.E., & Miller, P.C., 2008; Robertson, C.V. & Marino, F.E., 2015) or fixed intensity (Nielsen, B. et al., 2001; Nybo, L. & Nielsen, B., 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004) in nature and are primarily performed on a cycle ergometer, and at times, involve an exertional heat stress (Nielsen, B. et al., 2001; Nybo, L. & Nielsen, B., 2001). The sites chosen for analysis typically follow the international 10-20 system and vary from a combination of the frontal (F3, F4, F7, F8), motor (C3, C4) and parietal regions (P3, P4), excluding the midline (Bailey, S.P., Hall, E.E., Folger, S.E., & Miller, P.C., 2008); only 1 electrode at the frontal (F3) region (Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004), or 2 electrodes at the frontal (F3, F4) regions (Nielsen, B. et al., 2001); 1 cm in front of CZ, F3 and the visual
cortex (OZ), to represent the motor area, prefrontal cortex and visual cortex, respectively (Nybo, 2001 #84), or finally, at all frontal sites (F3, F4, FZ, F7, F8) at the left and right prefrontal cortices (Fp1 and Fp2, respectively), and at the motor cortex (C3, CZ and C4) (Robertson, C.V. & Marino, F.E., 2015). Further to this discrepancy, most literature does not elaborate on the reasoning behind choosing the sites of interest except to designate specific regions of the brain and the practice of averaging several sites together or using only a single electrode is dependent on the designated outcomes of the respective research question and hypotheses.

Nevertheless, incremental exercise protocols have revealed changes in EEG that are related to the intensity of exercise; however results are somewhat varied. One study reported a large increase in activity at the ventilatory threshold (Bailey, S.P., Hall, E.E., Folger, S.E., & Miller, P.C., 2008), although this has been contradicted by another study showing a decrease after the respiratory compensation ratio in the PFC, while that in the MC was maintained (Robertson, C.V. & Marino, F.E., 2015). During prolonged exercise, arousal is reflected in an increase in β activity, whereas fatigue is thought to be represented by a maintenance or reduction in β activity with a concurrent maintenance or increase in α wave activity (Brownsberger, J., Edwards, A., Crowther, R., & Cottrell, D., 2013; Gaoua, N., Grantham, J., Racinais, S., & El Massioufi, F., 2012; Nielsen, B. et al., 2001; Schneider, S. et al., 2009). Fixed intensity exercise is reported to result in decreasing β waves as an individual reaches volitional exhaustion, while α waves typically remain the same or increase slightly (Nielsen, B. et al., 2001; Nybo, L. & Nielsen, B., 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004; Robertson, C.V. & Marino, F.E., 2015). This change in α/β ratio activity has been accepted as an index of fatigue in exercise literature measuring EEG to date (Nybo, L. & Nielsen, B., 2001).

Cortical activity has also been implicated in mood and exercise affect. Studies of EEG activity show that exercising at either preferred velocities or high velocities is most beneficial for mood (Schneider, S. et al., 2009). Furthermore, evidence suggests a predictive effect of EEG from left frontal activity on affect responses of tiredness and calmness during recovery from
exercise (Hall, E.E., Ekkekakis, P., & Petruzzello, S.J., 2007). Passive whole body hyperthermia under light sedation has been shown to alter cortical activity (Dubois, M. et al., 1980), while heat stress has also been shown to decrease cognitive function even when \( T_c \) does not change significantly (Gaoua, N., Grantham, J., Racinais, S., & El Massioui, F., 2012). This highlights the integration of thermoregulatory and neural areas, which are stimulated even during heat stress alone.

**CORTICAL ACTIVITY DURING HEAT STRESS**

When thermal load is combined with exercise, changes in the EEG are exacerbated and apparently correlate with RPE and core temperature during fixed intensity exercise (Nielsen, B. et al., 2001; Nybo, L & Nielsen, B, 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004). Nielsen *et al.* (2001) report a ~150% increase in \( \alpha \) wave activity in heated (42°C) compared to only ~50% increase from baseline in the control condition (18°C). \( \beta \) activity increased initially in both heat stress (~100%) and control (~150%), but decreased back to baseline levels by the end of exercise, while in the control condition it was remained at ~50% greater than baseline. When they compared these changes from 2 min into exercise rather than baseline, they showed only ~25% increase in \( \alpha \) in both conditions, which was not significant, while \( \beta \) activity was mostly maintained in the control condition and decreased significantly from baseline in the hot condition (Nielsen, B. et al., 2001). These changes resulted in a significant increase in \( \alpha/\beta \) ratio in the hot condition, which was highly correlated to the change in oesophageal temperature (Nielsen, B. et al., 2001). These findings were supported by significant ~180-220% increase in \( \alpha/\beta \) ratio by the same group, which also resulted in a significant positive correlation with the core temperature in each of the measured sites (Nybo, L. & Nielsen, B., 2001).

**NEUROMUSCULAR DRIVE**

During exercise to volitional exhaustion at a fixed intensity, integrated (i) EMG signals increase as peripheral fatigue ensues. An increase in neural drive is observed in an effort to maintain the same force in lieu of peripheral fatigue (St Clair Gibson, A. & Noakes, T.D., 2004). In contrast, iEMG
signals tend to decrease with central fatigue, while self-paced exercise fosters the dynamic behaviour of muscle recruitment (Billaut, F. et al., 2010; Castle, P.C., Maxwell, N., Allchorn, A., Mauger, A.R., & White, D.K., 2011) based on performance and perception at any given time (St Clair Gibson, A. & Noakes, T.D., 2004). Indeed, iEMG decreased in self-paced exercise after 2 km into a 5 km running TT (Billaut, F. et al., 2010). This reduction was countered by an increase in the final 0.5 km, correlated with an end spurt and increased pace (Billaut, F. et al., 2010). It is suggested, therefore, that neuromuscular recruitment follows a similar pattern to the pacing strategy (Billaut, F. et al., 2010; Kay, D. et al., 2001; St Clair Gibson, A. & Noakes, T.D., 2004). Tucker et al. (2006) also highlighted the dynamic patterns associated with power output during a 20km time trial. They report that power does not change over time in a monotonic manner, but that it has dynamic fluctuations throughout, yet allowing an end spurt in the final 2km of the TT (Tucker, R. et al., 2006).

Lander et al., (2009) report an increase in iEMG during exercise of the same intensity when it is externally paced, compared to when it is internally paced. They also reported that PO dynamics had much more variability within the internally regulated protocol compared to the externally regulated protocol, while a time trial condition showed even greater variability than both submaximal conditions (Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009).

In heat stress, iEMG is has been shown to be reduced in anticipation of the protocol, or due to the thermal load (Tucker, R., Rauch, L., Harley, Y., & Noakes, T., 2004). Tucker et al. (2006) report that in a clamped RPE 16 protocol, iEMG decreased significantly in heat stress, but that it was only significantly different to the cool condition at 60, 90 and 100% of the trial completed. These studies largely support a pattern in the iEMG that follow that of the pacing strategy in self-paced exercise, although when it is attenuated in heat stress, however, further research is necessary to improve our present understanding of how all of the integrated systems work together and change dynamically over the duration of a self-paced exercise, and how processing of the signalling occurs and manifests as a perception.
## Table 2.1. Physiological responses to self-paced exercise

<table>
<thead>
<tr>
<th>Study Details</th>
<th>Methods</th>
<th>Conditions</th>
<th>Power Output or Performance</th>
<th>Cardiovascular</th>
<th>Thermoregulatory</th>
<th>Metabolic</th>
<th>Neuromuscular</th>
<th>Cerebral Blood Flow/Cortical Activity</th>
<th>Inflammatory/Endocrine</th>
<th>Perceptual</th>
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<tbody>
<tr>
<td><strong>Self-Paced Clamped RPE Protocol</strong></td>
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<tr>
<td>Edwards &amp; Lander (2012)</td>
<td>Clamped RPE - 20min @ RPE=15</td>
<td>Rowing (R) or Treadmill (TM)</td>
<td>Work Intensity ↑ TM</td>
<td>HR ↑ TM</td>
<td>-</td>
<td>VO2 ↔</td>
<td>La↑ ↑ TM</td>
<td>-</td>
<td>-</td>
<td>RPE ↔</td>
</tr>
<tr>
<td>Lander et al. (2009)</td>
<td>Row – Self paced 5,000metre Clamped RPE =15 or same workload</td>
<td>SubRPE or SubEXT or MAX (table only compares SubRPE to SubEXT)</td>
<td>PO ↔, except greater variability in SubRPE</td>
<td>HR ↔</td>
<td>Tc ↓ SubRPE</td>
<td>Tsk ↔</td>
<td>VO2 ↔</td>
<td>La↑ ↑ SubEXT</td>
<td>VL and BB ↑ SubEXT</td>
<td>-</td>
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<tr>
<td><strong>Self-Paced Time Trial – Ambient or Different Environmental Conditions</strong></td>
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<tr>
<td>Marino et al. (2001)</td>
<td>Run – 8km TT post 30min @70% peak TM run speed</td>
<td>H: 35°C or C: 15°C (results reflect 8km TT only)</td>
<td>Perf ↓ H</td>
<td>HR ↔</td>
<td>Te ↑ H</td>
<td>Tsk ↑ H</td>
<td>RER ↑ H</td>
<td>NH4↑ ↑ H</td>
<td>La↑ ↓ H</td>
<td>-</td>
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<tr>
<td>Study Details</td>
<td>Methods</td>
<td>Conditions</td>
<td>Power Output or Performance</td>
<td>Cardiovascular</td>
<td>Thermoregulatory</td>
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<tr>
<td>Ostrowski et al. (1998)</td>
<td>Run – Self-paced Marathon Race</td>
<td>N/A</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>IL-6 ↑</td>
<td>IL-1ra ↑</td>
</tr>
<tr>
<td>Periard et al. (2011)</td>
<td>Cycle – 40km TT</td>
<td>H: 35°C Or N: 20°C</td>
<td>PO ↓ H</td>
<td>HR ↔</td>
<td>SV ↓ H</td>
<td>Tsk ↑ H</td>
<td>Trec ↑ H</td>
<td>VO2 ↑ H</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Tatterson et al. (2000)</td>
<td>Cycle -30min TT</td>
<td>H: 32°C Or N: 23°C</td>
<td>PO ↓ H</td>
<td>HR ↑ H; peak ↔</td>
<td>Trec ↔</td>
<td>Tsk ↑ H</td>
<td>La↑ H</td>
<td>0-10min; ↓ H 20-30min</td>
<td>-</td>
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</tr>
<tr>
<td>Tucker et al. (2004)</td>
<td>Cycle – 20km TT</td>
<td>H: 35°C Or C: 15°C</td>
<td>PO ↓ H</td>
<td>HR ↔</td>
<td>Trec ↑ H at end</td>
<td>Tsk ↑ H</td>
<td>-</td>
<td>VL ↓ H</td>
<td>-</td>
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</tr>
<tr>
<td>Billaut et al. (2010)</td>
<td>Run – 5km TT</td>
<td>N/A</td>
<td>↑ 0-0.5km ↔ 0.5-4.5km ↑ 4.5-5km</td>
<td>↑ 0-1.5km ↔ 1.5-4km ↑ 4.5-5km</td>
<td>-</td>
<td>-</td>
<td>↑ 0-0.2km ↔ 2-4.5km ↑ 4.5-5.0km</td>
<td>Cox ↑ ([HbO2], [HHb], [THb]) 0-2.5km; Cox ↔ all 2.5-4.5km; Cox ↔ with deoxy ([HbO2]; ↑[HHb]; ↔[THb]) 4.5-5.0km</td>
<td>-</td>
<td>RPE ↑</td>
</tr>
<tr>
<td>Study Details</td>
<td>Methods</td>
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<td>Power Output or Performance</td>
<td>Cardiovascular</td>
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<tr>
<td>Schneider et al. (2009)</td>
<td>Run – Different intensities &amp; distances</td>
<td>Low (50-55% VO2peak); High (80-85% VO2peak); Preferred (SP)</td>
<td>Per ↑ High, SP</td>
<td>HR ↑ High; SP</td>
<td>-</td>
<td>La↑ High, SP</td>
<td>-</td>
<td>α1 ↑ Post Low; β2 ↓ Post High &amp; SP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carter et al. (2004)</td>
<td>Cycle – TT complete ~914kJ</td>
<td>Rinse 6.4% CHO or Water Every 12.5% completed</td>
<td>PO ↑ CHO</td>
<td>HR ↔</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>RPE ↔</td>
</tr>
<tr>
<td>Castle et al. (2012)</td>
<td>Cycle – 30 min TT</td>
<td>N: 22°C H: 31°C DEC: deceived Tc and ambient temp CON: no deception</td>
<td>PO ↓ CON-H</td>
<td>HR ↑</td>
<td>Tc ↔</td>
<td>Tsk ↓ H-CON and H-DEC</td>
<td>-</td>
<td>VL and RF ↔</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kay et al. (2003)</td>
<td>Cycle – 60min TT with 1 min all out sprint @10min intervals</td>
<td>N: 20°C or H: 33°C Fluid: F No Fluid: NF</td>
<td>PO ↔ all conditions</td>
<td>HR ↔ all conditions</td>
<td>Trec ↔ all conditions</td>
<td>-</td>
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</tr>
<tr>
<td>Pageaux et al. (2014)</td>
<td>Run – 5km TT</td>
<td>Mental Inhibition task (I) Control (C)</td>
<td>Perf ↓ I</td>
<td>HR ↔</td>
<td>-</td>
<td>La↑ ↔</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>RPE ↑</td>
</tr>
<tr>
<td>Mauger et al. (2010)</td>
<td>Cycle – 16.1km TT</td>
<td>ACT or PLAC</td>
<td>Perf ↑ ACT</td>
<td>HR ↑ ACT</td>
<td>-</td>
<td>La↑ ↔</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>RPE ↔ Pain ↔</td>
</tr>
<tr>
<td>Study Details</td>
<td>Methods</td>
<td>Conditions</td>
<td>Power Output or Performance</td>
<td>Cardiovascular</td>
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<tr>
<td>Robson Ansley et al. (2004)</td>
<td>Run – 10km TT</td>
<td>rhIL-6 or PLA</td>
<td>Perf ↓ rhIL-6</td>
<td>HR ↔</td>
<td>Tc ↔</td>
<td>La⁻ ↔</td>
<td>BG ↔</td>
<td>-</td>
<td>-</td>
<td>IL-6 ↑ rhIL-6</td>
</tr>
<tr>
<td>Robson Ansley et al. (2009)</td>
<td>Run – 90min TT</td>
<td>8% CHO ingestion or PLAC; + every 20min</td>
<td>Perf ↑ CHO</td>
<td>HR ↔</td>
<td>-</td>
<td>La⁻ ↔</td>
<td>BG ↑ CHO</td>
<td>-</td>
<td>-</td>
<td>IL-6 ↓ CHO</td>
</tr>
<tr>
<td>Whitham and McKinney (2007)</td>
<td>Run - 45min TT post 15min @65% VO2max</td>
<td>Rinse 6% CHO or PLAC pre and every 6min</td>
<td>PO ↔</td>
<td>HR ↔</td>
<td>-</td>
<td>VO2 ↔</td>
<td>RER ↔</td>
<td>-</td>
<td>-</td>
<td>RPE ↔</td>
</tr>
</tbody>
</table>

ACT: acetaminophen; BB: biceps brachii; EXT: external; MAP: mean arterial pressure; Perf: performance; PLA: placebo; Q: cardiac output; rhIL-6: recombinant human IL-6; sub: submaximal; SV: stroke volume; Tc: core temperature; Trec: rectal temperature; Tsk: skin temperature; TT: time trial; VL: vastus lateralis
METHODS OF STUDYING PERCEPTION AND FATIGUE

Several groups have since employed exercise protocols such as a time trial or clamped RPE protocols to highlight behaviour modifications to self-paced exercise. A time trial protocol enables applicability to field based scenarios where a clamped RPE protocol might not unless an individual races based on their perceptual feelings as opposed to strict physiological feedback – something that is becoming increasingly common in coaching practise today (Herman, L., Foster, C., Maher, M., Mikat, R., & Porcari, J., 2006; Suzuki, S., Sato, T., Maeda, A., & Takahashi, Y., 2006). While a TT, especially one performed in the lab with no physiological feedback, relies on pacing strategies based on how an individual is feeling, performance is the major outcome. In contrast, a clamped RPE protocol uses a method which places the perceptual feeling at the starting point, commanding the individual to respond to it and therefore eradicates perception from the equation, thereby highlighting the regulatory mechanisms that evolve to the perceptual phenomenon (Allport, F.H., 1955). This method is a useful tool when discerning how dynamic physiological systems integrate, inform perception and modify behaviour (Allport, F.H., 1955).

Clamped RPE protocols have only been used minimally in exercise research in which the RPE is designated as the intensity to be maintained. In this type of protocol, the individual self-selects the pace from the beginning, and it is assumed that the pace they choose indeed equates to the designated RPE (Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006). Clamped RPE protocols have primarily been used with the aim to determine how heat stress effects power output when the protocol is clamped at a designated intensity (Hartley, G.L. & Cheung, S.S., 2013), and how the physiological demands are changed between internal self-paced and external fixed intensity protocols (Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009), or between different modes of exercise (Edwards, A. & Lander, P., 2012). Furthermore, clamped RPE protocols have been employed which alter from ambient to warm temperature during cycling exercise in an attempt to isolate the dynamic
integration of thermophysiological afferents and the associated behaviour changes to maintain homeostasis (Hartley, G.L., Flouris, A.D., Plyley, M.J., & Cheung, S.S., 2012; Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011). With the main goal to maintain the designated RPE throughout the whole exercise protocol, behavioural modifications are made via regulation of power output and involve all the systems. Hence the culminating changes within systems equate to the designated RPE at any given time.

In contrast, a TT type of self-paced protocol requires individuals to perform to their optimal ability in the exercise test while employing pacing strategies as they deem fit (Jeukendrup, A., Saris, W., Brouns, F., & Kester, A.D., 1996). TT protocols are beneficial for identifying physiological responses during maximal performance, often being used pre and post an intervention to determine if the intervention had an effect (Jeukendrup, A., Saris, W., Brouns, F., & Kester, A.D., 1996). Contrary to a clamped RPE protocol, a TT highlights changes in physiological systems when an individual is aiming to perform their best. Still, it is assumed that during a TT, an individual is left with only minimal amounts of energy available in the tank by the time they reach the finish, and hence physiological changes are true reflections of the overall intensity, demands and performance decrements, if any (Tucker, R. & Noakes, T.D., 2009).

The RPE has long been used as a qualitative descriptor during exercise and is highly correlated with heart rate (Borg, G.A., 1982). Borg’s RPE scale, specifically tracks feelings within HR within 60-200, and therefore ranges from 6-20 in perceived levels (Borg, G.A., 1982). Notably, the RPE scale enables individuals to exercise based on how they feel, which are often used in training to minimize the development of overtraining or fatigue (Herman, L. et al., 2006; Impellizzeri, F.M., Rampinini, E., Coutts, A.J., Sassi, A., & Marcora, S.M., 2004). The RPE on any given day may be low prior to starting an exercise task, however, other stressors or signals can play into the perceptual basis of how an individual feels, and hence, may be reflected in the RPE reported. Exercising based on the RPE enables individuals to
modulate pace and performance as necessary based on how they are feeling at any given time.

The RPE in self-paced time trials often reflect an increase in effort, in accordance with power output in the initial stages of the protocol, followed by a plateau while power output is either maintained or reduced, and culminates with an end spurt in which both RPE and power output increase to near maximal levels (Périard, J.D. et al., 2011; Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000). At times, however, a linear increase across the whole duration of the protocol has also been reported (Billaut, F. et al., 2010; Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004; Froyd, C., Millet, G.Y., & Noakes, T.D., 2013; Mauger, A.R., Jones, A.M., & Williams, C.A., 2010), thus highlighting a disassociation between power output and RPE. It is suggested, therefore, that RPE at the beginning of an exercise task is not directly related to physiological changes in any one area, but rather from multiple sensory signals (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011; St Gibson, A. et al., 2006; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006), while the initial pace is believed to be chosen based on anticipatory regulation and perhaps external, often thermal stimuli (Billaut, F. et al., 2010; Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006).

Internal regulation of exercise pace and performance stress the dependency between physiological systems, especially in central processing of the peripheral changes that occur at any given time, and also allow individuals the chance to make a change if necessary. Hence, this opportunity enables the individual to exercise to their greatest capacity with operational decision making about modifying their behaviour if and when the RPE gets to be too great. Exercise protocols that clamp the RPE at a high intensity (≥15), further support anticipatory regulation for selecting the initial pace as there is an almost immediate decline in power output within the first few minutes (Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006). Interestingly, perceptually based protocols have been reported to be less physiologically demanding than those that are externally regulated and matched for overall
workload (Lander, P.J., Butterly, R.J., & Edwards, A.M., 2009). The physiological responses to self-paced exercise within individual systems, and their response to different intensities and environmental loads, can help to elucidate how they are able to work together and provide afferent feedback for informational processing. While much is still yet to be discovered, evidence suggests that most systems work in a dynamic non-linear fashion in response to numerous mediators, and hence integration during exercise is complicated and even more so under environmental stress (Glass, L., 2001; St Clair, G.A., Lambert, M.I., & Noakes, T.D., 2001; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006).

THE FEELING OF FATIGUE AND MOTIVATIONAL FACTORS

Although integration of sensory signals underpins fatigue in the present thesis, motivational factors should not be discounted. In fact, work completed by Marcora et al., (2009) supports the psychobiological theory in which both mental and motivational factors mediate exercise behaviour, performance and fatigue (Marcora, S.M., Staiano, W., & Manning, V., 2009). Often, it is assumed that the complex integrated systems and psychobiological theories of fatigue stand alone as they are two different entities – the former highlighting peripheral aspects that inform the brain of changes, and the later highlighting mental and psychological aspects that contribute to fatigue at any given time. It is the assumption of the present thesis, however, that these two models fit perfectly together – for no system within the body truly stands a chance on its own. In fact, it is the aim of the present thesis to explain how all of these models fit into one broader model of transient central sensitivity that may regulate acute exercise tolerance and performance as it does chronic pain and fatigue. In order to do this, we focus on a particular biomarker, IL-6 that is known to contribute afferent signals in disease and pain, and is the primary focus of the neuroinflammatory model of acute fatigue during exercise (Vargas, N.T. & Marino, F., 2014).
IL-6 was first identified as an immune mediator which, along with other cytokines, activates the acute-phase inflammatory response (Shek, P.N. & Shephard, R.J., 1998). The pro-inflammatory actions of IL-6 involve activating T-cells and promoting B-cells and other inflammatory mediators to eliminate damaged tissue or foreign material from an immune challenge (Shek, P.N. & Shephard, R.J., 1998). However, IL-6 also mediates stress and inflammation in the periphery by directly signalling the hypothalamic pituitary adrenal (HPA)-axis and inducing adrenal corticosteroids which act to attenuate the inflammatory response, hence also rendering it a molecule with anti-inflammatory properties (Chesnokova, V. & Melmed, S., 2002). IL-6 has since been identified as a regulator of blood glucose metabolism at rest (Tsigos, C. et al., 1997) and during exercise (Jeukendrup, A.E. et al., 1999; McConell, G., Fabris, S., Proietto, J., & Hargreaves, M., 1994) and in the febrile response as it is an endogenous pyrogen (Leon, L.R., 2002; Leon, L.R., White, A.A., & Kluger, M.J., 1998). With the multiple responsibilities that IL-6 has within the body, and increased plasma concentrations during exercise (Febbraio, M.A. et al., 2004; Ostrowski, K., Rohde, T., Zacho, M., Asp, S., & Pedersen, B.K., 1998; Robson-Ansley, P., Barwood, M., Eglin, C., & Ansley, L., 2009; Starkie, R., Arkinstall, M., Koukoulas, I., Hawley, J., & Febbraio, M., 2001), recent focus has shifted to the ability for IL-6 to provide afferent signalling and potentially contribute to the feelings of fatigue during exercise.

THE NEUROINFLAMMATORY MODEL OF FATIGUE

Despite significant evidence supporting the complex systems theory (St Clair Gibson, A. & Noakes, T.D., 2004), the overall integration of the individual physiological systems and their contributions to fatigue have yet to be fully elucidated. The field of immunology has, for years, highlighted the inflammatory response, cytokine release and neural regulation during disease and sickness as important contributors to fatigue (Olofsson, P.S., Rosas-Ballina, M., Levine, Y.A., & Tracey, K.J., 2012; Vitkovic, L. et al., 2000; Watkins, L.R., Maier, S.F., & Goehler, L.E., 1995). In fact, Olofsson et al. (2012) suggest the existence of an inflammatory reflex, acting as a “danger signal” via locally released cytokines and prostaglandins which
signal vagal afferent nerves and transmit information to areas within the brain stem, thalamus, hypothalamus and into higher centres. Importantly, this reflex alters the autonomic nervous system with increased sympathetic outflow and changes in heart rate variability (Olofsson, P.S., Rosas-Ballina, M., Levine, Y.A., & Tracey, K.J., 2012). The ability for inflammatory processes to signal from within the periphery adds to the evidence and robustness of the neuroinflammatory theory in disease and the role it may play within the complex integrated systems theory of fatigue in exercise.

**FATIGUE DURING DISEASE – IMPLICATIONS OF INTERLEUKIN-6**

Fatigue is classified as a symptom in sickness and disease and, combined with feelings of nausea, loss of appetite and apathy towards normal social and physical environments, is part of a cluster of behaviours termed ‘sickness behaviours’ (Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., & Kelley, K.W., 2008). Fatigue from disease and sickness can either be chronic or acute. Fatigue that is chronic in nature is stimulated by an unremitting condition, and has daily and long term consequences for sufferers (Heesen, C. et al., 2006). Conversely, acute sickness, such as the influenza virus can cause fatigue which is transient in nature and subsides when the body has eliminated the virus, or soon after, typically within 7-10 days (Eccles, R., 2005).

Immune glands such as the bone marrow, hepatocytes, thymus and spleen are specific to the innate immune response, house mast cells and macrophages, and are activated when foreign material (i.e. a virus) is identified in the body (Hubbard, J.L. & Mechan, D.J., 1997; Watkins, L.R., Maier, S.F., & Goehler, L.E., 1995, p. 357). For instance, during the influenza virus, the release of mast cells and macrophages cause the production of inflammatory cytokines such as interleukin (IL)-1, TNF-α and IL-6 to aid in systemic defence and prevent further infection (Hubbard, J.L. & Mechan, D.J., 1997). An increase in these cytokines are thought to be largely responsible for sending signals to the brain and producing all or some of the ‘sickness behaviours’ previously mentioned (Dantzer, R. et al.,
Mechanisms of cytokine induction during the influenza virus have been investigated using both in vitro and in vivo methods. It has been shown that in human monocyte, rat alveolar macrophage and murine macrophage cell cultures, the influenza virus causes the release of interferon (IFN)-α, TNF-α, IL-1 and IL-6, though the magnitude of the response is likely dependent on the microenvironment (Van Reeth, K., 2000). In a study looking at the effects of the rimantadine treatment for the influenza A virus, post intranasal inoculation of the virus in human volunteers caused a peak in IL-6 concentrations between day 2 and 3, while IL-8 peaked between days 4 and 5 (Skoner, D.P., Gentile, D.A., Patel, A., & Doyle, W.J., 1999). All values returned to near baseline levels by day 7. This study also aimed to determine the correlation between systemic symptoms (headache, chills, muscle ache, joint pain, sweats and fever) and cytokine release. A significant correlation was found between early (day 2) IL-6 concentrations and symptom scores (Skoner, D.P., Gentile, D.A., Patel, A., & Doyle, W.J., 1999). While fatigue was not directly measured, a clear link between cytokines and sickness behaviours has been documented in this study.

Research has also reported increases in circulating serum cytokines IL-6, IL-1α, TNF-α and TNF-beta (β) in some people with chronic fatigue syndrome (Borish, L. et al., 1998; Buchwald, D., Wener, M.H., Pearlman, T., & Kith, P., 1997; Chao, C.C., Gallagher, M., Phair, J., & Peterson, P.K., 1990; Gupta, S., Aggarwal, S., See, D., & Starr, A., 1997; Moss, R., Mercandetti, A., & Vojdani, A., 1999; Patarca, R., Klimas, N., Sandler, D., Garcia, M.N., & Fletcher, M.A., 1996; Robson-Ansley, P., Cockburn, E., Walshe, I., Stevenson, E., & Nimmo, M., 2010), while similar findings have been shown in several autoimmune diseases including rheumatoid arthritis, experimental allergic encephalomyelitis and multiple sclerosis (Ishihara, K. & Hirano, T., 2002; Rovaris, M. et al., 1996), all of which are associated with high levels of symptomatic fatigue. With this compelling evidence, it is hard to exclude IL-6 and other cytokines from possible mechanisms of fatigue. Although the release of cytokines, and specifically IL-6
prolonged submaximal exercise is caused by a different type of homeostatic imbalance (Gray, S.R. et al., 2009b; Robson-Ansley, P. et al., 2009), it is reasonable to believe that the mechanisms producing fatigue may be similar.

**CENTRAL SENSITISATION IN DISEASE AND EXERCISE**

When inflammatory cytokines act on the CNS system, they can play a role in changes in excitation and inhibition owing to either top-down or bottom-up mechanisms. These changes are recognised primarily as being chronic adaptations to external environmental stimuli and internal sensory stimuli, ultimately affecting the pain neuromatrix within the CNS (Nijs, J. et al., 2012). The chronic adaptations are classified as central sensitisation (CS) and are characterised by a heightened state of activity and reduced tolerance to the stimuli (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008; Nijs, J. et al., 2012). Specifically, CS involves an increase in neuronal excitation with a concomitant decrease in inhibition (Nijs, J. et al., 2012). The sensitisation can occur anywhere within the CNS in efferent and afferent spinal pathways and at lower and higher cortical areas, thus making it challenging to pinpoint exactly where it manifests (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008; Nijs, J. et al., 2012). Furthermore, CS is only implicated in chronic adaptations and not in transient fatigue as yet. Nevertheless, studying the neuromatrix of pain and fatigue has facilitated our understanding of numerous factors that contribute to the perceptual sensations, with an ultimate goal to remedy them. While CS is a well-established model for pain and fatigue in clinical populations, it is also a promising theory in terms of transient fatigue during exercise due to integration of the physiological feedback and information processing that occurs within the central regions. Furthermore, IL-6 and other inflammatory mediators such as prostaglandins and cyclo-oxygenase-2, can sensitise nerve fibres (Andratsch, M. et al., 2009; Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008) and therefore supports the notion of CS as a mechanism of acute fatigue.

**FATIGUE DURING EXERCISE – IMPLICATIONS OF METABOLIC CHANGES AND INTERLEUKIN-6**
Similar to acute illness, exercise induced fatigue is ephemeral, though it can become persistent in instances of overtraining. The overtraining-cytokine hypothesis suggests that continual release of cytokines due to stress, limited recovery time and altered sleep, causes an elevated inflammatory profile and is the culprit of chronic fatigue in athletes (Kreher, J.B. & Schwartz, J.B., 2012; Robson-Ansley, P., Blannin, A., & Gleeson, M., 2007). In individual bouts of acute exercise, fatigue is transient and stimulated by global homeostatic perturbations that occur in response to high levels of prolonged exertion (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005). This fatigue is largely dependent on the training status of the individual, workload, intensity and duration of the exercise, and can lead to volitional exhaustion.

In prolonged submaximal exercise, rapid changes in previously discussed metabolites are contested by glycolysis and the use of mitochondrial respiration and oxidative phosphorylation in order to maintain adequate supplies of ATP (Holloszy, J., 2008). Furthermore, glucose regulation during prolonged, submaximal exercise is shown to be regulated tightly by the release of IL-6 from glycogen breakdown, which stimulates hepatic glucose release (Febbraio, M.A. et al., 2004; Helge, J.W. et al., 2003). In normothermic exercise conditions, IL-6 is released in an intensity and duration dependent fashion. The total concentration of circulating IL-6 during exercise is primarily supplied by muscle glycogen break down (Febbraio, M. et al., 2003; Starkie, R. et al., 2001; Steensberg, A., van Hall, G., et al., 2001), and neuroendocrine responses (Steensberg, A., Toft, A.D., Schjerling, P., Halkjær-Kristensen, J., & Pedersen, B.K., 2001) that may in turn help to regulate the neuroinflammatory response from within the hypothalamic pituitary adrenal (HPA)-axis (Steensberg, A., Toft, A.D., et al., 2001).

Short bouts of repeated high intensity exercise increases circulating IL-6 (Leggate, M., Nowell, M.A., Jones, S.A., & Nimmo, M.A., 2010). In one study involving high intensity interval training (HIIT), participants completed 10 x 4 min intervals at 85-90% $\text{VO}_2\text{max}$ with 2 minute rest periods in between or moderate 60% $\text{VO}_2\text{max}$ cycling for the same workload.
(Leggate, M., Nowell, M.A., Jones, S.A., & Nimmo, M.A., 2010). IL-6 increased by ~ 6 pg·mL⁻¹ in the HIIT protocol, whereas in the moderate cycling protocol, it only reached ~ 3 pg·mL⁻¹ greater. IL-6 is also increased in prolonged exercise with total concentrations correlated to the intensity and overall workload (Leggate, M., Nowell, M.A., Jones, S.A., & Nimmo, M.A., 2010; Ostrowski, K., Schjerling, P., & Pedersen, K.B., 2000). Robson-Ansley et al. (2009), report a six fold increase in plasma IL-6 concentrations following intense mountain bike exercise. Similarly, in overweight sedentary populations and sedentary indigenous populations, moderate-vigorous aerobic exercise (80% maximum heart rate) has been shown to increase plasma IL-6 two and five fold, respectively (Mendham, A., Coutts, A., & Duffield, R., 2012; Mendham, A., Donges, C., Liberts, E., & Duffield, R., 2011). Moreover, other studies involving healthy, fit populations report five (Starkie, R. et al., 2001) and 9-10 fold (Ostrowski, K. et al., 1998; Starkie, R. et al., 2001) increases in plasma IL-6 during 1hr cycle and running, respectively. The multiple responsibilities that IL-6 has within the body have highlighted its potential to signal afferent pathways and contribute to fatigue during exercise too (Gray, S.R. et al., 2009b; Robson-Ansley, P. et al., 2009).

Early evidence suggested that cytokines released during prolonged submaximal exercise may have similar mechanistic manifestations of fatigue as both the metabolic response to short duration intense exercise, and the cytokine response to disease. For instance, the injection of rhIL-6 has been reported to induce a higher perception of fatigue at rest, and exacerbates fatigue during exercise (Robson-Ansley, P.J., Milander, L.d., Collins, M., & Noakes, T.D., 2004). This study reported that time to complete a 10 km run, and perceptions of fatigue, were significantly increased when participants received an injection of rhIL-6 prior to the run (Robson-Ansley, P.J., Milander, L.d., Collins, M., & Noakes, T.D., 2004). In another study of repeated endurance exercise over a 6 day mountain bike race, IL-6 levels were significantly correlated to sensations of fatigue in later part of the race (Robson-Ansley, P. et al., 2009). It is clear that IL-6 plays a seemingly undisputed role in many metabolic and inflammatory
pathways during exercise and disease which are known to be up-regulated during exertional heat stress.

**INTERLEUKIN-6 RELEASE DURING EXERTIONAL HEAT STRESS**

When exercise is performed with an additional heat stress or environmental load, the IL-6 response is exacerbated for several reasons. Exercise in the heat is known to increase intestinal wall permeability and release lipopolysaccharides (LPS, endotoxins) into the circulatory system (Zuhl, M. et al., 2014). In untrained individuals, plasma endotoxin is increased greater during a given exercise protocol than in trained individuals (Selkirk, G.A., McLellan, T.M., Wright, H.E., & Rhind, S.G., 2008). It is believed that endurance training increases the LPS-neutralising capacity through increased immunoglobulins (Ig) G and M, among other mechanisms (Selkirk, G.A., McLellan, T.M., Wright, H.E., & Rhind, S.G., 2008). Indeed, it has been shown that endurance athletes have a higher level of anti-LPS IgG at rest which is thought to be a self-protective mechanism increasing endotoxin tolerance due to repetitive small bouts of stimulus during training (Selkirk, G.A., McLellan, T.M., Wright, H.E., & Rhind, S.G., 2008). Nevertheless, the release of LPS, combined with lymphocyte toll like receptor-4 (TLR4) and cluster of differentiation-14 (CD14) receptors, increases the production of pro-inflammatory cytokines, including IL-6 and TNF-α (Rhind, S.G. et al., 2004; Zuhl, M. et al., 2012). LPS induced cytokinesis is reported to occur between 39.0-39.5˚C and can lead to systemic endotoxemia (Rhind, S.G. et al., 2004). The exacerbated concentration of IL-6 is primarily due to its role in eliminating the endotoxins (Starkie, R.L., Hargreaves, M., Rolland, J., & Febbraio, M.A., 2005; Zuhl, M. et al., 2014), although hormonal, thermal and metabolic responses are likely to contribute as well (Rhind, S.G. et al., 2004). Starkie *et al.* (2005) report a significant increase in core temperature (~ 36.5 – 39.1˚C) and a 4fold increase from ~0.5 – 4 pg·mL⁻¹ in IL-6 after 90 min of cycling at 70% VO₂max in 35°C. In contrast, ambient temperatures of 15°C only resulted in negligible changes (~ 0.5 – 1 pg·mL⁻¹). Similarly, Rhind *et al.* (2004) showed that 40 min of cycling at 65% VO₂max in hot (39°C) water immersion increased core temperature to 39.1˚C while IL-6 increased 150%
from 2.5 – 4 pg·mL\(^{-1}\), again with negligible changes in the cold water immersion (15°C) condition (Rhind, S.G. et al., 2004).

In the event that endotoxins are not removed from the circulation, exertional heat stroke, and potential death may ensue due to multi-organ failure from an increasingly complex systemic inflammatory environment (Lim, C.L. & Mackinnon, L.T., 2006). Accordingly, IL-6 has also been implicated as a thermal sensor within the muscle (Welc, S.S. et al., 2012), and in the core, specifically known to signal core body temperature changes during exercise, heat shock and illness (Lim, C.L. & Mackinnon, L.T., 2006; Rhind, S.G. et al., 2004). Indeed, it has been shown that centrally injecting IL-6 and IL-1β into conscious rats both act to increase body temperature and decrease wheel running (Harden, L.M., Plessis, I.d., Poole, S., & Laburn, H.P., 2008). Furthermore, when the two cytokines are injected together, they induce fever and anorexia (Harden, L.M., Plessis, I.d., Poole, S., & Laburn, H.P., 2008). The signalling abilities of IL-6 make it an important molecule in bi-directional brain communication and signalling from the periphery to the brain, followed by appropriate modifications for systemic or whole body preservation. Interestingly, there are common receptors for IL-6 located throughout the body, which allow similar actions on tissues regardless of the origin of cytokine response (Andratsch, M. et al., 2009; Taga, T. & Kishimoto, T., 1997).

**IMPLICATIONS OF INTESTINAL WALL PERMEABILITY DURING EXERTIONAL HEAT STRESS**

The effects of LPS leakage during exertional hyperthermia are further attenuated by vascular reactivity, namely stimulating the production of prostaglandins (PGE) (Bradford, C.D., Cotter, J.D., Thorburn, M.S., Walker, R.J., & Gerrard, D.F., 2007). PGE are known to be pyrogenic modulators and send information to the brain to alter the thermoregulatory set point when needed (Bradford, C.D. et al., 2007). PGE, like cytokines, have the ability to cross the blood brain barrier (BBB), which is also reported to be increasingly permeable during endurance exercise, especially in heated environments (Watson, P., Shirreffs, S.M., & Maughan, R.J.,
Furthermore, PGE, like IL-6, are suggested to be chemo-sensitive mediators of the vagus nerve (Goehler, L.E. et al., 2000).

PGE are rapidly produced at the site of inflammation, and are thought to regulate channels in the periphery that sensitize nociceptive pathways from the periphery to the brain (Ito, S., Okuda-Ashitaka, E., & Minami, T., 2001). PGE also increase during inflammation in the gut through breakdown of the lipid membrane that makes up the epithelial cell wall via the enzymes cyclooxygenase 1 and 2 (COX-1 and COX-2) (Ito, S., Okuda-Ashitaka, E., & Minami, T., 2001; Zuhl, M. et al., 2012). Hence, COX-1 and 2 are important mediators in thermoregulatory processes during exertional heat stress. Indeed, it has been reported that manipulating the development of PGE using COX-inhibitors, can change the inflammatory environment and alter the thermoregulation during exercise accordingly. A study of marathon runners found significant increases in PGE following completion of the race (Demers, L.M., Harrison, T.S., Halbert, D.R., & Santen, R.J., 1981), with another study also reporting increases in PGE in acute bouts of exercise in both untrained and trained individuals (Sinzinger, H. & Virgolini, I., 1988).

PGE acts as noxious stimuli and therefore have the potential to contribute to CS. Importantly, for PGE to exert its desired effects it requires the signalling from IL-6 and the specific molecules needed. For instance, circulating cytokines like IL-6 have been shown to stimulate cerebral vascular cells on the blood brain barrier (BBB) (Cao, C., Matsumura, K., Yamagata, K., & Watanabe, Y., 1996) and release PGE into brain (Engblom, D. et al., 2014). Likewise, rodent models confirm an in COX-2 mRNA in the cerebral vascular cells in the presence of systemic LPS, IL-1β, and TNF-α (Cao, C., Matsumura, K., Yamagata, K., & Watanabe, Y., 1996; Lacroix, S. & Rivest, S., 1998). Increases in IL-6 and PGE within the brain have been reported to effect nociceptive pathways (Hori, T., Oka, T., Hosoi, M., & Aou, S., 2006; Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008), potentially contributing to CS and fatigue, however the receptor molecules for IL-6 are necessary for the biochemical signalling to occur.
INTERLEUKIN-6 SIGNALLING MOLECULES

IL-6 signalling can occur at local or systemic levels (Rose-John, S., 2012). For instance, IL-6 has been shown to directly signal the HPA-axis in a febrile response using IL-6 knockout mice and cecal ligation (Leon, L.R., 2002). It is also a regulator of blood glucose homeostasis where circulating IL-6 signals the release of hepatic glucose in states of hypoglycaemia (Febbraio, M.A. et al., 2004), and is implicated in arthritis and other chronic inflammatory disease, signalling tissues and exacerbating inflammatory activity (Nowell, M.A. et al., 2003), and pain (Boettger, M.K. et al., 2010). Importantly, circulating IL-6 can signal nociceptive muscle fibres (Welc, S.S. et al., 2012; Zhang, Y., Pilon, G., Marette, A., & Baracos, V.E., 2000), sensory nerves (Alexander, G.M., Peterlin, B.L., Perreault, M.J., Grothusen, J.R., & Schwartzman, R.J., 2012; Andratsch, M. et al., 2009; Hoheisel, U., Unger, T., & Mense, S., 2005), and the circumventricular organs (Roth, J., Harre, E.M., Rummel, C., Gerstberger, R., & Hubschle, T., 2004), all effecting the CNS and perhaps CS.

IL-6 signals tissues through both classical and trans-signalling pathways (Kovacs, E., 2001). Classical signalling occurs when IL-6 binds to a membrane bound IL-6 receptor (R), which then signals through an associated membrane bound glycoprotein (gp) 130 receptor (Kallen, K.-J., 2002) (Fig 2-1a). Membrane bound IL-6R, however, is only available on a small number of, primarily, immune system tissues such as hepatocytes, monocytes and neutrophils, compared to the gp130 receptor which is located ubiquitously throughout the body (Kallen, K.-J., 2002). However, a soluble (s) receptor (R) (sIL-6R) is formed through alternative splicing (Horiuchi, S. et al., 1994; Lust, J.A. et al., 1992) or shedding from the membrane bound IL-6R (Mülberg, J. et al., 1993), which enables further signalling. Trans-signalling occurs when sIL-6R binds to IL-6, and forms a sIL-6R/IL-6 complex (Fig 2-1b). This complex then binds to a membrane bound gp130 receptor to initiate the designated response (Rose-John, S. & Neurath, M.F., 2004). Trans-signalling is known to occur in inflammatory arthritis (Emery, P. et al., 2008; Nowell, M.A. et al., 2003) and other autoimmune diseases (Ishihara, K. & Hirano, T., 2002; Mihara, M.,
Hashizume, M., Yoshida, H., Suzuki, M., & Shiina, M., 2012) and is implicated in both inflammation at the local area and in pain (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008).

Trans-signalling, however, can be inhibited by the antagonistic soluble receptor, sgp130 (Rose-John, S., 2012), which does not act on IL-6 alone (Jostock, T. et al., 2001). Inhibition of trans-signalling using sgp130 to neutralise sIL-6R/IL-6 signalling in inflammatory conditions has been shown to reduce inflammation and pain in arthritis (Boettger, M.K. et al., 2010; Emery, P. et al., 2008). Importantly, research suggests that the sIL-6R/IL-6 complex can bind to both the membrane bound gp130 receptor and sgp130, although sgp130 binds to the it with a greater affinity (Jostock, T. et al., 2001). Therefore, it is posited that only in the event that there is a disproportionate increase in sIL-6R over that of sgp130, will an overall systemic response occur, otherwise, trans-signalling is likely limited to local areas (Jostock, T. et al., 2001). Systemic signalling is often a severe response such as that which occurs during sepsis and heat stroke, and so it is likely that local, trans-signalling through the gut, muscle, and other organs with sensory nerve fibres are responsible for signalling the CNS, prompting a change in behaviour in order to prevent a septic-like response to extreme exertional heat stress (Lim, C.L. & Mackinnon, L.T., 2006).
Figure 2-1. **IL-6 receptor binding.** gp130 receptors are found universally on tissues in the body. 

- **a** depicts the binding of free circulating IL-6 to soluble IL-6 receptor (sIL-6R), to form the sIL-6R/IL-6 complex that acts on gp130 receptors on tissues. 
- **b** depicts circulating IL-6 binding to a membrane bound IL-6 receptor (IL-6R), to form the IL-6R/IL-6 complex. Both the sIL-6R/IL-6 and the IL-6R/IL-6 complexes act on gp130 receptors to signal a cascade of events in the respective cell tissue. gp = glycoprotein; IL = interleukin; s = soluble; R = receptor. Figure depicted as published (Vargas, N.T. & Marino, F., 2014).

The Exercise Response of the Soluble Interleukin-6 Receptors

Most studies report increases in receptor concentrations alongside plasma IL-6 during prolonged exercise (Gray, S.R. et al., 2009a; Gray, S.R., Robinson, M., & Nimmo, M.A., 2008). For instance, 60 min of prolonged cycling at 90% LT resulted in a 5-fold increase in IL-6, a 1.2-fold increase in sIL-6R and a 2.1-fold increase in the sIL-6R/IL-6 complex immediately after exercise (Gray, S.R. et al., 2009a). Similarly, cycling at the same intensity to volitional exhaustion resulted in a 76% increase in IL-6 and a 10% increase in both sIL-6R and sgp130 (Gray, S.R., Robinson, M., & Nimmo, M.A., 2008). Interestingly, despite high intensity interval training (HIIT) stimulating a significantly greater IL-6 response than moderate intensity cycling, the sIL-6R and sIL-6R/IL-6 complex response was not reported to differ between the two (Robson-Ansley, P., Blannin, A., & Gleeson, M., 2007). Notably, both protocols were matched for workload, which suggests the type of exercise is not the determining factor in sIL-6R release, but the overall work completed is likely to be a key factor (Robson-Ansley, P., Blannin, A., & Gleeson, M., 2007). Finally, in a study
conducted during a mountain bike event covering 468 km in 6-days, IL-6 was elevated at baseline only after the first day, but increased significantly by the end of every day (Robson-Ansley, P. et al., 2009). On the other hand, sIL-6R was not elevated at baseline after the first day, but was in each consecutive day (Robson-Ansley, P. et al., 2009). Notably, individuals reported greater levels of fatigue at baseline on days 4, 5 and 6, which was highly correlated to sIL-6R, therefore, the authors concluded that sIL-6R may be associated with perceptions of fatigue at rest (Robson-Ansley, P. et al., 2009).

In contrast, some studies have revealed no changes in receptor concentrations despite significant increases in IL-6. For instance, cycling at 90% of LT to volitional exhaustion did not stimulate an increase in sIL-6R or sgp130 in individuals with chronic fatigue syndrome or healthy controls, despite an increase in IL-6 (Robinson, M. et al., 2010). Similarly, 60 min cycling at 70% VO$_{2\text{max}}$ in thermoneutral (20°C) and cold (0°C) conditions, induced a significant increase in IL-6, while neither sIL-6R or sgp130 were altered by exercise or environment (Patterson, S., Reid, S., Gray, S., & Nimmo, M., 2008). The former study, specifically, suggests that sIL-6R, may not be related to perceptions of fatigue, as it would be expected that individuals with chronic fatigue syndrome would have had greater levels compared with healthy controls. Nevertheless, the evaluation of the literature thus far, and the inherent assumptions in terms of signalling abilities highlights the need for further evidence to support each claim.

**INTERLEUKIN-6 SIGNALLING MOLECULES IN EXERTIONAL HEAT STRESS**

Notably, sIL-6R and sgp130 responses have not been studied during exertional heat stress. It has been reported, however, that individuals suffering from clinical heat stroke (diagnosed by a core temperature >40.1°C and neurological abnormalities) have resting sIL-6R concentrations that are attenuated compared to those suffering heat stress, potentially due to the binding of sIL-6R to IL-6 (Hammami, M.M. et al., 1997), although these data were collected from migrants suffering from heat exposure, and thus there may have been variables that were not completely controlled for.
Unfortunately, the discrepancies between results in the aforementioned studies, combined with the lack of sgp130 concentration data, limit the interpretation of whether IL-6 signalling can be implicated in the down regulation of CNS function when fatigue ensues in heat stress. Nevertheless, due to the known decrements in performance in exertional heat stress (González-Alonso, J. et al., 1999; Kay, D. et al., 2001; Starkie, R.L., Hargreaves, M., Rolland, J., & Febbraio, M.A., 2005; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006), it is plausible that the exacerbated IL-6 response can be implicated in transient exercise induced fatigue and central sensitisation.

**TRANS-SIGNALLING OF AFFERENT NEURONS THROUGH GLYCOPEPTIDE 130**

The spinothalamic pathway is a sensory pathway from the periphery to the brain through which pain and temperature information ascend nerves in the spinal cord to the thalamus of the brain via nociceptive fibres (Bear, M.F., Connors, B.W., & Paradiso, M.A., 2006). Acid sensitive ion channels (ASICs) on the dendrites of some nerve fibres are responsible for responding to an accumulation of metabolites, proteases, cytokines and other substances and, at threshold, send signals to the CNS via these pathways (Chester, A.R. & Kathryn, H.G., 1997; Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008; Kennedy, D.S., McNeil, C.J., Gandevia, S.C., & Taylor, J.L., 2013; Luc Darques, J., Decherchi, P., & Jammes, Y., 1998). Similar to chemosensory pathways, hypothetically, all peripheral nerves are capable of being immunosensitive.

Immke and McCleskey (2001) found that decreased pH levels (~7.0) activate ASIC channels, while the presence of lactate attenuates the depolarization of nerve fibres by a further 70%. Similarly, the release of La during intense exercise signals nociceptors, as studies in mice show activation of both sustained current and intermittent current neurons when injected with saline of pH 6.0 (Gautam, M., Benson, C.J., & Sluka, K.A., 2010). During high intensity exercise, it is common for muscle pH to reach levels as low as 6.2 (Street, D., Bangsbo, J., & Juel, C., 2001; Yquel, R.J.,
Arsac, L.M., Thiaudiere, E., Canioni, P., & Manier, G., 2002). Thus, it is possible that the activation of ASIC channels during exercise is responsible for neural input and even feelings of fatigue.

Similar mechanisms of neural signalling are possible for IL-6 and other cytokines. An early study found the intensity of hyperalgesia (as measured by reaction time in hind paws of rats) to increase significantly within the first 1hr post injection of IL-6 and TNF-α into the hind paw (Cunha, F.Q., Poole, S., Lorenzetti, B.B., & Ferreira, S.H., 1992). More recent studies in rats have found that, while TNF-α has an acute desensitizing effect on low and high threshold mechanosensitive neurons (LTM and HTM, respectively), IL-6 may have a slight sensitizing effect on LTM, though this was only shown in 2 out of 7 LTM neurons (Hoheisel, U., Unger, T., & Mense, S., 2005).

Moreover, studies have also shown that nerve damage (Vissers, K.C., De Jongh, R.F., Hoffmann, V.L., & Meert, T.F., 2005) and some chronic inflammatory diseases, like rheumatoid arthritis (Andratsch, M. et al., 2009), are reported to have high local concentrations of IL-6, which is upregulated at peripheral nerve endings through gp130 receptors on nociceptive fibres (Andratsch, M. et al., 2009), and through either gp130 or IL-6R at the dorsal root ganglion (DRG) of the spinal tract (Vissers, K.C., De Jongh, R.F., Hoffmann, V.L., & Meert, T.F., 2005). One of the most compelling pieces of evidence has come from a study which reports that TNF-α and IL-1β enhance excitatory pathways while IL-6 suppresses inhibitory pathways at the DRG, which can lead to overall increased sensitisation in the central regions (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008). As such, it is possible that upregulation of gp130 receptors and IL-6R in exercise may produce a similar response as that which occurs during disease. Additionally, Andratsch et al. (2009) provide evidence of gp130’s role in inducing pain through selective deletion of gp130 from peripheral sensory neurons in mice. They determined that the gp130 receptor both mediates and contributes to chronic inflammatory pain through activating nociceptive nerve fibres. In this study, it was found that deleting the gp130 receptor in sensory nerves decreased heat hyperalgesia.
and pathological pain (Andratsch, M. et al., 2009). Assuming that the same response occurs in humans, it is important to identify possible mechanisms of how and where the information is processed and whether the manifestation of fatigue is similar to that of hyperalgesia.

**INTERLEUKIN-6 INFLUENCE AT OTHER CENTRAL REGIONS**

Plasma IL-6 also exerts effects on the blood brain barrier (BBB) and circumventricular organs (CVOs) in the brain, though evidence largely supports the latter of the two. The CVOs pose a more probable area of action as they do not have a BBB that prevents large molecules, like IL-6 and other cytokines from passing (Vitkovic, L. et al., 2000). An early study by Vallieres and Rivest (1997) profiled basal levels of IL-6, IL-6R and gp130 mRNA in rat brains compared with levels induced by LPS, and a pro-inflammatory mediator, IL-1β. Interestingly, prior to the injection of LPS and IL-1β, there was no IL-6 mRNA present in the rat brain. Concentrations of IL-6 mRNA increased rapidly and selectively post injection, while IL-6R and gp130 were both present at basal levels and up-regulated post injection (Vallières, L. & Rivest, S., 1997). Concentrations of IL-6R and gp130 were found to be the highest in the sensory CVOs such as the organum vasculosum of the lamina terminalis (OVLT), the area postrema (AP), the subfornical organ (SF) and the median eminence (ME) (Vallières, L. & Rivest, S., 1997). A similar study looking at cytokines IL-1β and TNF-α found an increased concentration of IL-β and TNF-α mRNA at the CVOs, but not at the BBB after low doses of systemically induced LPS (Quan, N., Stern, E.L., Whiteside, M.B., & Herkenham, M., 1999).

While the aforementioned studies suggest an ability to modulate neuronal activity through CVOs in rats, human evidence remains limited for ethical and technical reasons. Though not direct evidence, Nybo et al. (2002), determined concentrations of IL-6 in cerebral blood during prolonged cycling (2 hr total with 1hr rest in between) in normothermic and hyperthermic environments at ~50% VO$_{2\text{max}}$. As expected, there was a significant increase in plasma IL-6 after the first and second hour of exercise; however, cerebral IL-6 kinetics did not follow the same pattern.
The first hour of exercise only induced a small release of IL-6 from the brain, followed by an increase in the arterial-venous difference, indicating a larger net release of IL-6 at the end of the second hour cycling bout. While the authors conclude that the limited release of IL-6 from the brain discounts IL-6 in the periphery as a neuro-modulator and mediator of central fatigue, this statement is arguably flawed as, theoretically, IL-6 could stimulate neural activity in ways that may not cause a release of cerebral IL-6 directly. Unfortunately, the authors did not report any measures indicating whether or not the release of IL-6 in the periphery or the brain affected performance. Again, while not contributing directly to the evidence of cytokines acting at the CVOs, this study does highlight possible effects of the feedback mechanisms of plasma IL-6, and feed-forward mechanisms of cerebral IL-6 released during prolonged exercise. Further studies looking at the contribution of circulating plasma and cerebral IL-6 on fatigue during prolonged exercise would shed further light on this.

**THE NEUROINFLAMMATORY MODEL OF FATIGUE, PERCEPTION OF EFFORT AND EXERCISE REGULATION**

Collectively, the aforementioned research suggests a potential for IL-6 to contribute to fatigue through sensitising afferent fibres, or through other mechanisms of signalling the brain. Its role in inflammation, thermal regulation, and association with sickness behaviours, highlights its ability to provide bi-directional communication from the periphery to the brain, and to alter perceptions at any given moment. Considering its release in exercise, the neuroinflammatory model of acute fatigue during exercise appropriately suggests IL-6 can provide afferent signals, sensitising the CNS, and ultimately alter the perception of effort and modify the regulation of power output at any given time (Fig 2-2). Importantly, IL-6 is unlikely to be the primary source of fatigue, but rather a subset or single model that represents one piece of the large theory of complex integrated systems of fatigue (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005). It would be worth investigating whether manipulating IL-6 can affect the regulation of power
output, performance or perception of effort and hence, help to further determine the significance of IL-6 on acute exercise fatigue.

Figure 2-2. The neuroinflammatory model for acute fatigue during exercise.

Current models of fatigue outline different sensory feedback that may decrease exercise affinity, but none so far have focused on the effect that IL-6 may have on perceptions of fatigue. The above model outlines a theoretical cascade of events that may play a role in sending afferent feedback during exercise altering our perceptions of fatigue and ability to maintain desired exercise intensity. Figure depicted as published (Vargas, N.T. & Marino, F., 2014).

**MANIPULATING THE RELEASE OF INTERLEUKIN-6 DURING EXERCISE**

In an attempt to further the understanding of the implications of IL-6 release on perceptions of fatigue, manipulating the inflammatory, neuroendocrine and metabolic pathways is applicable as IL-6 is a common mediator between the systems. Despite the favourable arguments for the neuroinflammatory model of acute fatigue during exercise, there is limited evidence which directly supports or refutes the ability for IL-6 to contribute to fatigue. Furthermore, the limited research that has been conducted in the
area is a largely variable, primarily due to the theoretical aspect and numerous physiological variables that are difficult to control. Nevertheless, it is common practice to take anti-inflammatory medications to aide with inflammation and muscle soreness that occurs post exercise, however, few studies have investigated the performance aspect of taking anti-inflammatory drugs prior to exercising. Still, this is one way that the cytokine may be manipulated, which can help in determining the contribution of IL-6 to fatigue. Another, perhaps less direct way, is through attenuation of the IL-6 response through carbohydrate ingestion.

**Nonsteroidal Anti-Inflammatory Intake Prior to Exercise**

Acetaminophen, ibuprofen and refocoxib are common non-steroidal anti-inflammatory drugs (NSAIDs) that have been used as an intervention in the exercise science literature. NSAIDs work by inhibiting cyclo-oxygenase (COX) pathways, which are an integral aspect of creating prostaglandins (PGEs) (Anderson, B.J., 2008; Ito, S., Okuda-Ashitaka, E., & Minami, T., 2001; Tsuboi, I., Tanaka, H., Nakao, M., Shichijo, S., & Itoh, K., 1995). PGEs possess numerous characteristics and functions in the human body including vasodilation, inhibiting platelet aggregation, fever generation, hyperalgesia, increasing gastrointestinal smooth muscle contraction, among others (Anderson, B.J., 2008). Importantly, PGEs are also associated with the release of inflammatory cytokines, such as IL-6, which can be blocked by the inhibition of COX pathways using NSAIDs (Reents, S., 2000).

Acetaminophen is believed to work through inhibiting COX-1 and COX-2 pathways, however, it is also believed to act primarily in a central manner through interference with descending serotonergic pain pathways which possess numerous PGE receptors (Anderson, B.J., 2008). Most notably, acetaminophen does not possess strong anti-inflammatory properties unlike ibuprofen due to its relatively weak action on COX-2 pathways in comparison (Anderson, B.J., 2008; Mitchell, J.A., Akarasereenont, P., Thiemermann, C., Flower, R.J., & Vane, J.R., 1993). Conversely, Ibuprofen is a dual COX-1 and COX-2 inhibitor through substrate competition with
arachidonic acid (Mitchell, J.A. et al., 1993), while refocoxib is selective for only COX-2 pathways (Pinheiro, R. & Calixto, J., 2002).

Peripheral inflammation is associated with the release of COX-2 in the spinal cord (Ito, S., Okuda-Ashitaka, E., & Minami, T., 2001) and gut (Zuhl, M. et al., 2012), releasing PGE, and consequently sensitising receptors located on sensory neurons (Ito, S., Okuda-Ashitaka, E., & Minami, T., 2001). PGE release is inhibited through NSAIDs by blocking the COX enzymes, hence minimising the stimuli that work on sensory receptors and pain pathways (Reents, S., 2000). Additionally, because PGE are implicated in the febrile response, the inhibition of COX pathways can help to eliminate fever and maintain a thermoneutral state (Reents, S., 2000). Rather than altering the inflammatory response directly, NSAIDs primarily target PGE as a sensory mediator thereby possessing a central analgesic effect (Jurna, I. & Brune, K., 1990).

Two studies have reported on benefits of acute ingestion of NSAIDs on exercise performance. A field based study showed that acetaminophen reduces the rating of perceived exertion at the lactate threshold, hence increasing exercise tolerance (Garcin, M. et al., 2005). Similarly, Mauger et al., (2010) found that acetaminophen leads to faster 16.1km cycling time trial performance. Individuals reported no change in perceived pain or exertion, despite a significantly increased heart rate and reduced time to complete the trial (Mauger, A.R., Jones, A.M., & Williams, C.A., 2010). In contrast to literature that suggests performance benefits from taking NSAIDs, it has also been suggested that acute NSAID therapy can have adverse effects when exercising. Van Wjick et al. (2011) determined that ingesting 400 mg of Ibuprofen twice before cycling at intensities ~70% VO2max for 30 min, resulted in a significant increase in intestinal permeability as measured by levels of plasma intestinal fatty acid binding protein (I-FABP) which increased from 328 pg·mL⁻¹ at baseline to 875 pg·mL⁻¹, a peak difference in 400 pg·mL⁻¹ from control conditions. Additionally, the implications of gastrointestinal compromise that may accompany acute Ibuprofen therapy prior to exercise is likely to be
exacerbated by heat stress as the permeability of the intestinal wall is further compromised in heated environments.

In contrast to the effects of acute ingestion, Bradford et al. (2007) studied the effects of chronic intake of refocoxib (50mg/day for 6 days), a specific COX-2 inhibitor on acute pyrogenic effects of exercise and heat stress. Results suggest that chronic intake of refocoxib attenuates core temperature and heat strain at a given intensity (Bradford, C.D. et al., 2007). Specifically, the author’s had the individuals run for 45 min at ~75% VO2max, followed by 45 min cycling at the same intensity, and found that total body temperature was 0.33°C lower by the end of the cycling period in the refocoxib trial compared with placebo. In addition, cardiac strain was blunted in with refocoxib, however there was a similar increase in TNF-α and IL-10 in both conditions (Bradford, C.D. et al., 2007). The authors concluded, therefore, that blocking PGE synthesis during exercise can lead to a fever-like increase in temperature, independent of endogenous heat production and thermal stress (Bradford, C.D. et al., 2007), which could be detrimental to performance.

**CARBOHYDRATE INGESTION AND RINSING DURING EXERCISE**

Carbohydrate supplementation during prolonged endurance exercise has continually showed improvements in performance for various reasons. It was initially believed that carbohydrate (CHO) ingestion during exercise delayed fatigue because of the extra energy available through blood glucose metabolism during prolonged strenuous exercise (Coyle, E.F., 1992). In one study, participants ingested 300ml of a sweetened placebo or a 10% glucose polymer solution prior to beginning, and every 15 minutes during exercise starting at 90% VO2max and dropping in power over 60 min. Although PO was greater in the glucose trial after 40 min of exercise, continual supplementation with glucose every 15 min did not increase performance further (Anantaraman, R., Carmines, A., Gaesser, G., & Weltman, A., 1995). In contrast, another study showed, that CHO supplementation is beneficial whether it is done before, during, or in combination, when cycling for prolonged durations at 70% VO2max (Sherman, W.M., Peden,
The authors report that CHO supplementation produced a total workload that was 19-46% greater than in control conditions with an 18-48% increase in time to exhaustion (Sherman, W.M., Peden, M.C., & Wright, D.A., 1991).

In terms of self-paced exercise, most studies have used a time trial protocol to examine effects of CHO supplementation. It has been shown that ingesting larger quantities (1.3mL) of 6.4% CHO had greater performance benefits (6.3% quicker) than when only small quantities were ingested (200mL) (Below, P.R., Mora-Rodríguez, R., González-Alonso, J., & Coyle, E.F., 1995). Another study found that both 26 g/hr and 72 g/hr glucose supplementation during a pre-time trial (2 hr moderate cycling test) had a similar effect on physiological responses in terms of plasma osmolality, volume, rectal temperature, lactate, RPE and heart rate; during the 4.8 km time trial, there was an improvement in performance of the with both doses of CHO supplementation compared to a control (Murray, R., Paul, G.L., Seifert, J.G., & Eddy, D.E., 1991).

Further, self-paced time trial studies have also reported improvements with CHO supplementations, but combining CHO with further aides was often not found to be beneficial. For instance, in a 100km time trial, ingestion of CHO or CHO and triglycerides significantly improved performance compared to a sweetened placebo (Angus, D.J., Hargreaves, M., Dancey, J., & Febbraio, M.A., 2000). Similarly, a CHO and electrolyte treatment significantly increased performance in a ~1hr time trial performance compared to a placebo (Jeukendrup, A., Brouns, F., Wagenmakers, A.J.M., & Saris, W.H.M., 1997), with similar findings when supplementing with a either high or low glycaemic index CHO during a 64km cycling time trial (Earnest, C.P. et al., 2004). Evidence suggests that there is no difference in muscle glycogen utilisation when supplementing with CHO, however, there is a significant change in blood glucose levels when CHO is not supplemented (Fielding, R. et al., 1985), hence suggesting a sparing effect of glycogen metabolism in the presence of available BG.
Effects of carbohydrate supplementation also have clear benefits when exercising in heat stress at moderate (65% VO$_{2\text{max}}$) and high (73% VO$_{2\text{max}}$) intensities. A study by Carter et al. (2003), reported that when a 6.4% carbohydrate (CHO) solution was ingested during the cycling trial, time to exhaustion was significantly greater in both CHO trials irrelevant to the intensity, which was further accompanied by lower RPE in the CHO compared to the placebo trials (Carter, J., Jeukendrup, A.E., Mundel, T., & Jones, D.A., 2003). Because plasma glucose levels remained stable, and there was no difference in body temperature between the two trials, the authors concluded that further research is warranted in terms of the effect that CHO supplementation has on central responses (Carter, J., Jeukendrup, A.E., Mundel, T., & Jones, D.A., 2003).

Centrally speaking, CHO has also been shown to have performance enhancing mechanisms even when it is only rinsed in the mouth rather than ingested (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004; Pottier, A., Bouckaert, J., Gilis, W., Roels, T., & Derave, W., 2010; Sinclair, J. et al., 2014). Most studies of CHO rinsing have employed self-paced time trial types of protocols that last ~1hr. Studies have reported an increase in performance using glucose and maltodextrin solution, where participants completed the time trial on average ~1 or 2 min quicker, respectively (Chambers, E.S., Bridge, M.W., & Jones, D.A., 2009). Another study found that participants cycled on average, 7 W greater and finished the designated amount of work almost 2 min quicker than when a placebo was rinsed (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004). Likewise, in a time to completion test of a set amount of work, participants cycled on average over 2 min quicker when rinsing a CHO-electrolyte solution, compared to placebo (Pottier, A. et al., 2010). Interestingly, in this study, participants also completed 2 trials that involved ingesting the same CHO-electrolyte solution or a placebo and found that ingestion of the solution did not increase performance over ingesting the placebo. The authors conclude that the ergogenic effect may be due to the time the solution was kept in the mouth and the greater availability of sensing the solution when rinsing in central regions, however this warrants further study (Pottier, A. et al., 2010).
An interesting thing to note from most of the CHO rinse studies is that performance is enhanced regardless of an increase in lactate compared to placebo, while RPE does not tend to differ (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004; Pottier, A. et al., 2010). Furthermore, it has been suggested that the duration that a solution is kept in the mouth can have an effect, with 5s rinse not resulting in a significant difference between CHO and PLAC (although the majority of participants did perform better), while 10s did significantly improve performance (Sinclair, J. et al., 2014).

The mechanisms behind the ergogenic effect are generally thought to be due to activation of pathways of reward and motor control in the brain (Chambers, E.S., Bridge, M.W., & Jones, D.A., 2009). Notably, the ingestion of CHO during exercise is known to blunt the release of IL-6 and hence alter glucose regulation due to increased blood glucose availability. Indeed numerous studies have shown that IL-6 is attenuated in contracting skeletal muscle (Febbraio, M. et al., 2003) and circulation (Nehlsen-Cannarella, S.L. et al., 1997; Robson-Ansley, P., Barwood, M., Eglin, C., & Ansley, L., 2009; Starkie, R. et al., 2001) when glucose is supplemented throughout an exercise protocol. Despite the overwhelming evidence for benefits of supplementing with CHO during exercise, many studies confirm that, although there may be a delay when supplementing with CHO, fatigue still ensues. Hence, the integration of other physiological systems cannot be discounted for contributing to sensations of fatigue during endurance exercise.

**PROCESSING SENSORY INFORMATION IN THE BRAIN AND PERCEPTIONS OF FATIGUE**

Emotional processing begins with a stimulus input, which triggers output circuits in response (Phelps, E.A. & LeDoux, J.E., 2005). Cytokine release during exercise initiates afferent signals to the brain, or acts at the CVOs, to trigger output circuits from neuronal propagation through diffusion through the cerebral spinal fluid, neuronal projections and gap junctions (Vitkovic, L. et al., 2000). It is believed that information processing within the thalamus generates the ultimate perceptual feeling at any given time (St
Gibson, A. et al., 2006) and this feeling is the driving force for the effort put forth, thus creating the dynamic behaviour loop of perception – action – perception (Warren, W.H., 2006). The exact mechanisms of informational processing leading to cognitive perception have yet to be elucidated and perhaps never will be. However, it has been shown that both nociceptive and non-nociceptive sensory information are processed within the primary and secondary somatosensory cortices from the thalamus, in a concomitant manner (Liang, M., Mouraux, A., & Iannetti, G.D., 2011). This result suggests equal importance between both types of afferent signalling. Hence, it is likely that all afferent signals are equal, but may differ based on intensity and threshold for the designated signal within the CNS.

Appreciating the complexity of the human brain, the details of processing emotions are yet to be fully rectified. Cabanac (2006) suggests that our knowledge of the world is provided by various senses which are filtered twice during afferent processing. Firstly, he suggests that the afferent information is filtered by the narrow physical/chemical window of our senses and again through the biological and cultural programming within our brains (Cabanac, M., 2006). In terms of exercise regulation, numerous factors are can be attributed to the abundant afferent processing including sensations from muscular exertion such as heart rate and respiration, muscle temperature, and changes in metabolites or other internal influences (Cabanac, M., 2006).

In relation to the present thesis, it is likely that cytokine signals induce a cascade of other molecular and cellular activities to regulate brain function, and affect both autonomic and behavioural functions, including emotions (Vitkovic, L. et al., 2000). While details are largely beyond the scope of this paper, readers are directed to the review of cytokine signal propagation in the brain by Vitkovic et al. (2000). Still, with advancing technology, we are able to make theoretical suggestions about where and the sequence in which areas of the brain are engaged in processing information. Functional magnetic resonance imaging (fMRI), EEG and magnetoencephalography (MEG) have shown that nociceptive signals from αδ and C fibres are sent from the thalamus and processed in the primary (S1) and secondary (S2)

Changes in neuronal activity through EEG during different tasks, especially in response to cognitive demands and fatigue have been previously identified, prompting further research in fatigue and exercise. Research regarding exercise induced changes in EEG has largely focused on pinpointing changes in alpha and beta EEG waves at the onset of fatigue (Hall, E.E., Ekkekakis, P., & Petruzzello, S.J., 2007; Nielsen, B. et al., 2001; Nybo, L. & Nielsen, B., 2001). What remains elusive is whether cytokine release in the periphery can act as a stimulus through signal propagation to actually alter the alpha and beta waves recorded through EEG signals.

CONCLUSIONS

While not a single physiological system can account for modifications which might lead to reductions in performance during exercise, it is likely that the efferent and afferent signalling from all internal and external stimuli culminate in a perceptual feeling and modification of behaviour, or ceased exercise to avoid extreme danger to the system or organism as a whole. Considering the evidence that IL-6 is released during exercise and augmented in heat stress, the signalling pathways of IL-6 that lead to altered cortical inhibitory or excitatory mechanisms might highlight the contribution of circulating IL-6 to CNS function during exercise. Furthermore, understanding the implications of central sensitisation from IL-6 in exertional heat stress may help to explain heat sensitivity and extreme fatigue that is known to occur in some autoimmune conditions such as multiple sclerosis (Marino, F.E., 2009; Nelson, D.A. & McDowell, F., 1959). Hence, further research is warranted to elucidate the role of IL-6 in afferent signalling and its potential to instigate transient and chronic perceptions of fatigue and behaviour modifications in exercise and disease alike. Furthermore, as fatigue is unlikely to be caused by one system alone, as posited in the complex integrated systems theory of fatigue, studying the interactions of other physiological systems, especially neuromuscular, cardiovascular, metabolic, inflammatory and thermoregulatory, may help to
provide a greater understanding of why we feel fatigue and how the perception itself arises.

Finally, the notion of central sensitisation is a promising theory in terms of changes in the CNS that may allude to decreased tolerance to exercise as excitatory and inhibitory pathways are altered and reduce the threshold for fatigue. Although central sensitisation is largely implicated in chronic pain and fatigue, the acute sensitisation that has been shown to occur through afferent signalling shouldn’t be overlooked in terms of the manifestations of the feeling of acute, transient fatigue. Notably, the release of cytokines, especially, IL-6 has been shown to sensitise nerve fibres and hence the potential for IL-6 released during exercise could indeed alter the overall state of the brain, which may be reflected in cortical activity measured through electroencephalography. Future research should therefore attempt to identify specific neuronal fibres that are sensitive to increases in IL-6 and other cytokines present during prolonged, submaximal exercise. In addition, there is much to discover with respect to the interaction between perceptions of fatigue during prolonged exercise and altered neuronal activity, especially in motor and sensory cortices. Elucidating the link between the cytokine release and manifestations of fatigue may help improve our understanding of both exercise induced fatigue, and that resulting from disease that is accompanied by increased or chronic inflammatory states.
3. **CORTICAL ACTIVITY DURING SELF-PACED EXERCISE IN THERMONEUTRAL AND HEATED ENVIRONMENTS — EVIDENCE FOR ACUTE CENTRAL SENSITISATION**

**STUDY 1**
ABSTRACT

Changes in cortical activity measured using EEG may reflect an acute central sensitisation during self-paced exercise. This study evaluated changes in alpha (α), beta (β) and the α/β ratio during exercise across prefrontal (PFC) frontal (FC), motor (MC) and parietal (PC) cortices. Seven active males completed 4 separate 60 min cycling trials in thermoneutral (NORM) or heat stress (HOT) environments set at a RPE 16 (HIGH), RPE 12 (LOW) intensity. Power output revealed HIGH was greater than LOW in the respective HOT (p=0.002) and NORM conditions (p<0.001), while NORM HIGH was greater than HOT HIGH (p=0.01). α waves increased in the PFC (p<0.001) during HOT HIGH compared to HOT LOW exercise. FC (p<0.001), MC (p<0.001) and PC (p=0.01) also showed increases in α waves in HIGH compared to LOW. PFC, MC and PC all had an increase in β waves in HIGH compared to the respective LOW protocol (p<0.01), while FC showed a main effect for HIGH greater than LOW (p<0.001) and HOT greater than NORM (p=0.001). Across all conditions, α/β ratio decreased compared to baseline (p<0.05). PFC showed a greater change in α/β ratio in HIGH compared to LOW (p=0.01). In FC (p=0.01) and MC (p=0.04), HOT and NORM NIGH and HOT LOW all had greater changes that NORM LOW, while in PC, HOT had greater changes than NORM (p=0.001). The data demonstrates a significant reduction in power output during high intensity exercise in heated conditions, accompanied by exacerbated α and β activity. These findings do not support conventional decreased arousal owing to increased α/β ratio, but may be indicative of central sensitisation to heat stress from increased afferent sensory information and a concomitant increase in efferent drive in an effort to maintain the designated RPE.
INTRODUCTION

Central regulation of performance during sport and exercise is well acknowledged as a neuroprotective mechanism by which fatigue ensues to prevent cellular damage (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005; Marino, F.E., 2004; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006; Vargas, N.T. & Marino, F., 2014). There are two well-known theories to central regulation that are believed to offer neuroprotection including the critical limiting temperature (CLT) (Nielsen, B. et al., 1993) and selective brain cooling (SBC) (Marino, F., 2011). However, it has been suggested that neither offer conclusive evidence for reduced exercise performance, especially during hyperthermia. Therefore, it is likely that other physiological phenomena exist which helps to determine initial self-selected exercise pace and modulates the pace through excitatory or inhibitory signalling as a neuroprotective mechanism when exercise intensity or environmental stress reaches a critical point (Marino, F., 2011).

Central sensitisation is a well-known theory that refers to altered sensory processing in the brain and overall hyperactivity in the central nervous system, owing to both top down and bottom up mechanisms (Nijs, J. et al., 2012). Central sensitisation is largely implicated in pain models through an overactive pain neuromatrix, combining activity in areas of the brain not involved in acute pain (various brain stem nuclei, dorsolateral frontal cortex and the parietal associated cortex), in addition to those likely to be (insula, anterior cingulated cortex and prefrontal cortex) (Nijs, J. et al., 2012). Central activity is further exacerbated through afferent input from peripheral stimuli such as metabolites (Kennedy, D.S., McNeil, C.J., Gandevia, S.C., & Taylor, J.L., 2013), cytokines (Andratsch, M. et al., 2009) and thermal stress (Nomoto, S., Shibata, M., Iriki, M., & Riedel, W., 2004). The combination of all stimuli can lead to increased acute and chronic responsiveness, which in turn decreases tolerance and arousal, manifesting in behavioural changes and fatigue (Craig, A., Tran, Y., Wijesuriya, N., & Nguyen, H., 2012; Nijs, J. et al., 2012). Central sensitisation, therefore, is
an appealing physiological adaptation that may be missing in the place of central regulation of performance during exercise and heat stress.

Cortical activity at higher levels within the brain can provide an indication of excitability and can be measured though recording brain waves using electroencephalography (EEG). In any given recording, high frequency beta (β) waves (12.5-35 Hz) are believed to be indicative of arousal (Schneider, S. et al., 2009) and directional flow from the cortex to the muscle (Mima, T., Matsuoka, T., & Hallett, M., 2001), and are predominant during cognitively engaging tasks and exercise alike (Brownsberger, J., Edwards, A., Crowther, R., & Cottrell, D., 2013). Mid-range alpha (α) waves (7.5-12.5 Hz) are predominant in relaxation or resting states, while slow theta (θ) waves (3.5-7.5 Hz) are linked to calmness, meditation, drowsiness and sleep (Schneider, S. et al., 2009). Contrary to β and γ activity, α and theta (θ) waves are believed to represent information flow that is non-directional and can be attributed to sensory stimuli in subcortical areas in the brain (Mima, T., Matsuoka, T., & Hallett, M., 2001; Mima, T. et al., 2000).

Arousal is typically defined as an increase in β activity, whereas fatigue is thought to be represented by a maintenance or reduction in β activity with a concurrent maintenance or increase in α wave activity (at times added to θ) (Brownsberger, J., Edwards, A., Crowther, R., & Cottrell, D., 2013; Gaoua, N., Grantham, J., Racinais, S., & El Massiou, F., 2012; Nielsen, B. et al., 2001; Schneider, S. et al., 2009). During fixed intensity endurance exercise, β waves have been shown to decrease as an individual reaches volitional exhaustion, while α waves typically remain the same or increase slightly (Nielsen, B. et al., 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004; Robertson, C.V. & Marino, F.E., 2015). During exercise in the heat, changes in the EEG are exacerbated and apparently correlated with changes in the rating of perceived exertion (RPE) and core temperature (T<sub>c</sub>) (Nielsen, B. et al., 2001; Nybo, L & Nielsen, B, 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004). Even without exercise, passive whole body hyperthermia under light sedation has been shown to alter cortical activity (Dubois, M. et al., 1980), while heat stress has also been shown to decrease cognitive function even when core temperature does not change.
significantly (Gaoua, N., Grantham, J., Racinais, S., & El Massioui, F., 2012), therefore highlighting sensitivity to thermal sensations even in the absence of exertion. Similarly, it has been suggested that self-selected pace is chosen based upon thermal stimuli from skin receptors in the initial stages of exercise which suggests a physiological sensitivity to the environment prior to commencing an exercise task (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011).

Although these previous studies are indicative of cerebral perturbations to both heat stress and exercise, no study thus far has used a self-paced exercise model to explore arousal or central sensitisation through cortical activity. In fact, only one study has examined EEG changes in respect to mental fatigue and short (10 min) self-paced exercise task, and found that increased mental fatigue (self-reported) and higher β activity pre-exercise, results in reductions in total work during the self-paced task (Brownsberger, J., Edwards, A., Crowther, R., & Cottrell, D., 2013). Consequently, the present study aimed to determine whether or not there is evidence of decreased arousal in the EEG owing to decreased β and increased α wave activity during prolonged, fatiguing, self-paced exercise. Furthermore, we explored whether these changes are exacerbated in heat stress and during high intensity compared to low intensity exercise. We employed a self-paced cycling protocol in which the rating of perceived exertion (RPE) was clamped at a low (12) or high (16) intensity, according to the Borg RPE Scale (Borg, G.A., 1982). Participants were required to cycle for a 60 min without physiological feedback or knowledge of the time during the trials.

We hypothesised that there would be an increase in α activity, a concomitant decrease in β activity and an increase in the α/β ratio in high intensity exercise as PO decreased. A further hypothesis was that activity in individual EEG bandwidths over the course of the protocol would be both intensity and environmentally dependent and that changes in α and β waves would be greatest in the heat stress high intensity condition, with comparable changes between the thermoneutral high intensity and heat stress low intensity conditions, and fewest changes in the thermoneutral low intensity condition.
METHODS

ETHICAL APPROVAL

The respective Institutional Research and Human Ethics Committee approved all methods and procedures and the study conformed to standards set by the latest revision of the Declaration of Helsinki. All participants provided verbal and written consent prior to participating in the study.

SUBJECTS AND STUDY DESIGN

The sample comprised of seven healthy, recreationally active males. Their age, height, body weight and maximal oxygen consumption ($\text{VO}_{2\text{max}}$) (mean ± SD) were 24.48 ± 5.5 years, 183.1 ± 8.75 cm, 81.17 ± 12.48 kg, 44.04 ± 5.09 mL/(kg·min$^{-1}$), respectively. All participants were screened for current and previous exercise and disease history using the adult pre-screening exercise tool (APSS) (Exercise Sport and Science Australia, 2011). Exclusion criteria included any previous or current history of epilepsy, motor neuron disease, bipolar disorder, dementia, Alzheimer disease, brain damage, and tumour or other injury or medications likely to alter the neurophysiological state of the brain.

Subjects completed 5 sessions consisting of 1 x familiarisation and 4 x experimental trials performed in a randomised crossover design, separated by at least 7-10 days. All trials were completed in a climate chamber in ambient conditions for the respective trial of either thermoneutral (22°C) or hot (35°C) environments at 60% relative humidity (Rh).

FAMILIARISATION TRIAL

Subjects reported to the laboratory for a familiarisation session consisting of an explanation of requirements for the study and equipment used. This session included measures of body mass, height and a graded exercise test (GXT) to determine $\text{VO}_{2\text{max}}$ and peak power output (PO) on a cycle ergometer (Veletron DynaFit Pro, RacerMate Inc., WA, USA). All measurements pertaining to seat and handle bar positioning were recorded.
for future use to decrease within-subject variability on equipment. Following 2 min rest, participants were fitted with head gear and were instructed to sit quietly on the cycle ergometer for 1 min while resting metabolic data was recorded. In the final 15 seconds of the rest minute, participants were told they would begin the VO$_{2\text{max}}$ test in shortly and were counted them in from 5 sec. The test commenced at 100W and increased by 20W every minute with cadence maintained at 70 rpms for the whole time. The test ceased when participants voluntarily stopped or when they could no longer maintain a cadence above 70 rpm for at least 5 sec. After sufficient recovery, participants were asked to cycle on the ergometer in the heat chamber for 30 min at variable RPE ratings to ensure understanding of both how to use the cycle ergometer and how perform the desired intensity using the RPE scale.

In order to ensure familiarity with the RPE protocol, individuals were asked to first remember how they felt whilst performing the VO$_{2\text{max}}$ test and recall how their rating of perceived exertion increased as the test got harder until they felt like were at an RPE of 20, or maximal exertion. After a general discussion, the individuals were required to cycle for 10 min at RPE 12, followed by an increase for 30 sec to RPE 14-15. They then brought their intensity back down to an RPE 12 for 2.5 min. At 15 min into the 30 min cycle, they were asked to cycle at an RPE of 16 for 10 min. They were then asked to increase their intensity to meet an RPE of 19-20 for 30 sec, followed by an immediate return to RPE 16 for 2.5 min. For the final 2 min of the 30 min block, they were asked to cycle at a comfortable pace while a discussion was had about how it is expected that their power would drop throughout the test, and that was okay, so long as they felt as though they were at the required RPE.

**Exercise Protocol**

Participants arrived at the laboratory following an overnight fast (10hrs), having avoided physical activity for at least 36 hr and caffeine and alcohol at least 24 hr prior. The 4 x exercise protocols required participants to cycle for 60 min and were conducted in either thermoneutral (22°C) or heat stress
(35°C) at either RPE of 12 (moderate-somewhat hard) or 16 (hard-very hard). For 30s at the end of every 10 min, subjects were instructed to either increase their pace and/or resistance to meet an RPE of 15 (RPE_{12} protocol) or to perform an all-out maximal sprint to meet an RPE of 20 (RPE_{16} protocol).

**DATA COLLECTION**

The present data are a subset of the data presented in Study 2 (Chapter 4) as a larger overall project. This data, however, has been extracted as it represents the culminated cortical activity of all physiological responses and it is important to firstly focus only on changes in cortical activity to become familiar with the theory of central sensitisation and culminated signals that occur during the exercise task. Hence, baseline measures prior to each exercise protocol consisted of resting HR, RPE and baseline EEG (2 min eyes open sitting still on the cycle ergometer). About 2min post the 60min cycle protocol, a final post EEG measure was taken (30s, eyes open, sitting still on the cycle ergometer).

Prior to the EEG recording, participants were reminded to refrain from talking or tensing any facial or neck muscles while sitting relaxed on the cycle ergometer with eyes open. Participants were given the same instructions for all snapshots (SS) during the cycling protocol but were required to continue pedalling.

EEG data were collected at 5 min intervals over the duration of the protocol, alternating between steady state (continuous cycling at designated RPE) or sprint SS. Starting at 4:30min, a 60s steady state SS was recorded every 10min and starting at 9min, a 90s SS (30s pre, 30s sprint and 30s post) was recorded every 10 min (Fig 3-1). For each steady state SS, participants were required to continue cycling at the designated RPE intensity with 2 notifications: when the SS started and stopped. For each sprint SS, they were instructed that there would be 4 x notifications: when the SS started, when to begin sprinting, when to stop sprinting and when the SS ended. In each instance when the SS started and stopped, the investigator spoke prior
to starting and after stopping the EEG recording so as to not compromise the data with auditory stimuli.

The cycle ergometer was situated facing a blank white wall in the climate chamber in order to eliminate any visual and auditory stimulus that could compromise EEG recordings. All computers for data collection were situated outside of the heat chamber where the chief investigator monitored participants and data collection during designated time points or SS during each test. The investigator entered the heat chamber immediately pre and post each SS to give instructions, collect RPE and HR readings.

Figure 3-1 A representation of the 60 min exercise protocol.

**POWER OUTPUT**

Power output was measured continuously through the Veletron cycle ergometer (RacerMate, Seattle, WA) at a sampling rate of 2,000Hz, along with heart rate, using a Polar HR Transmitter (Kempele, Finland). Data were saved as a .csv file and averaged over the first and second 30 min of the protocol in excel (Microsoft Office, 2013). Heart rate data were also manually recorded pre and post every snapshot using the accompanying sports watch (RS300X) for accuracy.
SKIN TEMPERATURE AND CORE TEMPERATURE

$T_{sk}$ was collected as a measure of thermal strain to confirm the physiological changes in the heat stress conditions. Four Thermodata TDHC thermologgers (Brisbane, QLD) were fixed to the skin (Opsite Flexifix, Smith & Nephew, AU) at the bicep, chest, mid-thigh and mid-calf area. One recording every minute was logged during the cycling protocol. Data were exported to excel and mean $T_{sk}$ for the respective trial was calculated using the following equation: $T_{sk}=0.2(T_{sk\,\text{thigh}} + T_{sk\,\text{calf}}) + 0.3(T_{sk\,\text{bicep}} + T_{sk\,\text{chest}})$ (Ramanathan, N.L., 1964).

Core temperature data were anticipated to further inform thermal response, however, due to logistical and technical difficulties, we were unable to continue with collection of this measure. Notably, participant compliance was the greatest obstacle for core temperature collection as it meant they had to wake up between 1:00-3:00am to ingest the pill. Whilst most were happy to do so, there were numerous instances where they didn’t hear their alarm, or forgot to set one, and hence, due to the expense and less than desired compliance, we determined that collecting core temperature many not be manageable for the present study.

ELECTROENCEPHALOGRAPHY SET UP AND DATA PROCESSING

EEG data were collected at 4 sites (Fp2, FZ, CZ, PZ) according to the international 10-20 system to represent the right prefrontal cortex (PFC) and midline of the frontal (FC), motor (MC) and parietal (PC) cortices, respectively, using a 20-channel wireless EEG system (BAlertX24, San Diego, CA) fitted to each subject based on the circumference of their head at a level just above their glabella, and the measure of the distance from the glabella to the occipital protuberance and between external acoustic meatus. The impedance of all EEG sites of interest were tested and maintained below 20kΩ as directed by the manufacturer and previous literature. Raw EEG signals were collected at a sample rate of 256Hz and referenced to the mastoid processes.
Data were cleaned (removal of eye blinks and baseline corrected) and quality checked using the manufacturer’s software (BAAlertLab, San Diego, CA) in 4 sites, PFC, FC, MC, and PC. Where <80% good data were reported in a respective SS, data were manually processed to determine if at least 20 epochs of good data were available. Data were also manually checked to identify if cross talk occurred in any SS between any sites. In the event that there was cross talk, or if less than 20 epochs of good data were available for analysis, data were rejected. A Kaiser window was applied, and mean power spectral density (PSD) for α (8-12 Hz) and β (13-30 Hz) waves for each 60s snapshot was computed after automated decontamination (removal of eye blink and other muscle artefact, and baseline correction) of the signals to eliminate known artefacts using in house proprietary algorithms (BAAlertLab, San Diego, CA). PSD values were transferred into an excel spread sheet (Microsoft Office, 2007) for analysis and determination of changes in the mean α, β, and α/β ratio at each site during each 60s SS.

**Statistical Analysis**

Prior to performing parametric analysis, tests of normality and homogeneity were performed on the data. While not every time point of the datum was completely normal and homogenous, the majority were, and thus parametric tests were considered applicable. Furthermore, the parametric test applied accounted for variability within the conditions. Data for PO, HR and Tsk represent absolute values, while data for EEG represent the magnitude of change from the first 60 s SS taken at 4 min 30 s into the protocol. Steady state measurements from each 10 min time point (5, 15, 25, 35, 45, 55), temperature (HOT, NORM) and intensity (HIGH, LOW) mean effects of the dependent variables (PO, HR, Tsk, α and β wave activity and α/β index in PFC, FC, MC, PC) were estimated using a three within-subject linear mixed-model analysis.

Mixed modelling was performed using IBM PASW statistical software (SPSS v. 20, Chicago, USA), in which the subject was treated as a correlated random effect, while temperature, intensity and time were treated
as fixed effects. This was to ensure that the model took into account subjects repeating each trial. Furthermore, the covariance was unstructured as the nature of the clamped RPE protocol assumes variability within each protocol for the respective subject. We felt this model was a more suitable fit for the present data compared to a conventional RM ANOVA as would otherwise be performed. Pairwise comparisons were identified for main effects and 2-way interactions. All tests were considered significant at an alpha level of $P<0.05$. Results are presented as mean ± SEM (Cumming, G., Fidler, F., & Vaux, D.L., 2007), unless otherwise specified.

RESULTS

POWER OUTPUT, HEART RATE AND SKIN TEMPERATURE

POWER OUTPUT

There was a significant intensity x time interaction ($p<0.001$), revealing that HIGH was greater to LOW intensity in the respective HOT ($p=0.002$) and NORM ($p<0.001$) conditions, while NORM was greater than HOT in the HIGH intensity condition, revealing that HOT HIGH decreased significantly compared to NORM HIGH ($p=0.01$) (Fig 3-2A)

HEART RATE

There was only a significant main effect for temperature ($p<0.001$) and intensity ($p<0.001$), revealing that HOT was greater than the respective NORM ($p<0.001$) and HIGH was greater than LOW ($p<0.001$), respectively (Fig 3-2B).

SKIN TEMPERATURE

There was a significant temperature x intensity interaction for $T_{sk}$ ($p<0.001$) revealing that HIGH was greater than the respective LOW intensity in HOT ($p=0.01$) and NORM ($p<0.001$) and that HOT was greater than NORM in the respective HIGH ($p<0.001$) and LOW ($p<0.001$) condition (Fig 3-2C).
Figure 3-2. A. Mean ± SEM Power Output (PO) (Watts). B. Mean ± SEM Heart Rate (HR) (beats/min). C. Mean ± SEM Skin Temperature (Tsk) (°C). All figures represent percent change from baseline in HOT or NORM environments at HIGH (16) or LOW (12) intensity. *NORM HIGH greater than HOT HIGH (p<0.05); †HIGH greater than LOW in respective HOT and NORM environment; (p<0.05); &HOT greater than NORM in respective HIGH and LOW intensity (p<0.05); ^HIGH greater than LOW (p<0.05); †HOT greater than NORM.
CORTICAL ACTIVITY

Prefrontal Cortex (PFC)

There was a significant temperature by intensity interaction (p<0.001) for α activity, revealing an increase in HOT HIGHT compared to HOT LOW (p<0.001). No differences were seen between thermoneutral conditions (p=0.7) (Fig 3-3A). PFC β waves showed a significant temperature by intensity interaction (p<0.001), revealing that HIGH intensity was greater than LOW intensity for the respective HOT (p<0.001) and NORM (p=0.002) conditions (Fig 3-4A). PFC α/β waves revealed a significant main effect for intensity (p=0.01), revealing that HIGH was greater than LOW (p=0.01) (Fig 3-5A).

Frontal Cortex (FC)

For FC α waves, there was a significant temperature x intensity interaction (p<0.001), revealing HIGH intensity greater than the respective LOW intensity in HOT (p<0.001) and NORM (p=0.04) (Fig 3-3B). FC β waves revealed a main effect for temperature (p<0.001) and intensity (p<0.001) revealing HOT was greater than NORM (p=0.001) and HIGH was greater than LOW (p<0.001) (Fig 3-4B). FC α/β ratio had a significant temperature x intensity interaction (p=0.01), revealing α/β ratio in NORM LOW showed fewer changes than HOW LOW (p<0.001) and both HIGH conditions (p=0.01) (Fig 3-5B).

Motor Cortex (MC)

In MC, α waves had was a significant temperature x intensity interaction (p<0.001), revealing that HOW HIGH and NORM HIGH were greater than their respective HOW LOW (p<0.001) and NORM LOW (p=0.04) (Fig 3-3C). In MC β waves there was also a significant temperature by intensity interaction (p=0.01). Similar to α waves, HIGH intensity was greater than LOW intensity for the respective HOT (p<0.001) and NORM (p<0.001) conditions (Fig 3-4C). MC α/β ratio revealed a significant temperature by intensity interaction (p=0.04), where NORM LOW had a fewer changes in α/β ratio compared to NORM HIGH (p<0.001) and HOW LOW (p<0.001) (Fig 3-5C).
In PC, α waves showed a significant temperature by intensity interaction (p=0.01) where HIGH intensity was greater than the respective LOW intensity in HOT (p<0.001) and NORM (p=0.01) (Fig 3-3D). There was also a significant temperature by intensity interaction for PC β waves (p=0.01), revealing that HIGH intensity was greater than respective LOW intensity in HOT (p<0.001) and NORM (p=0.04) (Fig 3-4D). There was only a main effect for temperature in PC α/β ratio (p<0.001), revealing greater changes in HOT than NORM (p=0.001) (Fig 3-5D).
Chapter 3 – Cortical activity and central sensitisation during self-paced exercise

**Figure 3-3** A. Mean ± SEM ∆PFC α. B. Mean ± SEM ∆FC α. C. Mean ± SEM ∆MC α. D. Mean ± SEM ∆PC α. All figures represent percent change from baseline in HOT or NORM environments at HIGH (16) or LOW (12) intensity. *HOT HIGH greater than HOT LOW (p<0.05); †HIGH greater than respective LOW intensity for HOT and NORM (p<0.05).
Figure 3-4  A. Mean ± SEM ΔPFC β.  B. Mean ± SEM ΔFC β.  C. Mean ± SEM ΔMC β.  D. Mean ± SEM ΔPC β. All figures represent percent change from baseline in HOT or NORM environments at HIGH (16) or LOW (12) intensity. †HIGH greater than respective LOW intensity for HOT and NORM (p<0.05); ‡HOT greater than NORM (p<0.05); †HIGH greater than LOW (p<0.05).
Chapter 3 – Cortical activity and central sensitisation during self-paced exercise

Figure 3-5 A. Mean ± SEM PFC α/β. B. Mean ± SEM FC α/β. C. Mean ± SEM MC α/β. D. Mean ± SEM PC α/β. All figures represent percent change from baseline in HOT or NORM environments at HIGH (16) or LOW (12) intensity. *NORM LOW greater than all conditions (p<0.05); †HIGH greater than LOW (p<0.05); ‡HOT less than NORM.
**DISCUSSION**

The primary purpose of the present study was to evaluate changes in EEG α and β wave activity and overall arousal indicated by the α/β ratio during high and low intensity cycling in heat stress and in thermoneutral environments. The novelty of the present study is that we employed an internally regulated clamped RPE protocol to account for the participant’s level of perceived exertion and regulation of power output and performance while monitoring brain wave activity using electroencephalography (Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006). This enabled us to evaluate the effect of different exercise intensities and environments on cortical activity during self-paced exercise where individuals were able to adapt to the demands of the intensity and environment internally. Furthermore, it provided an overall indication of the cumulative effects of self-paced exercise on cortical activity within the midline of the cortex throughout the exercise task.

The main findings were that cycling with the RPE clamped at high intensity lead to an increase in β activity across all measured sites (PFC, FC, MC and PC) in the high intensity compared to the respective low intensity conditions in thermoneutral and heat stress (Fig 3-4). Additionally, in the FC and PC, the heated conditions showed greater β activity than the thermoneutral conditions (Fig 3-4). Likewise, α activity was significantly increased in high compared to low intensity in the respective environments in all sites (Fig 3-3). Finally, there was a significant decrease in α/β ratio from baseline in the PFC in which high was greater than low intensity (Fig 3-5), while PC showed greater changes in heat stress compared to thermoneutral environments (Fig 3-5). Additionally, FC and PC showed significant changes in both low and high heat stress and high thermoneutral conditions, suggesting greater α/β ratio changes in all except the low, thermoneutral condition (Fig 3.5). Collectively, the data may represent central sensitisation during self-paced exercise in which there is a greater increase in α activity and β activity in high intensities, or the longer the duration required in the exercise task. Finally, the changes in the α/β ratio may
highlight changes in central sensitisation as there were greater changes in the conditions with greater power output, effort and thermal load.

During self-paced exercise, the power profile initially starts with a high self-selected pace, followed by almost immediate reductions in PO, and maintenance of dynamic PO based on internal and external regulatory factors, culminating with an end-spurt (Tucker, R. & Noakes, T.D., 2009). Hence, the CLT theory cannot explain the immediate reductions in power output that have been recorded prior to $T_c$ reaching ~40°C (Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006). Anticipatory regulation (Marino, F.E., 2004) may play a more significant role in the initial reduction in power output, but does not account for many of the other factors that regulate self-paced exercise throughout the task itself (Marino, F., 2011; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006). Although the complex integrated systems theory of fatigue and exercise regulation (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005) is robust, it is still missing a critical aspect in the culminating activity within the brain from both central and peripheral, internal, external, and motivational factors that inform the brain and are likely to alter cortical activity and influence exercise regulation throughout the task.

Cortical activity, at any given moment, is the end product of all efferent and afferent information that flows from and to the brain, respectively, and thus changes in brain waves measured may be indicative of exercise regulation and tolerance. Afferent feedback can alter cortical activity through neural pathways and increased sensory input that are either excitatory or inhibitory. However, reduced inhibition or increased excitation, in a chronic manner, can lead to central sensitisation, or an overall change in threshold that increases the sensitising effect of any given stimuli. For example, Kawasaki et al. (2008) report that peripheral inflammatory cytokines induce central sensitisation by increasing excitatory or decreasing inhibitory synaptic transmission at the dorsal root ganglion in the CNS, respectively. These changes ultimately affect the amount of signalling that reaches the subcortical regions of the brain to be processed and can therefore alter cortical activity. While literature focuses on central sensitisation in a
chronic sense, there are likely repeated acute sensitising situations that lead to this, and hence, any positive or negative stress, including exercise may be an example of acute central sensitisation that occurs.

In fixed intensity exercise, performance is limited by energy demands, cardiac and/or thermal strain, and motivational factors that ultimately lead to volitional exhaustion (Tucker, R. et al., 2006). These changes have also been associated with an increase in the α/β activity as an individual reaches the end point of exercise (Nielsen, B. et al., 2001; Nybo, L. & Nielsen, B., 2001) and are exacerbated in heat stress (Nielsen, B. et al., 2001). Acute central sensitisation during fixed intensity exercise models may, therefore, represent a change in inhibitory and excitatory signals when the culmination of physiological and psychological information reaches the point of threshold and results in decreased power output, reflected by an inverse relationship between the α/β ratio and power output over time. In self-paced exercise, however, power output is regulated through dynamic fluctuations within all physiological systems, combined with external motivational factors alike. Hence, cortical activity would be expected to also represent these dynamic fluctuations as was found in the present study.

Data in our study revealed that power output decreases immediately after 5min in the high intensity protocols in heat stress and thermoneutral environments, where the high intensity heated conditions resulted in significantly less power output than the respective thermoneutral condition overall (Fig 3-2). Contrary to previous research that has reported an increase in the α/β ratio over the duration of a fixed intensity protocol, data from this study reveals a general increase and maintenance of both α and β activity over the duration of each protocol, however, the ratio between the two decreased and was maintained compared to baseline by 5 min in all regions of the brain (Fig 3-5). The undulating dynamic pattern of α and β activity is likely due to the culmination of all internal and external signals that modulate self-paced exercise.

Nevertheless, while all exercise conditions provoked some change in α and β activity, the ratio between the two was markedly different based on
intensity in the PFC and on environment in the PC (Fig 3-5). Furthermore, in the FC and MC, the low intensity thermoneutral condition showed significantly fewer changes in $\alpha/\beta$ compared to all other conditions. An understanding of the changes in $\alpha/\beta$ ratio may come from further insights into the specific bandwidths themselves. $\beta$ activity in all brain regions showed a significant increase in high intensity compared to the respective low intensity condition for both heat stress and thermoneutral conditions. Consequently, and as has been previously reported, (Mima, T., Matsuoka, T., & Hallett, M., 2001), the increased $\beta$ activity could indicate directional flow from cortical areas to the muscle as this would suggest increased efferent drive and align with the greater power output in high intensity conditions compared to their respective low intensity conditions (Fig 3-2A).

While $\beta$ activity may represent efferent drive, it has been suggested that $\alpha$ activity represents subcortical and cortical informational processing in a non-directional manner (Mima, T., Matsuoka, T., & Hallett, M., 2001; Mima, T. et al., 2000). Similar to $\beta$ activity, $\alpha$ activity increased in high compared to the respective low intensity in heat stress and thermoneutral conditions across all sites, except PFC, which only showed a difference in the heat stress conditions (Fig 3-3A), all which would suggest greater afferent stimuli from mechano/chemoreceptors during high intensity and heat stress overall. An acute central sensitisation may therefore reduce exercise tolerance and require a higher level of activity within the cortical regions of the brain to exercise at high intensities or under heat stress. Notably, the $\alpha/\beta$ ratio in the somatosensory area (PC) of the brain was significantly greater in heat stress compared to thermoneutral conditions. This may further highlight an area in which temperature signals are processed and can influence decision making at the PFC and subsequent modifications to power output stemming from the FC and MC.

In conclusion, we found an immediate increase in $\alpha$ and $\beta$ wave activity that is maintained throughout the protocol despite apparent decreases in power output over the duration of the protocol in the high intensity conditions. It seems plausible that the upregulation and maintenance of $\alpha$ and $\beta$ waves in
high intensity compared to the respective low intensity conditions in heat stress and thermoneutral environments reflects an acute sensitisation that occurs as a neuroprotective mechanism for maintaining overall homeostasis in the presence of a potentially dangerous stimuli, such as over exertion and heat stress. These findings do not support conventional measures of decreased arousal in exercise and heat stress, but may be indicative of modulating performance in self-paced exercise as a neuroprotective mechanism.

The changes in cortical activity in this study are a reflection of the cumulative effect of efferent and afferent signalling that culminates in a perceptual feeling and subsequent behaviour modification. The following chapter presents data collected at the same time the EEG in the present study was collected and hence, aims to provide further insight into the peripheral and central changes occurring may have influence the cortical activity and lead to the apparent central sensitisation overall.

LIMITATIONS TO THE STUDY

The above study has several limitations that need be acknowledged. First and foremost, the sample size was limited and thus likely rendered the study underpowered. A G*power analysis a priori calculation was performed prior to data collection for the study and the designated sample size was 11 with a large effect (0.4), alpha level of 0.05; beta of 0.95; 1 group within repeated measures; with 7 measurements all together. Out of 15 participants who were recruited, 7 participants completed all 5 trials (familiarisation + 4 experimental trials) and hence this was the data that was used for analysis. 2 more participants dropped out after 2 trials due to work commitments and moving. A further 3 participants were recruited but also pulled out after undergoing the first trial. Unfortunately, the timeline for completing all three studies and performing blood analysis required the study be cut short at only 7 participants. Nevertheless, the data is still an important beginning to further understanding how different environments and exercise intensities can impact cortical activity and thus warrants further research.
Another limitation to the study resides in the lack of core temperature data. We believe, however, that this data would not have provided much information as the core temperature data that was collected in chapter 5 shows that when performing self-paced cycling in heated environments at a fixed RPE of 16, core temperature did not increase above 39°C. Hence, while core temperature increased, it was clear that it remained well below levels of exertional hyperthermia as has been seen in fixed intensity protocols were individuals were not able to employ pacing strategies.

Finally, EEG measures come with inherent limitations. Movement artefact, eye blinks and other EMG activity can compromise the data, especially during a cycling exercise task and at intensities that were required in the present study, in conjunction with heat stress. The delimitation in this lies in the instructions given to the participants when snapshots of EEG data were taken, as well as in the processing of the data. Specifically, participants were reminded every time prior to an EEG snapshot that they need to maintain a position looking straight ahead and without clenching their jaw muscles or neck and upper back muscles. Likewise, they were reminded to move as little as possible during the snapshots with the exception of continuing to pedal. During processing of the data, procedures were developed from our lab to ensure quality of data through both the provided software and manually checking it after decontamination (see appendix 10).

**CHAPTER SUMMARY**

- There is an overall change in cortical activity during an internally regulated, high intensity, self-paced exercise protocol in heat and thermoneutral environments compared to the respective low intensity protocol.

- Significant increases in α activity in high intensity compared to low intensity exercise in the respective environments in all sites suggest an increase afferent signalling to subcortical areas.

- An increased β wave activity in all sites in high intensity compared to respective low intensity conditions in heated and thermoneutral
environments suggests an increase in efferent drive in an attempt to maintain the designated high RPE.

- Changes lead to a significantly greater change in $\alpha/\beta$ ratio where both high intensity and the heated low intensity conditions tended to have a greater change. This may be indicative of increased sensory and decreased efferent activity in FC, MC and PFC, while in the sensory cortex, thermal load in heat stress may have caused a greater $\alpha/\beta$ ratio than the respective thermoneutral conditions.

- The cumulative changes in EEG may be representative of all internal and external sensory afferent information and efferent information, identifying the underpinning metabolic, sensory and central contributions to the cumulative EEG recordings during the exercise task may further elucidate what is contributing to the overall state of the brain in any given region during exercise.
4. **Effects of IL-6 and other peripheral and central changes on regulation of power output during internally regulated self-paced exercise**

**Study 2**
ABSTRACT

Perception of effort is influenced by integration of central and peripheral factors that are augmented during intense exercise and exacerbated in heat stress. This study evaluated specific factors that contribute to decreased power output during varied exercise intensities and ambient temperatures. Seven active males completed 4 separate 60min cycling trials in thermoneutral (NORM) or heat stress (HOT) environments set at a rating of perceived exertion (RPE) of 16 (HIGH), or 12 (LOW). Measures of cerebral total haemoglobin concentration (HbTot), the difference in haemoglobin concentration (HbDiff) electromyography (EMG), blood glucose (BG), lactate (La), plasma interleukin (IL)-6, soluble (s) IL-6 receptor (R) and sgp130 were taken. IL-6 (p=0.002) and sIL-6R (p=0.002) increased in HIGH during the second half of the protocol, while sgp130 increased in all conditions during the protocol (p=0.01). PO decreased significantly in HOT compared to NORM in the second half of the protocol (p=0.005). HbTot (p=0.004) and EMG were attenuated in HOT HIGH (p=0.02) in the first and second half of the protocol, respectively. The data suggests that central sensitisation, signified by the attenuated HbTot and EMG activity in heat stress, in conjunction with exacerbated IL-6 and La signaling in high intensity, contributes to perception of effort and subsequent power output during an internally regulated exercise task.
INTRODUCTION

The data from study one was a subset of data from the present study in which we aimed to highlight the central changes that occurred in the EEG during each high or low intensity clamped RPE protocol in thermoneutral and heated environments. The overall purpose of this was to show culminated responses in cortical activity, which are a direct reflection of the state of internal and external signals processed at any given time during exercise. The present study, therefore, is an attempt to elucidate changes that occur in the periphery and how they may ultimately effect the cortical activity that was previously reported.

The behavioural dynamics theory posits that adaptive behaviour is characterised on at least two levels – by perception and action (Warren, W.H., 2006). When applied to an exercise paradigm, the interplay between perception and action occurs through informational processing in which stimuli from physiological or environmental factors affect changes at higher levels within the brain (St Gibson, A. et al., 2006). Perception of exercise intensity is influenced by previous experience, current demands of the given task and dynamic peripheral and central systemic changes contributing to sensory awareness and modification of exercise behaviour. Behaviour modification can be likened to changes in performance in a self-paced exercise task as perceptions of increased intensity beyond a point of comfort, leads to a reduction in power output, or conservation of energy (behaviour modification) in order to complete the desired task (Warren, W.H., 2006). Perception, however, is not influenced by intensity-dependent physiological changes alone, but also through external environmental (Schlader, Z.J., Prange, H.D., Mickleborough, T.D., & Stager, J.M., 2009; Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011) and motivational factors (Marcora, S.M., Staiano, W., & Manning, V., 2009).

When self-paced exercise is clamped at a specific intensity based on a rating of perceived exertion (RPE), dynamic physiological systems become targeted to maintain the level of intensity as power output is modified,
depending on the information received from within these control systems (St Gibson, A. et al., 2006). The oscillatory changes in physiological systems are well regulated and integrated in an effort to maintain homeostasis (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005; St Gibson, A. et al., 2006). Thus, the level of oscillation at any given time depends on chemical, mechanical and thermal stimuli due to the level of exercise intensity, concurrent demands of the external environment and individual participant variability (Havenith, G., Coenen, L.J.M., Kistemaker, L., & Kenney, L.W., 1998). Cardiovascular, metabolic, inflammatory, and cerebral alterations are likely to respond on a spectrum according to internal and external demands in which lower intensity induces smaller changes, while the magnitude of change is exacerbated with increasing intensity, and even more so with increased heat stress.

Heart rate and associated cardiac output responds to exercise intensity in a linear fashion with increasing demands of effort (Nadel, E.R., Cafarelli, E., Roberts, M.F., & Wenger, C.B., 1979) and is amplified in heat stress due to decreased plasma volume and increased blood perfusion to the skin (Nadel, E.R., Cafarelli, E., Roberts, M.F., & Wenger, C.B., 1979; Sawka, M.N., Leon, L.R., Montain, S.J., & Sonna, L.A., 2011). Increased cardiac output therefore is required to meet metabolic demands for oxidative and glycolytic pathways fuelling energy for muscular work. When glycogen stores are depleted, blood glucose is up-regulated to provide extra energy that would otherwise not be available. In effect, these responses are all tightly regulated by enzymes and humoral mediators, one important one being the biomarker, interleukin (IL)-6 (Febbraio, M. et al., 2003; Shephard, R.J., 2002). To further the complexity of the systems, IL-6 is also implicated in temperature regulation (Kozak, W. et al., 1998; Leon, L.R., White, A.A., & Kluger, M.J., 1998), as an inflammatory mediator (Heinrich, P.C. et al., 2003), and is believed to be a source that causes fatigue and overall sickness behaviours (Maier, S.F. & Watkins, L.R., 1998; Robson-Ansley, P.J., Milander, L.d., Collins, M., & Noakes, T.D., 2004). In these instances, IL-6 can stimulate nociceptive fibres in the periphery, which provide sensory information to central regions and can therefore alter the neuronal excitability within the central nervous system (Andratsch, M. et al., 2009;
Richter, F. et al., 2010). IL-6, however, requires its soluble (s) receptor (R), sIL-6R, to signal systemically, but is also regulated by an antagonist soluble receptor, s-glycoprotein (gp) 130 (Kovacs, E., 2001). Both receptors have been shown to increase when blood plasma concentrations of IL-6 are increased, thus providing regulation of IL-6 bioactivity at any given time.

All of the physiological and sensory signals that reach the brain from the periphery and external environment culminate in efferent drive that is increased, maintained, or reduced at any given time during a self-paced exercise task. Indeed, it has been shown that increased metabolic by-products such as prostaglandins increase activity in high frequency spectra of a brain electrocorticogram (Wallenstein, M.C., 1985). Furthermore, cortical activity is altered during prolonged exercise, and exacerbated in heat stress, with an increase in alpha wave and concomitant decrease in beta wave activity, suggesting diminished arousal (Nybo, L. & Nielsen, B., 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004). Likewise, cerebral oxygenation is believed to be indicative of neuronal activity and is increased in self-paced endurance activity (Billaut, F. et al., 2010), although heat stress has been shown to attenuate this response (Rasmussen, P. et al., 2010). Evidently, dynamic changes within the periphery and central region occur with the integration of the systems, likely influencing perception at any given time.

Still, our understanding of the changes in each of these peripheral and central regions and their interaction with one another remains limited. While we have studied each individual system to exhaustion, no previous studies have evaluated the extent that each changes over the duration of exercise and how they may be associated with one another. Furthermore, none so far have evaluated how a designated perceptual feeling is maintained through internal regulation and integration within the physiological systems, especially during different intensities and environments. Consequently, the present study aimed to illustrate how the cardiovascular, metabolic, inflammatory and neuromuscular systems change when the perception of effort is clamped during exercise of different
intensities and environmental stress. We were particularly interested in the magnitude of change in plasma IL-6, sIL-6R and sgp130, and the associated power output. Furthermore, we aimed to determine whether other physiological responses including the change in heart rate (HR), skin temperature (Tsk), neuromuscular recruitment (EMG), Lactate and blood glucose, and cerebral measures of HbTot and HbDiff were associated with the regulation of power output as well. We employed a 60 min clamped RPE protocol at either a low intensity (RPE 12) or high intensity (RPE 16) during either thermoneutral or heat stress and required participants to cycle without explicit physiological feedback or knowledge of the time during the trials.

METHODS

Generally speaking, the methodology for this study is the same as reported in Study 1 as data were collected at the same time. However, for different variables that are not explained in the previous study, the methodology will be explained in detail here.

ETHICAL APPROVAL

The Institutional Research and Human Ethics Committee approved all methods and procedures and the study conformed to standards set by the latest revision of the Declaration of Helsinki. All participants provided verbal and written consent prior to participating in the study.

SUBJECTS AND STUDY DESIGN (AS REPORTED IN CHAPTER 3)

The sample comprised of seven healthy, recreationally active males. Their age, height, body weight and VO2max (mean ± SD) were 24.48 ± 5.5 years, 183.1 ± 8.75 cm, 81.17 ± 12.48 kg, 44.04 ± 5.09 mL/(kg·min⁻¹), respectively. All participants were screened for current and previous exercise and disease history using the Adult pre-screening exercise tool (APSS) (ESSA, 2011). Exclusion criteria included any previous or current history of epilepsy, motor neuron disease, bipolar disorder, dementia, Alzheimer disease, brain damage, and tumour or other injury or medications likely to alter the neurophysiological state of the brain. Further exclusion
criteria were any reoccurring or recent (< 3 weeks prior to participation) bout of influenza illness, recent surgical procedures, cholesterol lowering, anti-inflammatory, or any other medications known to interfere with a normal inflammatory response or; those with rheumatoid arthritis, recent and/or current periodontal disease, and any other conditions associated with an altered inflammatory state.

Subjects completed 5 sessions consisting of 1 familiarization and 4 experimental trials performed in a randomized crossover design, separated by at least 7-10 days. All trials were completed in a climate chamber to ambient conditions for the respective trial of either thermoneutral (22°C) or hot (35°C) environments at 60% relative humidity (Rh).

**Familiarisation Trial (As Reported in Chapter 3)**

Subjects reported to the laboratory for a familiarisation session consisting of an explanation of requirements for the study and equipment used. Subjects also completed baseline testing consisting of mass and height measures and a VO$_{2max}$ test to determine fitness level and peak power output (PPO) on a cycle ergometer (Veletron DynaFit Pro, RacerMate Inc., WA, USA). All measurements were recorded for future use to decrease within-subject variability on equipment. Participants completed a 5 min warm up at light-moderate intensity. Following 2 min rest, participants were fitted with head gear and were instructed to sit quietly on the cycle ergometer for 1 min while resting metabolic data was recorded. In the final 15 seconds of the rest minute, participants were told they would begin the VO$_{2max}$ test in shortly and were counted them in from 5 sec. The test commenced at 100W and increased by 20W every minute with cadence maintained at 70 rpms for the whole time. The test ceased when participants voluntarily stopped or when they could no longer maintain a cadence above 70 rpm for at least 5 sec. After sufficient recovery, they were asked to cycle on the ergometer in the heat chamber for 30 minutes at variable RPE ratings to ensure understanding of both how to use the cycle ergometer and how perform the desired intensity using the RPE scale.
In order to ensure familiarity with the RPE protocol, individuals were asked to first remember how they felt whilst performing the VO$_{2\text{max}}$ test and recall how their rating of perceived exertion increased as the test got harder until they felt like they were at an RPE of 20, or maximal exertion. After a general discussion, the individuals were required to cycle for 10 min at RPE 12, followed by an increase for 30 sec to RPE 14-15. They then brought their intensity back down to an RPE 12 for 2.5 min. At 15 min into the 30 min cycle, they were asked to cycle at an RPE of 16 for 10 min. They were then asked to increase their intensity to meet an RPE of 19-20 for 30 sec, followed by an immediate return to RPE 16 for 2.5 min. For the final 2 min of the 30 min block, they were asked to cycle at a comfortable pace while a discussion was had about how it is expected that their power would drop throughout the test, and that was okay, so long as they felt as though they were at the required RPE.

**EXERCISE PROTOCOL (AS REPORTED IN CHAPTER 3)**

Participants arrived at the laboratory following an overnight fast (10hrs), having avoided physical activity for at least 36hr and caffeine and alcohol at least 24hr prior. The 4 x exercise protocols required participants to cycle for 60min and were conducted in either thermoneutral (22°C) or heat stress (35°C) at either RPE of 12 (moderate-somewhat hard) or 16 (hard-very hard). For 30s at the end of every 10 min, subjects were instructed to either increase their pace and/or resistance to meet an RPE of 15 (RPE$_{12}$ protocol) or to perform an all-out maximal sprint to meet an RPE of 20 (RPE$_{16}$ protocol).

**DATA COLLECTION (AS REPORTED IN CHAPTER 3)**

Baseline measures for each experimental session consisted of resting HR and RPE, body mass, cerebral oxygenation via near-infrared spectroscopy (NIRS) measures (2min eyes open sitting still on the cycle ergometer) and venous blood measures for lactate (La$^-$), glucose, IL-6, sgp130 and sIL-6R. NIRS and electromyography (EMG) data were collected at 5min intervals over the duration of the protocol, alternating between steady state.
(continuous cycling at designated RPE) or sprint snapshots (SS). Starting at 4:30, a 60s steady state SS was recorded every 10min and starting at 9min, a 90s SS (30s pre, 30s sprint and 30s post) was recorded every 10min. For each steady state SS, participants were required to continue cycling at the designated RPE intensity with 2 notifications: when the SS started and stopped (Fig 1). For each sprint SS, they were instructed that there would be 4 notifications: when the SS started, when to begin sprinting, when to stop sprinting and when the SS ended.

Blood draws were repeated half way through and immediately post the 60 min cycle protocol. HR and skin temperature (Tsk) were recorded continuously throughout the exercise protocol. A post exercise NIRS (30s, eyes open and sitting still on the cycle ergometer) measure was taken immediately post the final blood draw.

![Figure 4-1 A representation of the 60 min exercise protocol.](image)

**POWER OUTPUT AND HEART RATE (AS REPORTED IN CHAPTER 3)**

Power output was measured continuously through the Veletron cycle ergometer (RacerMate, Seattle, WA) at a sampling rate of 2,000Hz, along with heart rate, using a Polar HR Transmitter (Kempele, Finland). Data
were saved as a .csv file and averaged over the first and second 30 min of the protocol in excel (Microsoft Office, 2013). Heart rate data were also manually recorded pre and post every snapshot using the accompanying sports watch (RS300X) for accuracy. Heart rate data were also manually recorded pre and post every snapshot using the accompanying sports watch (RS300X).

**RATING OF PERCEIVED EXERTION (RPE) (AS REPORTED IN CHAPTER 3)**

RPE was manually recorded pre and post every snapshot using the Borg RPE scale (Borg, G.A., 1982).

**SKIN TEMPERATURE (AS REPORTED IN CHAPTER 3)**

Four Thermodata TDHC thermologgers (Brisbane, QLD) were fixed to the skin (Opsite Flexifix, Smith & Nephew, AU) at the bicep, chest, mid-thigh and mid-calf area. One recording every minute was logged during the cycling protocol. Data were exported to excel and mean skin temperature for the respective trial was calculated using the following equation:

\[ T_{sk} = 0.2(T_{sk \text{ thigh}} + T_{sk \text{ calf}}) + 0.3(T_{sk \text{ bicep}} + T_{sk \text{ chest}}) \]

as previously reported (Ramanathan, N.L., 1964).

Core temperature data were anticipated to further inform thermal response, however, due to logistical and technical difficulties, we were unable to continue with collection of this measure. Participant compliance was the greatest obstacle for core temperature collection as it meant they had to wake up between 1:00-3:00am to ingest the pill. Whilst most were happy to do so, there were numerous instances where they didn’t hear their alarm, or forgot to set one, and hence, due to the expense and less than desired compliance, we determined that collecting core temperature may not be manageable for the present study.

**ELECTROMYOGRAPHY**

EMG signals were collected during the cycling protocol from the vastus lateralis (VL) muscle. An 8mm Ag/AgCl electrode was placed on the patella to ground the signals. The VL was prepped by shaving, abrading
and alcohol-swabbing the site thoroughly to rid of hair and dead skin cells that could cause artefact. The lead was placed on the VL approximately 10 cm above the proximal lateral border of the patella, on the belly of the muscle. Signals were sampled at 2000 Hz using pre-amplified single differential surface recording electrodes (model DE-2.1, Delsys, Boston MA) with a bar configuration of 10mm x 10mm and bandwidth of 20-450Hz connected to an amplifier with a gain of 1000V/V and a common rejection ratio of >120dB (Bagnoli-8, Delsys, Boston MA).

EMG signal processing involved importing the data file to a custom developed software program incorporating a 3rd order Butterworth bandpass filter set at 20 and 450 Hz, for low to high, respectively. Signals were then processed to isolate the start and end of each contraction based on a 200% deviation from the baseline signals. The start and end data points of each contraction were identified, and then used to selectively capture the EMG signal spanning each contraction. EMG contraction signals were stored to a 2-dimensional array and later saved to a subject, trial and SS-specific data file. Each EMG contraction signal was then processed for root mean square (rms) and mean frequency, with this final data also saved to a data file for later mean data collation.

**NEAR INFRARED SPECTROSCOPY**

CBF was measured using NIRS (Niro-200x, Hamamatsu Photonics, Hamamatsu, Japan). The optodes were placed on the left prefrontal cortex (PFC), 40 mm apart, using double side adhesive tape. Black rubber was placed over the optodes to shield them from the light. Data were obtained at a frequency of 60Hz during the cycle protocol using wavelengths of 735, 810 and 850nm to calculate the change in oxyhaemaglobin ($\Delta$HbO$_2$) and deoxyhaemoglobin ($\Delta$HHb), total haemoglobin ($\Delta$Hb$_{Tot}$ = [HbO$_2$] + [HHb]) and the difference in haemoglobin concentration ($\Delta$Hb$_{Diff}$ = [HbO$_2$] – [HHb]), as these have been shown to be reliable indicators of tissue de-oxygenation and cerebral blood flow (Billaut, F. et al., 2010). Data were imported into an excel spread sheet (Microsoft Office, 2007) and manually
time averaged for every SS to determine changes in the above measures over the duration of the exercise protocol.

**Blood Measures**

15mL blood was collected at each time point using a standard 22guage cannula (Becton Dickinson, NJ, USA). 10mL was transferred immediately to an ethylenediaminetetraacetic acid (EDTA) tube and centrifuged at 4°C, 3500 rpm\(^{-1}\). After centrifuging for 15 min, 1 mL of plasma was immediately aliquoted into 2 microfuge tubes and stored at -80°C. Blood samples were analysed in duplicate using Merck Millipore Multiplex Assay human cytokine/chemokine magnetic bead panel kit for IL-6, sgp130 and sIL-6R (HCYTOMAG-60K & HSCRMAG-32K, Magpix, Luminex, Austin TX). The other 5 mL of blood was transferred to a potassium oxalate/sodium fluoride tube (BD Vacutainer® Plus Fluoride/Oxalate) to cease metabolic break down of glucose and lactate for in house analysis (ABL825 Flex Analyser, Radiometer Medical ApS, Bronshoj, Denmark), within 4hr of collection time for La\(^{-}\) and BG levels.

**Statistical Analysis**

Prior to performing parametric analysis, tests of normality and homogeneity were performed on the data. While not every part of the datum was completely normal and homogenous, the majority were, and thus parametric tests were considered applicable. Furthermore, the parametric test applied accounted for variability within the conditions. Data represent the magnitude of change (Δ) between the first half (0-30min (EX)) and second half (30-60 min (PT)) of exercise, as normalised to the first 5 min SS, except for blood data. Time (EX, POST), temperature (HOT, NORM) and intensity (HIGH, LOW) mean effects of the dependent variables (PO, HR, EMG, \(T_s\), La\(^{-}\), BG, IL-6, sIL-6R, sgp130, Hb\(_{Tot}\), Hb\(_{Diff}\)) were estimated using a three within-subject linear mixed-model analysis.

Mixed modelling was performed with IBM PASW statistical software (SPSS v. 20, Chicago, USA), in which the subject was treated as a correlated random effect, while temperature, intensity and time were treated
as fixed effects. This was to ensure that the model took into the account subjects repeating each trial. Furthermore, the covariance was unstructured as the nature of the clamped RPE protocol assumes variability within each protocol for the respective subject. We felt this model was a more suitable fit for the present data compared to a conventional RM ANOVA as would otherwise be performed. Pairwise comparisons were identified for main effects and 2-way interactions. All tests were considered significant at an alpha level of $P<0.05$.

Results are presented as mean ± SEM (Cumming, G., Fidler, F., & Vaux, D.L., 2007), unless otherwise specified. Results for the sprint data are not included to reduce complexity of the present paper. Furthermore, each figure includes results for the mean data and a corresponding individual figure so as to highlight some of the differences in variables amongst individuals. Standardised effect sizes (ES; Cohen’s $d$) analyses were used in interpreting the magnitude of differences between conditions. An ES was designated as trivial ($d<0.20$), small ($d=0.20-0.49$), moderate ($d=0.50-0.79$) or large ($d>0.80$).

**RESULTS**

**PERFORMANCE**

The $\Delta$PO is presented in Fig 4-2A. Individual responses are presented in Figure 4-2B. There was a significant temperature x time interaction for $\Delta$PO ($p=0.005$), indicating that PO in both HOT conditions significantly decreased compared to NORM conditions in the second half of the protocol compared to the first ($p<0.001$; $d=1.3$).

Furthermore, ES revealed large decreases in $\Delta$PO in both high intensity protocols in the first half ($d=1.2-2.0$). Decreases in $\Delta$PO in the second half of the protocols revealed large reductions in both HOT conditions ($d=1.2-1.9$) and in NORM HIGH ($d=0.8$), while only trivial to moderate reductions in $\Delta$PO occurred in the first half for HOT LOW. NORM LOW only had trivial changes in $\Delta$PO across the whole protocol ($d=0.14-0.4$).
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Figure 4-2. A. Mean ± SEM ∆PO between the first half (0-30min) and the second half (0-60min) of cycling and B. Absolute Mean ± SEM PO for cycling in HOT HIGH (HS_{16}), NORM HIGH (NT_{16}), HOT LOW (HS_{12}) and NORM LOW (NT_{12}). *Significant interaction for temperature and time (p=0.005), HOT decreased compared to NORM (p<0.001).

a Large ES HOT HIGH (d>0.08).
b Large ES HOT LOW (d>0.08).
c Large ES NORM HIGH (d>0.08).

*Large ES NORM LOW (d>0.08).
PHYSIOLOGICAL MEASURES

BLOOD MEASURES

LACTATE

There was a significant intensity x time interaction (p=0.003), revealing a significant increase in lactate in the first half of the protocol for HIGH intensity compared to LOW intensity (p<0.001; $d=1.1$). However, all concentrations of La returned to near baseline levels within the second half of the protocol (Fig 4-5A&B). This was supported by a large increase in the first half across all conditions ($d=1.6-4.4$), followed by a large decrease across all conditions in the second half ($d=1.7-4.0$).

GLUCOSE

There was only a main effect for temperature, revealing an increase in glucose levels over the duration of the protocol in HOT compared to NORM conditions (p=0.02) (Fig 4-5C&D). ES revealed only trivial to moderate changes for all conditions across all time points ($d=0.1-0.7$).
Figure 4-3. Mean ± SEM ∆Lactate; B. Mean ± SEM Absolute Lactate. C. Mean ± SEM ∆Glucose; B. Mean ± SEM Absolute Glucose between the first half (0-30min) and the second half (0-60min) of cycling in HOT HIGH (HS_{16}), NORM HIGH (NT_{16}), HOT LOW (HS_{12}) and NORM LOW (NT_{12}). *Significant interaction, HIGH greater than LOW in the first half of the protocol (p<0.05); †Main effect for temperature, HOT greater than NORM (p<0.05). a Large ES HOT HIGH (d>0.08). b Large ES NORM HIGH (d>0.08). c Large ES HOT LOW (d>0.08). d Large ES NORM LOW (d>0.08).
INTERLEUKIN-6

There was a significant intensity x time interaction for ∆IL-6 (p=0.05), revealing that IL-6 increased significantly in HIGH intensity compared to LOW intensity in the second half of the protocol (p=0.002; d=1.4) (Fig 4-6A&B). Furthermore, ES revealed a large increase for HOT HIGH and HOT LOW in the first half of the protocol (d=1.1-1.7), while all except HOT LOW showed a large increase in the second half of the protocol (d=0.8-1.5).

SOLUBLE INTERLEUKIN-6 RECEPTOR

ΔsIL-6R also had a significant intensity x time interaction (p=0.01), revealing that sIL-6R increased greater in HIGH intensity compared to LOW intensity in the second half of the protocol (p=0.002; d=1.4) (Fig 4-6C&D). ES also revealed a large increase in sIL-6R for all except HOT HIGH in the first half of the protocol (d=1.3-1.5). In the second half of the protocol, HOT HIGH had a large increase (d=1.1), while HOT LOW had a large decrease (d=0.8).

SOLUBLE GLYCOPROTEIN 130

There was only a main effect for intensity for ∆sgp130, revealing that changes in HIGH were greater than LOW for the duration of the protocols (p=0.01). All conditions showed a large increase in the first half of the protocol (d=0.9-2.1), but there was a plateau across all in the second half of the protocol (d<0.07).
Figure 4-4. A. Mean ± SEM ∆Interleukin-6; B. Mean ± SEM Absolute Interleukin-6; C. Mean ± SEM ∆Soluble IL-6 receptor; D. Mean ± SEM Absolute Soluble Interleukin-6 Receptor; E. Mean ± SEM ∆Soluble Glycoprotein130; F. Mean ± SEM Absolute Soluble Glycoprotein130 between the first half (0-30min) and the second half (0-60min) of cycling (A,C,E) and over the duration of the protocol (B,D,F) in HOT HIGH (HS16), NORM HIGH (NT16), HOT LOW (HS12) and NORM LOW (NT12). *Significant interaction, HIGH increased greater than LOW in the second half of the protocol (p<0.05). †Main effect for intensity, HIGH greater than LOW (p<0.05). a Large ES HS16 (d>0.08). b Large ES NT16 (d>0.08). c Large ES HS12 (d>0.08). d Large ES NT12 (d>0.08).
**HEART RATE**

$\Delta HR$ revealed main effects for both intensity ($p<0.001$) and time ($p=0.01$) (Fig 4-3A&B). Overall $\Delta HR$ was greater in HIGH intensity compared to LOW intensity ($p<0.001$) and decreased in the second half compared to the first half ($p=0.01$). ES revealed a large increase in HR for HOT HIGH, HOT LOW and NORM LOW in the first half of the protocol ($d=1.4=3.0$), while NORM HIGH only had a small increase ($d=0.3$). In the second half of the protocol, there was a large decrease for HOT HIGH ($d=1.0$), while the rest of the protocols only had trivial to moderate effects over time ($d<0.80$).

**SKIN TEMPERATURE**

There was a significant interaction for temperature, intensity and time ($p=0.02$) with pairwise comparisons revealing that in the HO HIGH increased greater than NORM HIGH in the second half of the protocol ($p=0.01, d=1.4$), while there was a similar trend for HOT LOW to increase greater than NORM LOW, although there was only a small ES ($p=0.06, d=0.2$) (Fig 4-3C&D). ES for $T_{sk}$ revealed a large increase across all conditions in the first half ($d=2.3-4.7$) and in the second half ($d=0.8-1.1$), with the exception of NORM LOW which decreased slightly in the second half ($d=0.14$).

**ELECTROMYOGRAPHY**

There was a significant temperature by intensity interaction ($p=0.04$), revealing NORM HIGH increased greater than HOT HIGH in the second half of the protocol ($p=0.2, d=1.0$) (Fig 4-3E&F). ES for EMG revealed large increases across all conditions except NORM LOW in the first half of the protocol ($d=0.9-3.0$), followed by a further increase in the second half of the protocol in NORM HIGH only ($d=1.6$).
Figure 4.5. A. Mean ± SEM ∆Heart Rate; B. Mean ± SEM Absolute Heart Rate; C. Mean ± SEM ∆Tsk; D. Mean ± SEM Absolute Tsk; E. Mean ± SEM ∆EMG; F. Mean ± SEM Absolute EMG between the first half (0-30min) and the second half (0-60min) of cycling (A,C,E) and over the duration of the protocol (B,D,F) in HOT HIGH (HS₁₆), NORM HIGH (NT₁₆), HOT LOW (HS₁₂) and NORM LOW (NT₁₂). ** Significant interaction, HOT HIGH greater than NORM HIGH (p<0.05). *Significant interaction, NORM HIGH greater than HOT HIGH (p<0.05). †Significant main effect for intensity (p<0.05); ‡Significant main effect for time (p<0.05). Large ES HOT HIGH (d>0.08). Large ES NORM HIGH (d>0.08). Large ES HOT LOW (d>0.08). Large ES NORM LOW (d>0.08).
**TOTAL HEMOGLOBIN**

There was a significant 3-way interaction for temperature, intensity and time, revealing a greater increase in $\text{Hb}_{\text{Tot}}$ for NORM HIGH compared to HOT HIGH in the first half of the protocol, although there was only a trivial ES associated with this ($p=0.004; d=0.1$). Similar trends occurred in the respective low intensity conditions, however a large ES was associated with this ($p=0.08; d=0.8$) (Fig 3-4A&B). All conditions had a large increase in $\text{Hb}_{\text{Tot}}$ in the first half of the protocol ($d=1.0-2.4$). All conditions also saw a large decrease in the second half ($d=0.8-1.6$), except NORM LOW ($d=0.04$).

**HEMAGLOBIN DIFFERENCE**

There was only a main effect for time, revealing that the haemoglobin difference decreased over the second half of the protocol in all conditions ($p=0.001$) (Fig 3-4C&D). ES revealed a large increase in haemoglobin difference in the first half of the protocol ($d=2.3-2.7$), followed by a large decrease in only the NORM conditions the second half of the protocol ($d=1.2-1.6$).
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Figure 4-6. A. Mean ± SEM Δ[Hb Total]; B. Mean ± SEM Absolute [Hb Total]; C. Mean ± SEM Δ[Hb Difference]; B. Mean ± SEM Absolute [Hb Difference] between the first half (0-30min) and the second half (0-60min) of cycling (A&C), and over the duration of the protocol (B&D) in HOT HIGH (HS16), NORM HIGH (NT16), HOT LOW (HS12) and NORM LOW (NT12). *Significant interaction, HbTot greater in NORM HIGH compared to HOT HIGH (p<0.05). †Main effect for time, HbDiff decreased over the second half (p<0.05). ‡Large ES HOT HIGH (d>0.08). §Large ES NORM HIGH (d>0.08). ¶Large ES HOT LOW (d>0.08). ‖Large ES NORM LOW (d>0.08).


**DISCUSSION**

The purpose of this study was to evaluate the magnitude of change within numerous physiological systems which highlight the interdependent nature of efferent and afferent signalling when perception of effort is the driving force behind intensity. The findings indicate that perception of effort is influenced by both internal and external factors of which sensitivity to the heat, combined with increased metabolic and inflammatory signalling, leads to the greatest reductions in power output.

**CENTRAL AND THERMOREGULATORY RESPONSES**

There is substantial evidence that central regions play a large role in reducing performance and exercise tolerance (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005; Marino, F.E., 2004; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006) especially during high intensity exercise (Edwards, A.M. & Polman, R.C., 2013). The central changes are often thought of in terms of anticipatory regulation or evolutionary protection (Marino, F., 2011; Marino, F.E., 2004), of which acute central sensitisation may play a role. Central sensitisation is associated with altered sensory processing in the brain and hyperactivity in the central nervous system caused by internal and external sensory stimuli (Nijs, J. et al., 2012). While the notion of central sensitisation is commonly linked to pain and fatigue models due to overactive neuromatrices, there is reason to associate it with exercise, especially in the heat, where a combination of internal and external stressors can overstimulate to the brain and cause sensitivity to the environment and exercise task. Indeed, it has been shown that even during passive heat stress, cerebral haemodynamics are greatly reduced due to decreased arterial pressure of carbon dioxide ($\text{PaCO}_2$) (Nelson, M.D. et al., 2011), which provides sensory information through peripheral and central chemoreceptors (Duffin, J. & Mateika, J.H., 2013). Likewise, cortical activity is markedly reduced during passive hyperthermia, also supporting central sensitivity to the environment (Dubois, M. et al., 1980).
Cerebral blood flow measured using the Kety-Schmidt technique is reduced by ~18% during submaximal exercise in hyperthermic environments (Nybo, L., Moller, K., et al., 2002; Rasmussen, P. et al., 2010; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004). In the present study, $Hb_{\text{Tot}}$ was attenuated by ~15% in the first 30 minutes in HS$_{16}$ compared to NT$_{16}$ (Fig 4-4A&B). In fact, $Hb_{\text{Tot}}$ in the HS$_{16}$ matched that of HS$_{12}$ in the first half of the protocol despite power output being on average ~50 W greater. Reductions in $Pa_{\text{CO}_2}$ are known attenuate total haemoglobin due to reduced cardiac output or increased hyperventilation during exercise in heat stress (Nybo, L., Moller, K., et al., 2002; Nybo, Lars & Nielsen, Bodil, 2001). While we didn’t directly measure $Pa_{\text{CO}_2}$, we also did not observe a decrease in heart rate or associated cardiac output in the first half of the protocol, thus it is plausible that hyperventilation lead to the reduction in $Hb_{\text{Tot}}$ in HS$_{16}$ during the first 30 min, whereby after, participants became aware of the increasingly overwhelming internal and external stimuli and altered their pacing strategy in order to complete the test, which would have reduced their hyperventilatory response and thereby maintained $Hb_{\text{Tot}}$ in the second half of the protocol (Edwards, A.M. & Polman, R.C., 2013).

Despite the significant reduction in cerebral blood flow in the heat, there were no significant changes in $[Hb_{\text{Diff}}]$ between conditions, although it did decrease across all conditions in the second half of the test. $[Hb_{\text{Diff}}]$ represents changes in the amount of oxygen vs. carbon dioxide present in the tissue. An increase in $[Hb_{\text{Diff}}]$ represents an increase in tissue oxygenation, while a decrease in $[Hb_{\text{Diff}}]$ can be interpreted as an increase in tissue deoxygenation. There was a large increase in tissue oxygenation over the first 30 min across all conditions, and thus a likely decrease in $Pa_{\text{CO}_2}$, which again supports the reduction in $Hb_{\text{Tot}}$ in HS$_{16}$ during the time. However, during the second half of the protocol, there was increased deoxygenation, potentially due to reductions in overall cerebral blood flow as a consequence of both heat and high intensity exercise.

During externally paced protocols, cardiac strain is increased in heat stress due to the loss of fluids, and reductions in blood plasma volume from sweating (Hargreaves, M., 2008). These changes can contribute to early
volitional exhaustion compared to thermoneutral environments. Interestingly, a reduction in performance was evident in the present study without the increase in cardiac strain in heat stress. Reductions in power output for HS$_{16}$ were accompanied by a reduced heart rate in the second half of the protocol, while NT$_{16}$ had an increase in heart rate during the second half of the protocol, with non-significant reductions in power output (Fig 4-2A&B). Furthermore, reduced performance was also evident in HS$_{12}$, with only trivial changes in heart rate which suggests a disconnect between power output and heart rate in the heat. Reductions in power output in high intensity exercise in the heat may contribute to a reduced heart rate, but that power reductions in low intensity exercise in the heat are independent of changes in heart rate. It seems, therefore, that modifications to power output may influence heart rate in a feed forward manner, but that the maintenance of heart rate is does not have the same effect of increasing power output, at least in the heat.

The maintained heart rate in the NT$_{16}$ protocol was accompanied by increased neuromuscular activity (Fig 4-3E), and therefore, increased force production which seemed to attenuate the decline in power output compared to the HS$_{16}$ protocol. This may further indicate an increased sympathetic drive that helped to sustain heart rate. The attenuated EMG activity in the first 30 min of HS$_{16}$ may again reveal implications of heat stress on perceptual effort. Reductions in power output between the two high intensity protocols in the first half were similar ($p>0.05$; $d=1.2-1.7$) with HS$_{16}$ decreasing by 11.5 ± 13.2% and NT$_{16}$ decreasing by 8.2 ± 14.2%. Although there were no significant differences found between conditions for EMG in the first 30 min, it increased in NT$_{16}$ (6.11±3.7%; $d=3.3$) compared to HS$_{16}$ (3.8 ±8.6%; $d=0.88$) in the second half of the protocol. Thus, it is interesting to note that EMG was blunted even in the first half of the protocol in HS$_{16}$ compared to NT$_{16}$, even though power output between the two conditions were not significantly different.

A previous study found that neuromuscular fatigue is evident during cycling in hot humid environments as EMG activity decreased during maximal sprint efforts, but that the final sprint returned to 90% of the initial sprint,
suggesting changes in neuromuscular recruitment, central or peripheral control systems, and anticipatory regulation that preserves enough energy for an end spurt in the final sprint (Kay, D. et al., 2001). Taking these findings into account, it is possible that participants responded in a similar manner in the present study, reducing EMG in the HS\textsubscript{16} trial respective to the NT\textsubscript{16} trial in the first 30 min, in anticipation of lasting the full 60 min. It is also likely that sensitisation from exacerbated peripheral changes, especially thermal and metabolic, in this condition, contributed to the reduction in power output and EMG in the second half of the protocol (Hunter, A., Gibson, C., Mbambo, Z., Lambert, M., & Noakes, T., 2002; Kay, D. et al., 2001; Sabiosky, J., Marino, F., Kay, D., & Cannon, J., 2003).

It has previously been suggested that during self-paced cycling, power output may be adjusted in an effort to minimize increases in $T_c$ by adjusting the pace to maintain a comfortable $T_c$ (Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000), or through anticipatory regulation that sets the pace at a level which will not exceed comfort from the beginning (Tucker, R. et al., 2006). While we don’t present $T_c$ data in the present study due to technical limitations, averages of some pilot testing performed (unpublished data) also indicate no difference in $T_c$ either the heat stress or thermoneutral high intensity protocol. This supports both theories in that anticipatory regulation may be at work, in conjunction with a self-selected, comfortable $T_c$ limit, subconsciously imposed by the individual.

Unfortunately, we don’t have any other measures of thermal stress or body weight to indicate the rate of internal heat storage or how much fluid individuals lost during the protocol which is a limitation to the present study. Nevertheless, we did find increased $T_{sk}$ in HS conditions compared to their respective NT conditions (Fig 4-3B), with greater changes in the second half compared to the first half of the protocol, and so it may be concluded that external thermal stimuli provides innocuous heat information for perceptual processing which may exacerbate the sensitising effect of heat stress.

**PERIPHERAL METABOLIC AND INFLAMMATORY RESPONSES**
Peripheral metabolic and inflammatory factors cannot be discounted in the regulation of exercise intensity, perception of effort and subsequent power output as they play an integral part in afferent signalling to central regions and in thermoregulatory processes alike. Contrary to previous literature that shows augmented blood lactate levels in heat stress (Febbraio, M.A., Snow, R.J., Hargreaves, M., et al., 1994; Fink, W.J., Costill, D.L., & Van Handel, P.J., 1975; Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000), we found no differences between NT\textsubscript{16} and HS\textsubscript{16}, although both high intensity conditions showed greater accumulation than low intensity conditions in the first half of the protocol (Fig 4-5A). It is possible that lactate levels remained similar between high intensity conditions because our participants completed a 30s sprint every 10 min.

Also of interest is the significant reduction in lactate in the second half of the protocol compared to the first for both HS\textsubscript{16} and NT\textsubscript{16}. Our best attempt to explain this lies in the reduction of PO in both protocols in the second half of the study. It is reasonable to conclude that perhaps the large increase in lactate in the first half initiated a perceptual response that lead to decreased power output in the second half. Metabolites, including lactate, can activate peripheral sensory neurons through acid sensing ion channels (ASIC) (Gautam, M., Benson, C.J., & Sluka, K.A., 2010; Krishtal, O., 2003), which have been shown to decrease voluntary activation within the muscle, along with other muscles in the exercising limb (Kennedy, D.S., McNeil, C.J., Gandevia, S.C., & Taylor, J.L., 2013). Afferent signalling from lactate therefore may be considered an additional contributor to acute central sensitisation during exercise in both heat and thermoneutral environments.

Contrary to blood lactate, blood glucose levels were heat rather than intensity dependent, but were not found to be significantly different between any conditions. Nevertheless, a trend towards a decrease in plasma glucose in NT\textsubscript{16} and NT\textsubscript{12} conditions suggests up regulation of blood glucose as an energy source. However, it is likely that, while blood glucose stores alone provided enough energy during the lower intensity condition (Romijn, J.A. et al., 1993), there was increased dependency on intramuscular glycogen in
the higher intensity condition, which may explain the concomitant increase in IL-6 in NT_{16} but not NT_{12} (Fig 4A). Contrary to thermoneutral conditions, prolonged cycling in heat stress is known to cause hyperglycaemia as glucose is released from the liver, which exceeds blood glucose uptake into the muscles (Hargreaves, M., 2008). Still, intramuscular glycogen breakdown simulates the catabolism of liver glycogen and subsequent release of glucose into the circulation (Febbraio, M.A. et al., 2004). The release of IL-6 in HS is exacerbated by neuroendocrine (Rhind, S.G. et al., 2004) and inflammatory responses as IL-6 is also known to act as a heat stress sensor (Welc, S.S. et al., 2012) and responds to circulatory assault from endotoxins released through compromised endothelial tissue (Selkirk, G.A., McLellan, T.M., Wright, H.E., & Rhind, S.G., 2008; Starkie, R.L., Hargreaves, M., Rolland, J., & Febbraio, M.A., 2005; van Wijck, K. et al., 2011). Notably, like lactate, IL-6 has also been implicated in periphery to brain communication, although signalling depends highly on its soluble receptors.

The sIL-6R and sgp130 receptors for IL-6 act as an agonist and antagonist for IL-6 signalling, respectively. sIL-6R increases trans-signalling in tissues with gp130 receptors, while sgp130 acts as an antagonist to the IL-6/sIL-6R complex, eliminating its signalling activity (Jones, S.A., Richards, P.J., Scheller, J., & Rose-John, S., 2005). Both soluble receptors have recently been shown to increase simultaneously with IL-6 during exercise in NT environments (Gray, S.R. et al., 2009a; Gray, S.R., Robinson, M., & Nimmo, M.A., 2008). There are a few pathways that can transmit signals from IL-6 to the brain. It has been reported that IL-6 alone may stimulate non-nociceptive, low threshold nerves, while the sIL-6R/IL-6 complex is likely to stimulate nociceptive fibres (Hoheisel, U., Unger, T., & Mense, S., 2005). Furthermore, an inflammatory reflex arc may also act as a pathway for periphery to brain communication (Olofsson, P.S., Rosas-Ballina, M., Levine, Y.A., & Tracey, K.J., 2012), and membrane bound gp130 is known to be present on circumventricular organs, allowing the signalling of different inflammatory mediators to pass through to central areas (Roth, J. et al., 2004). Our data show that in all exercise conditions, sIL-6R is released to a similar degree as IL-6, supported by previous literature which has found
it to have a linear relationship with IL-6 (Obreja, O., Schmelz, M., Poole, S., & Kress, M., 2002).

IL-6 can signal tissues at a local and systemic level, however the local level is more prominent as systemic signalling likely only occurs if there is a disproportionate increase in sIL-6R compared to sgp130 and an overwhelming inflammatory cascade like in sepsis (Jostock, T. et al., 2001). Unfortunately, it is virtually impossible to study signalling at the local tissues, at least in vivo. While our exercise protocols did not reach septic-like concentrations of plasma IL-6, it is still interesting to note the different patterns for the receptors that were released. We found that sIL-6R in NT<sub>16</sub> and HS<sub>16</sub> increased significantly greater than the respective low intensity conditions in the second half of the protocol. This may indicate an increase in availability, and thus receptor signalling within high intensity protocols. Agreeably, sgp130 showed increases across all protocols, with high intensity being greater than low intensity. However, there was no significant change in the second half of the protocol, and thus, the blunted response of sgp130, and concomitant rise in sIL-6R, could increase the potential for trans-signalling through sIL-6R/IL-6 on membrane bound gp130 receptors.

**CONCLUSIONS**

The complex integrated systems model of fatigue (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005) posits that all of the different systems within the body work together in order to maintain homeostasis at any given time. When homeostasis is altered by stress, environmental, exertional, or the like, the protective systems are either up or down regulated to either reset the homeostatic point at a new level, or attempt to bring the system down to a level with which it can sustain without damaging any one system. In addition to this, we present data which highlights a sensitising effect of all these physiological systems working together in order to modulate perception of effort at any given time. Our data clearly shows a sensitising effect of heat on perception of effort and regulation of power output, in addition to anticipatory regulation and integration of central and peripheral
systems employed during self-paced high intensity exercise, especially in heat stress, to preserve the overall organism.

Decreased cerebral haemodynamics and neuromuscular recruitment are two essential central mechanisms that likely regulate perception of effort and subsequent power output during HS. In the periphery, increased plasma IL-6 owing to intramuscular glycogen breakdown and signalling through sIL-6R/IL-6 molecules, in addition to lactate signalling through afferent nerves, may be responsible for periphery-to-brain communication providing additional sensitising effects. Most notably, our findings highlight the integral aspect of informational processing in higher central regions that are stimulated by the integrated physiological systems, and leads to reductions in power output in order to maintain a designated perception of effort.

LIMITATIONS TO THE STUDY

There are several limitations to the present study that need to be acknowledged. Namely, measures of EMG and NIRS can be affected by blood flow to the periphery, which can be exacerbated in heat stress. This physiological response may result in augmented tissue oxygenation signals (Davis, S.L., Fadel, P.J., Cui, J., Thomas, G.D., & Crandall, C.G., 2006). While this is an important limitation, previous research has, nevertheless, shown similar NIRS changes as what were seen in the present study and hence, we find the data convincing. Furthermore, in the heated condition, where it has been shown that signals are augmented specifically, there was actually a reduction in tissue oxygenation. Likewise, the nature of the repeated measures protocol required that each participant complete all trials and the data therefore provides a picture of average changes for each individual using the respective trial as its own control. Each of these factors lead us to believe the NIRS data is justified, noting that there may still be effects of skin blood flow during prolonged and/or high intensity exercise as suggested by Davis et al., (2006).

Another important limitation may reside in the lack of blood pressure data which could have provided an indication of perfusion pressure and vascular
resistance to accompany NIRS signals, specifically. Likewise, a measure of \( \text{PaCO}_2 \) or \( \text{PETCO}_2 \) could have indicated the levels at which O2 or CO2 were maintained within the blood and associated O2 or CO2 perfusion to the tissues. A final limitation to the present study includes a lack of thermoregulatory data, namely core temperature, although in pilot testing we did not see a significant change in \( T_c \) between high intensity exercise in heat or thermoneutral environments. It still would have been desirable to collect core temperature data to confirm these results in a greater number of participants. Nevertheless, we have seen that in high intensity exercise in heat stress using a self-paced fixed RPE protocol, core temperature does not increase over 39°C (i.e. Data in Chapter 5). Hence, it seems likely that the individuals use the self-pacing to minimise internal heat storage over the duration of the protocols.

**CHAPTER SUMMARY**

- A reduction in power output in heat stress and high intensity exercise suggests a significant sensitivity to heat in both central and peripheral regions during self-paced exercise.
- An attenuated neuromuscular drive and cerebral blood flow in the first 30 min when exercising at high intensities may also be indicative of acute central sensitisation to heat stress and exercise.
- Greater metabolic and inflammatory changes in high intensity exercise and likely contributed to exacerbated reductions in power output in the second 30 min of the exercise task.
- The findings indicate that perception of effort is influenced by both internal and external factors of which sensitivity to the heat, combined with increased metabolic and inflammatory signalling leads to reductions in power output and performance.
5. The effects of ibuprofen ingestion on the IL-6 response and regulation of power output during internally regulated self-paced exercise in the heat

Study 3
ABSTRACT

The aim of the present study was to determine if ingestion of Ibuprofen (IBU) prior to self-paced cycling in heat stress (HS) leads to increases in interleukin (IL)-6, and an associated decrease in power output (PO), changes in cortical activity or other peripheral and central factors that could influence regulation of power output. Eight males completed two 60min cycling trials at a rating of perceived exertion (RPE) of 16. Variables measured included PO, peripheral measures of heart rate (HR), core temperature (Tc) plasma IL-6, soluble (s) IL-6 receptor (R) and s glycoprotein (gp) 130 and central measures of electromyography (EMG), cerebral total haemoglobin (HbTot) and haemoglobin difference (HbDiff) in the prefrontal cortex (PFC), and electroencephalographic (EEG) measures of alpha (α) and beta (β) activity in the PFC, frontal (FC), motor (MC) and parietal (PC) cortices. PO was not different between IBU and PLAC and did not decrease significantly over 60 min (p>0.05). IL-6 was greater in the second half for both conditions (p=0.004), but ES suggest an exacerbated response for IBU (d=1.5), with no changes in sIL-6R or sgp130 (p>0.05). Tc was not different, although a moderate ES indicated an attenuated Tc in the IBU (d=0.5). α/β ratio increased in both conditions in the PFC (p=0.02), FC (p=0.01) and MC (p=0.01). Data suggests that IL-6 may signal central areas, but the opposing effect of an attenuated Tc may counteract this, reducing cortical activity overall. PLAC may have greater cortical activity due to increased afferent signalling. As none of the responses resulted in changes in performance, there are likely other mechanisms that influence regulation of power output, hence suggesting integration of numerous physiological systems in self-paced exercise task.
INTRODUCTION

It has been shown that an acute dose of a non-steroidal anti-inflammatory drug (NSAID), acetaminophen, leads to faster time trial performance in thermoneutral environments, with no change in perceived pain or exertion (Mauger, A.R., Jones, A.M., & Williams, C.A., 2010). Additionally, NSAIDs have been shown to reduce the rating of perceived exertion (RPE) at the lactate threshold (Garcin, M. et al., 2005). Furthermore, chronic intake (every day for 6 days) of another type of NSAID, refocoxib, lowers thermal and cardiovascular strain during exercise in the heat, independent of heat production (Bradford, C.D. et al., 2007). These findings suggest that NSAID ingestion, either in an acute or chronic manner, can limit heat strain and increase performance or exercise capacity at a given intensity.

Ibuprofen (IBU) is an NSAID which acts through non-selective inhibition of inflammatory proteins, known as prostaglandins (PGE), formed through arachidonic metabolism via the cyclooxygenase (COX) pathways (Spinas, G.A., Bloesch, D., Keller, U., Zimmerli, W., & Cammisuli, S., 1991). PGEs are known to send temperature signals to the hypothalamic-pituitary-adrenal (HPA)-axis in the brain to mediate inflammatory responses during heat stress (Spinas, G.A. et al., 1991). Spengler et al. (1989) reported that blocking PGE synthesis using a COX inhibitor in acute endotoxemia increases the inflammatory response of tumour necrosis factor (TNF)-alpha (α), a precursor to the interleukin (IL)-6 cytokine that has implications in behaviour modification and fatigue in diseased states (Dantzer, R. et al., 2008; Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008) and in exercise (Robson-Ansley, P., Blannin, A., & Gleeson, M., 2007; Robson-Ansley, P.J., Milander, L.d., Collins, M., & Noakes, T.D., 2004). It has also been shown that IBU exacerbates the release of IL-6 under endotoxemic conditions while blunting core temperature ($T_c$), further implicating the effects of PGE on temperature signalling to the brain (Spinas, G.A. et al., 1991). As mild endotoxemia is known to occur during exertional heat stress due to increased intestinal permeability (Bradford, C.D. et al., 2007; Selkirk, G.A., McLellan, T.M., Wright, H.E., & Rhind, S.G., 2008; Starkie, R.L., Hargreaves, M., Rolland, J., & Febbraio, M.A., 2005), and IBU has been
reported to further exacerbate this response during exercise (Nieman, D.C. et al., 2006), the ingestion of IBU prior to an acute bout of exercise in the heat may in fact have negative effects on performance through an exacerbated IL-6 response.

As with most biological markers, IL-6 and PGEs are not limited within thermal and inflammatory pathways within the body. Integration of the inflammatory and central nervous systems is evident through increased sensitisation of nociceptive fibres within a healthy joint after injecting TNF-α, or IL-6 and its soluble (s) receptor (R), sIL-6R (Brenn, D., Richter, F., & Schaible, H.-G., 2007; Richter, F. et al., 2010). Interestingly, sensitisation of sensory fibres by TNF-α was prevented when a COX-inhibitor was systemically provided, suggesting desensitisation or reduced neuronal excitation from PGE inhibition (Richter, F. et al., 2010). Inhibition of PGE using IBU has also been shown to have sedative effects on the central nervous system measured via electrocorticogram readings directly implanted into the neural tissue (Wallenstein, M.C., 1985). Likewise, exercise has been shown to alter cortical activity with increased alpha (α) and decrease beta (β) waves measured through electroencephalography (EEG), with heat stress exacerbating the ratio between the two. This α/β ratio is suggested to be an indicator of fatigue (Nybo, L. & Nielsen, B., 2001). Additionally, it is believed that α waves represent sensory type information that is non-directional and comes from numerous sources of afferent feedback, while β activity represents a uni-directional pattern of efferent signalling from cortical areas to the periphery (Mima, T., Matsuoka, T., & Hallett, M., 2001; Mima, T. et al., 2000), which may indicate how the brain is interpreting and responding to afferent signalling.

In addition to cortical activity, cerebral haemodynamics has previously been identified as a marker of neuronal activity, with limitations (Ogoh, S. & Ainslie, P.N., 2009; Perrey, S., 2008). Total haemoglobin content (HbTot) has been shown to increase and remain stable during a 5km running time trial until the final 0.5km, where deoxyhaemoglobin (HHb) increases in conjunction with increased effort, thus leading to reductions in the overall haemoglobin difference (HbDiff) (Billaut, F. et al., 2010). It has also been
shown that heat stress reduces cerebral oxygenation during fixed intensity, submaximal cycling (Rasmussen, P. et al., 2010). It could therefore be hypothesised that, in addition to exacerbating the IL-6 response during exertional heat stress, IBU concomitantly exerts effects in central regions, thereby altering information processing and overall perception of effort through altered in sensory feedback. Indeed, it is plausible that the combination of up regulated inflammatory processes and down regulated temperature stimuli and associated cortical activity may counteract one another and result in no changes in perceptions or performance.

The dynamic workings of PGEs within the physiological systems during exercise, and the corresponding changes that occur through PGE inhibition, in addition to changes incurred through heat stress, are prime examples of the dynamic behaviour theory (Warren, W.H., 2006) and integrated physiological at work. Still, the effects of taking an acute maximal dose (800mg) of IBU prior to perceived high intensity, self-paced exercise in environmental heat stress has yet to be determined. Therefore, the purpose of this study was to examine the effect of acute IBU ingestion during prolonged exertional heat stress in humans on the regulation of power output (PO), peripheral changes in heart rate (HR), inflammation and thermoregulation, and central changes in cerebral blood flow, neuromuscular and cortical activity. We hypothesised that IBU would reduce PO due to an increase in IL-6 and sIL-6R, despite having a blunting effect on Tc and HR. Furthermore, although a direct link between IBU and central changes has not been made in the literature, it was hypothesised that there would be an increase in α wave activity, along with changes in the α/β ratio and neuromuscular drive, while cerebral haemodynamics would remain stable overall either due to a primary or secondary effect of IBU administration.

METHODS

ETHICAL APPROVAL
All participants provided verbal and written consent prior to participating in the study. The study conformed to the standards set by the latest revision of the Declaration of Helsinki. The Research and Human Ethics Committee of the appropriate university approved all methods and procedures prior to commencing the study.

**SUBJECTS AND STUDY DESIGN**

Subject population sample comprised of eight healthy, recreationally active males. Their age, height, body weight and VO$_{2\text{max}}$ (mean ± SD) were 25 ± 3.8, 180.8 ± 2.5cm, 85.4 ± 6.9kg and 45.64 ± 7.7 mL·min$^{-1}$, respectively. All participants were screened for current and previous exercise and disease history using the adult pre-screening exercise tool (APSS) (ESSA, 2011). Exclusion criteria for the study included any previous or current history of epilepsy, motor neuron disease, bipolar disorder, dementia, Alzheimer disease, brain damage, and tumour or other injury or medications likely to alter the neurophysiological state of the brain. Further exclusion criteria consisted of any reoccurring or recent (< 3weeks prior to participation) bout of influenza illness, recent surgical procedures, cholesterol lowering, anti-inflammatory, or any other medications known to interfere with a normal inflammatory response or; those with rheumatoid arthritis, recent and/or current periodontal disease, and any other conditions associated with an altered inflammatory state.

Subjects completed 3 sessions consisting of 1 familiarisation and 2 experimental trials. Both trials were performed in a randomised, double-blind design, separated by at least 7-10 days. All were completed in a climate chamber with heat stress (35°C, 60% relative humidity (Rh)).

**FAMILIARISATION TRIAL**

Subjects reported to the laboratory for a familiarisation session consisting of detailed explanation of requirements for the involvement in the study and equipment used. Subjects also completed baseline testing which consisted of weight and height measures and a VO$_{2\text{max}}$ test to determine fitness level.
and peak power output (PPO) on a cycle ergometer (Veletron DynaFit Pro, RacerMate Inc., WA, USA). All ergometer measurements were recorded for future use to decrease within-subject variability on equipment. Following 2 min rest, participants were fitted with head gear and were instructed to sit quietly on the cycle ergometer for 1 min while resting metabolic data was recorded. In the final 15 seconds of the rest minute, participants were told they would begin the VO$_{2 \text{max}}$ test in shortly and were counted them in from 5 sec. The test commenced at 100W and increased by 20W every minute with cadence maintained at 70 rpm for the whole time. The test ceased when participants voluntarily stopped or when they could no longer maintain a cadence above 70 rpm for at least 5 sec. After sufficient recovery, participants cycled on the ergometer in the heat chamber for 30 minutes at variable RPE ratings to ensure understanding of both how to use the cycle ergometer and how it felt to perform the designated RPE 16 (hard-very hard) (Borg, G.A., 1982) intensity.

In order to ensure familiarity with the RPE protocol, individuals were asked to first remember how they felt whilst performing the VO$_{2 \text{max}}$ test and recall how their rating of perceived exertion increased as the test got harder until they felt like were at an RPE of 20, or maximal exertion. After a general discussion, the individuals were required to cycle for 10 min at RPE 12, followed by an increase for 30 sec to RPE 14-15. They then brought their intensity back down to an RPE 12 for 2.5 min. At 15 min into the 30 min cycle, they were asked to cycle at an RPE of 16 for 10 min. They were then asked to increase their intensity to meet an RPE of 19-20 for 30 sec, followed by an immediate return to RPE 16 for 2.5 min. For the final 2 min of the 30 min block, they were asked to cycle at a comfortable pace while a discussion was had about how it is expected that their power would drop throughout the test, and that was okay, so long as they felt as though they were at the required RPE.

**Exercise Protocol**

Participants were asked to arrive to the laboratory following an overnight fast (8hrs), having avoided physical activity for at least 36 hr and caffeine
and alcohol at least 24 hr prior. The two exercise protocols required participants to cycle for 60 min with a 30 s sprint every 10 min, after ingesting either 800mg Ibuprofen (IBU) or a placebo (PLA). Ibuprofen is rapidly absorbed from the upper GI tract with peak plasma concentration at around 1-2hrs (Rainsford, K.D., 2009), therefore the pills were ingested 1hr prior to exercise commencing, during which time all other data collection procedures were set up.

**DATA COLLECTION**

Baseline measures for each experimental session consisted of resting HR and RPE, body mass, cerebral oxygenation via near-infrared spectroscopy (NIRS) and cortical activity via electroencephalography (EEG) measures (2 min eyes open sitting still on the cycle ergometer) and venous blood measures for IL-6, sgp130 and sIL-6R.

EEG, NIRS and electromyography (EMG) data were collected at 5 min increments over the duration of the protocol, alternating between steady state (continuous cycling at designated RPE) or sprint snapshots (SS) (Fig 5.1). Starting at 4:30, a 60s steady state SS was recorded every 10 min and starting at 9min, a 90s SS (30s pre, 30s sprint and 30s post) was recorded every 10min. For each steady state SS, participants were required to continue cycling at the designated RPE intensity with 2 notifications: when the SS started and stopped. For each sprint SS, they were instructed that there would be 4 notifications: when the SS started, when to begin sprinting, when to stop sprinting and when the SS ended.

Blood draws were repeated half way through and immediately post the 60 min protocol. HR and skin temperature ($T_{sk}$) were recorded continuously throughout the exercise protocol. A post exercise NIRS and EEG (30s, eyes open and sitting still on the cycle ergometer) measure was taken immediately post the final blood draw.
Figure 5-1. A representation of the 60 min exercise protocol.

PERCEPTUAL AND PERIPHERAL MEASURES

Power output and Heart Rate
Power output was measured continuously through the Veletron cycle ergometer (RacerMate, Seattle, WA) at a sampling rate of 2,000 Hz, along with heart rate, using a Polar HR Transmitter (Kempele, Finland). Data were saved as a .csv file and averaged over the first and second 30 min of the protocol in excel (Microsoft Office, 2013). Heart rate data were also manually recorded pre and post every snapshot using the accompanying sports watch (RS300X) for accuracy.

Rating of perceived exertion (RPE)
RPE was manually recorded pre and post every snapshot using the Borg RPE scale (Borg, G.A., 1982).

Blood Samples
15mL blood was collected at each time point using a standard 22guage cannula (Becton Dickinson, NJ, USA). 10mL was transferred immediately to an ethylenediaminetetraacetic acid (EDTA) tube and centrifuged at 4°C, 3500 rpm⁻¹. After centrifuging for 15 min, 1 mL of plasma was immediately aliquoted into 2 microfuge tubes and stored at -80°C. Blood samples were analysed in duplicate using Merck Millipore Multiplex Assay
human cytokine/chemokine magnetic bead panel kit for IL-6 (HCYTOMAG-60K, Magpix, Luminex, Austin TX) sgp130 and sIL-6R (HSCRMAG-32K, Magpix, Luminex, Austin TX).

**THERMAL MEASURES**

**Core Temperature**

Core temperature was monitored through a telemetric core temperature pill (Jonah™ Core body temperature capsule, VitalSense Company, Inc., Bend, OR) ingested 6hrs prior to the cycling task. Signals were transmitted via Bluetooth using Equivital sensor belt (EQO2) and software (Hidalgo Limited, Cambridgeshire, UK). All data were saved to an excel spreadsheet for further processing after the testing session.

**Skin Temperature**

Four Thermodata TDHC thermologgers (Brisbane, QLD) were fixed to the skin (Opsite Flexifix, Smith & Nephew, AU) at the bicep, chest, mid-thigh and mid-calf area. One recording every minute was logged during the cycling protocol. Data were exported to excel (Microsoft Office, 2013) and mean skin temperature for the respective trial was calculated using the following equation: $T_{sk} = 0.2(T_{sk\thigh} + T_{sk\calf}) + 0.3(T_{sk\bicep} + T_{sk\chest})$, as previously reported in a study by Ramanathan (1964).

**CENTRAL MEASURES**

**Electromyography**

EMG signals were collected during the cycling protocol from the vastus lateralis (VL) muscle. An 8mm Ag/AgCl electrode was placed on the patella to ground the signals. The VL was prepped by shaving, abrading and alcohol-swatting the site thoroughly to rid of hair and dead skin cells that could cause artefact. The lead was placed on the VL approximately 10 cm above the proximal lateral border of the patella, on the belly of the muscle. Signals were sampled at 2000 Hz using pre-amplified single differential surface recording electrodes (model DE-2.1, Delsys, Boston MA) with a bar configuration of 10mm x 10mm and bandwidth of 20-
450Hz connected to an amplifier with a gain of 1000V/V and a common rejection ratio of >120dB (Bagnoli-8, Delsys, Boston MA).

EMG signal processing involved data file importing to a custom developed software program incorporating a 3rd order Butterworth bandpass filter set at 20 and 450 Hz, for low to high, respectively. Signals were then processed to isolate the start and end of each contraction based on a 200% deviation from the baseline signals. The start and end data points of each contraction were identified, and then used to selectively capture the EMG signal spanning each contraction. EMG contraction signals were stored to a 2-dimensional array and later saved to a subject, trial and SS-specific data file. Each EMG contraction signal was then processed for root mean square (RMS) and mean frequency, with this final data also saved to a data file for later mean data collation.

**Electroencephalography**

A 20-channel wireless EEG system (BAAlertX24, San Diego, CA), was fitted to each subject based on the circumference of their head at a level just above their glabella, and the measure of the distance from the glabella to the occipital protuberance and between external acoustic meatus. The impedance of all EEG sites of interest were tested and maintained below 20kΩ as directed by the manufacturer and previous literature. Raw EEG signals were collected at a sample rate of 256Hz and referenced to the mastoid processes.

Prior to every EEG recording, participants were reminded to refrain from talking or tensing any facial or neck muscles while sitting relaxed on the cycle ergometer with eyes open. For baseline and post cycle measures, 2 min and 30 s, respectively, of EEG of data were collected. Participants were given the same instructions for all SS during the cycling protocol and were required to continue pedalling and keep their eyes open.

Data were quality checked using the manufacturer’s software (BAAlertLab, San Diego, CA) in 4 sites, prefrontal cortex (PFC), midline frontal (FC), motor (MC), and parietal (PC) cortices. Where <80% good data were
reported, data were manually processed to determine if at least 30 epochs of
good data were available. If less than 30 epochs of good data were available
for analysis, data were rejected. A Kaiser window was applied, and mean
power spectral density (PSD) for α (8-12Hz) and β (13-30Hz) waves for
each 60s snapshot was computed after automated decontamination of the
signals to eliminate known artefacts using in house proprietary algorithms
(BAlertLab, San Diego, CA). PSD values were copied and pasted into an
excel spreadsheet (Microsoft Office, 2007) to determine changes in the
mean α, β, and α/β ratio at each site during each 60s SS.

Near Infrared Spectroscopy
CBF was measured using NIRS (Niro-200x, Hamamatsu Photonics,
Hamamatsu, Japan). The optodes were placed on the left prefrontal cortex
(PFC), 40 mm apart, using double side adhesive tape. Black rubber was
placed over the optodes to shield them from the light. Data were obtained at
a frequency of 60Hz during the cycle protocol using wavelengths of 735,
810 and 850nm to calculate the change in oxyhaemoglobin (ΔHbO₂) and
deoxyhaemoglobin (ΔHHb), total haemoglobin (ΔHbTot = [HbO₂] + [HHb])
and the difference in haemoglobin concentration (ΔHbDiff = [HbO₂] −
[HHb]), as these have been shown to be reliable indicators of tissue de-
oxgenation and cerebral blood flow (Billaut, F. et al., 2010; Hoshi, Y.,
2007; Rupp, T. & Perrey, S., 2007). NIRS data were collected in
accordance with the same conditions as previously described for EEG
recordings. Data were imported into an excel spreadsheet (Microsoft
Office, 2013) and manually time averaged for every SS to determine
changes in the above measures over the duration of the exercise protocol.

Statistical Analysis
Prior to performing parametric analysis, tests of normality and homogeneity
were performed on the data. While not every part of the data were
completely normal and homogenous, the majority were, and thus parametric
tests were considered applicable. Furthermore, the parametric test applied
accounted for variability within subjects who repeated the conditions. Data
represent the magnitude of change (Δ) between the first half (0-30min (EX))
and second half (30-60 min (PT)) of exercise. Time (EX, POST), and drug (PLAC, IBU) mean effects on the dependent variables (PO, HR, EMG, Tsk, Tc, IL-6, sIL-6R, sgp130, HbTot, HbDiff, α and β waves and α/β index in PFC, FC, MC, PC) were estimated using a two within-subject linear mixed-model analysis. Mixed modelling was performed with IBM PASW statistical software (SPSS v. 20, Chicago, USA), in which the subject was treated as a correlated random effect, while drug and time were treated as fixed effects. This was to ensure that the model accounted for subjects repeating each trial as the repeated measure. Furthermore, the covariance was unstructured as the nature of the clamped RPE protocol assumes variability within each trial for the respective subject. We felt this model was a more suitable fit for the present data compared to a conventional RM ANOVA as would otherwise be performed. Pairwise comparisons were identified for main effects and 2-way interactions. All tests were considered significant at an alpha level of $P<0.05$.

Results are presented as mean ± SEM (Cumming, G., Fidler, F., & Vaux, D.L., 2007), unless otherwise specified. Results for the sprint data are not included so as to reduce complexity of the present paper. Furthermore, each figure includes results for the mean data and a corresponding individual figure as to highlight some of the differences in variables amongst individuals. Standardised effect sizes (ES; Cohen’s $d$) analyses were used in interpreting the magnitude of differences between conditions. An ES was designated as trivial ($d<0.20$), small ($d=0.20-0.49$), moderate ($d=0.50-0.79$) or large ($d>0.80$).

**RESULTS**

**POWER OUTPUT**

There were no significant differences between PLAC and IBU for ∆PO, ($p>0.05$). Effect sizes revealed a large decrease in PO in the first half of the protocol for both PLAC and IBU ($d=1.1-1.3$). PO was maintained in the second half of the protocol with only a trivial effect in IBU ($d=0.05$), but a
small decrease in PLAC ($d=0.4$). There were only trivial effects between the protocols in each half ($d<0.02$) (Fig 5-2A&B).

**Rating of Perceived Exertion**

There were no significant differences between the ∆RPE ($p>0.05$). Effect sizes revealed a large increase in the first half of the protocol for both conditions ($d=1.8-2.5$), followed by only a small decrease in RPE in the second half for both conditions ($d=0.2-0.4$), with only trivial effects between conditions ($d<0.02$) (Fig 5-2C&D).
Figure 5-2. A. Mean ± SEM ΔPower Output; B. Mean ± SEM Absolute Power Output; C. Mean ± SEM ΔRPE; D. Mean ± SEM Absolute RPE. Figures A&C represent magnitude change between the first half (0-30min) and the second half (0-60min) of cycling while Figures B&D represent absolute changes over the duration of the protocol with a placebo (PLAC) or ibuprofen (IBU). ¹Large ES for PLAC (d>0.8); ²Large ES for IBU (d>0.8).
**BLOOD MEASURES**

**INTERLEUKIN-6**

There was a main effect for time for ΔIL-6, revealing a significant increase in the second half of the protocol in both conditions (p=0.004). ES revealed a large increase in PLAC in the first half of the protocol (d=0.9), while the second half saw only a large increase in IL-6 in IBU (d=1.5). There was a moderate ES between protocols for the second half of the protocol (d=0.7), suggesting a greater increase in IBU compared to PLAC (Fig 5-4A&B).

**SOLUBLE GLYCOPROTEIN 130 AND SOLUBLE INTERLEUKIN-6 RECEPTOR**

There was no treatment effect Δsgp130 or ΔsIL-6R. A large ES for the first and second half of both conditions revealed a large increase in the first half (d=1.07-2.3), and a large decrease in the second half (d=1.0-1.4) for both receptors, with only trivial to moderate differences between conditions (d=0.05-0.6), (Fig 5-4C&D).
Figure 5-3. A. Mean ± SEM ∆Interleukin-6; B. Mean ± SEM Absolute Interleukin-6 (pg/mL). C. Mean ± SEM ∆soluble IL-6 Receptor; D. Mean ± SEM Absolute Soluble IL-6 Receptor (pg/mL). E. Mean ± SEM ∆soluble glycoprotein130. F. Mean ± SEM Absolute Soluble glycoprotein130 (pg/mL).

Figures A&C represent magnitude change between the first half (0-30min) and the second half (0-60min) of cycling while Figures B&D represent absolute changes over the duration of the protocol with a placebo (PLAC) or ibuprofen (IBU). #Main effect for time (p<0.05); ^Moderate ES between conditions (d=0.5-0.79); a Large ES for PLAC (d>0.8); b Large ES for IBU. (d>0.8).
**Physiological Measures**

**Heart Rate**

There was no treatment effect on \( \Delta HR \) \((p>0.05)\). ES revealed a large increase in HR in IBU for the first half of the protocol \((d=2.7)\), while PLAC only had a moderate increase \((d=0.6)\). In the second half of the protocol, HR only showed a trivial increase in IBU \((d=0.02)\), while in the PLAC condition, it increased moderately \((d=0.5)\). Only a small effect was seen between conditions in the first and second half of the protocol \((d=0.3-0.4)\) (Fig 5-3A&B).

**Core Temperature**

There was no treatment effect on \( \Delta T_c \) \((p>0.05)\). ES revealed a large increase in \( T_c \) in the first half for both IBU and PLAC \((d=4.2-4.6)\). PLAC ES showed a small increase in the second half \((d=0.4)\), while IBU showed a small decrease \((d=0.3)\), revealing a moderate ES between the conditions in the second half \((d=0.5)\) (FIG 5-3C&D).

**Skin Temperature**

There was no treatment effect for \( T_{sk} \) \((p>0.05)\). ES revealed a large increase in the first half for both conditions \((d=4.2-4.6)\), and a large decrease in the second half compared to the first half in both conditions \((d=1.0-1.7)\). There was only a small effect between conditions in the second half of the protocol \((d=0.5)\).

**Thermal Sensation and Thermal Comfort**

There was no treatment effect for thermal sensation or thermal comfort \((p>0.05)\), with only trivial ES \((d=<0.2)\) between conditions.
Table 5-1. Average power output, heart rate and absolute values for thermoregulatory and blood responses

<table>
<thead>
<tr>
<th></th>
<th>IBU</th>
<th>PLAC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Power Output</strong> (W)</td>
<td>123.4 ± 29.9</td>
<td>121.4 ± 29.3</td>
</tr>
<tr>
<td><strong>Mean Heart Rate</strong> (beats·min⁻¹)</td>
<td>135 ± 17.1</td>
<td>138.9 ± 15.8</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Core Temperature</strong> (°C)</td>
<td>36.8 ± 0.3</td>
<td>37.8 ± 0.5</td>
<td>36.7 ± 0.1</td>
<td>37.8 ± 0.3</td>
</tr>
<tr>
<td><strong>Skin Temperature</strong> (°C)</td>
<td>34.3 ± 0.4</td>
<td>36.0 ± 0.3</td>
<td>34.1 ± 0.7</td>
<td>36.1 ± 0.3</td>
</tr>
<tr>
<td><strong>IL-6</strong> (pg·mL⁻¹)</td>
<td>0.8 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>1.08 ± 0.7</td>
<td>1.5 ± 1.1</td>
</tr>
<tr>
<td><strong>sIL-6R</strong> (pg·mL⁻¹)</td>
<td>3996.6 ± 695.3</td>
<td>4220.1 ± 979.08</td>
<td>4093.8 ± 1125.2</td>
<td>4655.6 ± 1116.5</td>
</tr>
<tr>
<td><strong>spp130</strong> (pg·mL⁻¹)</td>
<td>28139 ± 8161.8</td>
<td>27978 ± 7002.8</td>
<td>28983 ± 8256.6</td>
<td>31971 ± 8425.6</td>
</tr>
</tbody>
</table>
Figure 5-4. A. Mean ± SEM ΔHeart Rate; B. Mean ± SEM Absolute Heart Rate (beats/min); C. Mean ± SEM ΔCore Temperature; D. Mean ± SEM Absolute Core Temperature; E. Mean ± SEM ΔEMG. F. Mean ± SEM Absolute EMG. Figures A&C represent magnitude change between the first half (0-30min) and the second half (0-60min) of cycling while Figures B&D represent absolute changes over the duration of the protocol with a placebo (PLAC) or ibuprofen (IBU). *Main effect for drug (p<0.05); ^Moderate ES between conditions (d=0.5-0.79); †Large ES between conditions (d>0.8); aLarge ES for PLAC (d>0.8); bLarge ES for IBU (d>0.8).
**Electromyography**

There was a significant main effect for treatment for ∆EMG (p=0.05), revealing that PLAC was greater than IBU. ES revealed large increases in the first half of the protocol for both PLAC ($d=1.7$) and IBU ($d=4.4$), while there was only a trivial decrease for the second half for PLAC ($d=0.03$), and a large decrease for IBU ($d=1.6$), these were supported by a large effect between conditions in the second half of the protocol ($d=1.0$) (FIG 5-3E&F).

**Cerebral Hemodynamics**

**Total Hemoglobin**

There was a main effect for time for ∆Hb$_{\text{Tot}}$ (p<0.01), revealing a significant decrease in CBF in both conditions. There was a large increase in CBF for the first half of the protocol for both PLAC ($d=2.0$-2.5). Conversely, there was a large decrease for both conditions in the second half of the protocol ($d=0.8$-1.3) and only trivial effects between both conditions ($d<0.20$) (Fig 5-5A&B).

**Hemoglobin Difference**

There was no treatment effects found for ∆Hb$_{\text{Diff}}$. ES revealed a large increase for PLAC and IBU in the first half ($d=1.0$-2.4), while in the second half, the PLAC had only a small decrease ($d=0.3$) while IBU had a large decrease ($d=0.9$). Still, this difference only showed a moderate ES between conditions in the second half ($d=0.7$) (Fig 5-5C&D).
Chapter 5 – IL-6, Ibuprofen and heat stress during self-paced exercise

Figure 5-5. A. Mean ± SEM ∆[Hb Total]; B. Mean ± SEM Absolute [Hb Total]; C. Mean ± SEM ∆[Hb Difference]; D. Mean ± SEM Absolute [Hb Difference]. Figures A&C represent magnitude change between the first half (0-30min) and the second half (0-60min) of cycling while Figures B&D represent absolute changes over the duration of the protocol with a placebo (PLA) or ibuprofen (IBU). *Main Effect for time (p<0.05); †Large ES for PLAC; (d>0.8); ‡Large ES for IBU (d>0.8).
Electroencephalography

Prefrontal Cortex (PFC)

There was no treatment effect for $\Delta$PFC $\alpha$ waves. ES revealed a large decrease in IBU in the first half of the protocol ($d=0.8$), while PLAC only had a small decrease ($d=0.4$). The second half of the protocol revealed a large increase in PLAC ($d=0.9$), while there was only a trivial increase in IBU ($d=0.1$). These findings were supported by a moderate ES between conditions ($d=0.7$) (Fig 5-6A&B).

Similarly, there was no treatment effect for $\Delta$PFC $\beta$ waves ($p>0.05$), although there was a moderate decrease in IBU in the first half of the protocol ($d=0.7$) and only a trivial increase for PLAC ($d=0.1$). The second half of the protocol revealed a large decrease for IBU ($d=0.9$), but only a small decrease in PLAC ($d=0.2$), revealing a moderate difference between conditions ($d=0.6$) (Fig 5-6C&D).

There was, however, a main effect for time for $\alpha/\beta$ ratio, revealing an increase over the duration of the protocols in both conditions ($p=0.02$). ES revealed only trivial increases in the first half of the protocol for both conditions ($d=0.04-0.1$), but there was a large increase for PLAC ($d=0.8$), and a moderate increase for IBU ($d=0.6$) in the second half of the protocols, with only trivial differences between conditions ($d<0.20$) (Fig 5-6E&F).
Figure 5-6. A. Mean ± SEM ΔPFC α; B. Mean ± SEM ΔPFC α individual responses. C. Mean ± SEM ΔPFC β; D. Mean ± SEM ΔPFC β individual responses. E. Mean ± SEM ΔPFC α/β ratio. F. Mean ± SEM ΔPFC α/β ratio individual responses. All figures represent magnitude change between the first half (0-30min) and the second half (0-60min) of cycling with a placebo (PLAC) or ibuprofen (IBU). *Main effect for time (p<0.05); ^Moderate ES between conditions (d=0.50-0.79); a Large ES for PLAC (d>0.8); b Large ES for IBU (d>0.8).
There was a trend towards a main treatment effect for $\Delta FC$ $\alpha$ wave activity (p=0.08), although it didn’t quite meet significance. ES revealed only a small reduction in the first half of the protocol for PLAC ($d=0.5$), while there was a large reduction for IBU ($d=1.0$). PLAC had a large increase in the second half of the protocol ($d=0.8$), while IBU had only a small decrease ($d=0.3$). These differences were supported by a large ES between conditions in the second half of the protocol ($d=1.1$) (Fig 5-7A&B).

There was also no treatment effect for $\Delta FC$ $\beta$ wave activity. ES revealed a small decrease in the first half ($d=2.0$) and a large decrease in the second half ($d=1.7$) of the protocol, compared to trivial and small changes for PLAC ($d=0.03$ and 0.2, respectively). The decreased $\beta$ waves in IBU were supported by a large ES between conditions in the second half ($d=0.9$) (Fig 5-7C&D).

There was a main effect for time for $\Delta FC$ $\alpha/\beta$ ratio (p=0.01), with ES revealing a small reduction in the first half of the protocol for IBU ($d=0.6$), followed by a large increase in the second half of the protocol ($d=1.0$). Only trivial changes were seen in the first half of the protocol for PLAC ($d=0.1$), with a moderate increase in the second half ($d=0.7$), and only trivial changes between conditions ($d<0.20$) (Fig 5-7E&F).
Figure 5-7. A. Mean ± SEM ∆FC α; B. Mean ± SEM ∆FC α individual responses. C. Mean ± SEM ∆FC β; D. Mean ± SEM ∆FC β individual responses. E. Mean ± SEM ∆FC α/β ratio. F. Mean ± SEM ∆FC α/β ratio individual responses. All figures represent magnitude change between the first half (0-30min) and the second half (0-60min) of cycling with a placebo (PLAC) or ibuprofen (IBU). "Main effect for time (p<0.05); "Large ES between conditions (d>0.8); "Large ES for PLAC (d>0.8); "Large ES for IBU (d>0.8).
**Motor Cortex (MC)**

There was no treatment effect for $\Delta$MC $\alpha$ wave activity ($p>0.05$). ES for both PLAC and IBU revealed only small decreases in the first half of the protocol ($d=0.3-0.4$), while in the second half, PLAC showed a large increase ($d=0.8$), and a small decrease in IBU ($d=0.4$). This was supported by a large ES between conditions in the second half ($d=1.3$) (Fig 5-8A&B).

There was no treatment effect for $\Delta$MC $\beta$ wave activity ($p>0.05$), although ES revealed only a small decrease in the first half ($d=0.2$) and a large decrease in the second half ($d=1.0$) of the protocol for IBU, compared to changes in PLAC which were only trivial to small ($d=0.02-0.4$). There was only a small ES between conditions ($d=0.3$) (Fig 5-8C&D).

There was a main effect for time for $\Delta$MC $\alpha/\beta$ ratio ($p=0.01$), and a trend towards a main treatment effect ($p=0.08$). ES revealed only trivial changes in the first half of the protocol for both IBU and PLAC ($d=0.09-0.12$), while PLAC had a large increase ($d=0.9$) compared to IBU ($d=0.5$) in the second half of the protocol. There was a large ES between conditions for the second half of the protocol revealing an increase in PLAC compared to IBU ($d=0.8$) (Fig 5-8E&F).
Chapter 5 – IL-6, Ibuprofen and heat stress during self-paced exercise

Figure 5-8. A. Mean ± SEM ΔMC α; B. Mean ± SEM ΔMC α individual responses. C. Mean ± SEM ΔMC β; D. Mean ± SEM ΔMC β individual responses. E. Mean ± SEM ΔMC α/β ratio. F. Mean ± SEM ΔMC α/β ratio individual responses. Figures A&C represent magnitude change between the first half (0-30min) and the second half (0-60min) of cycling while Figures B&D represent absolute changes over the duration of the protocol a placebo (PLAC) or ibuprofen (IBU). *Main effect for time (p<0.05); †Large ES between conditions (d>0.8); a Large ES for PLAC (d>0.8); b Large ES for IBU (d>0.8).
PARIELT CORTEX (PC)

There was no treatment effect for ΔPC α wave activity (p>0.05). ES revealed only a trivial change in the first half of the protocol for the PLAC (d=0.1), followed by a moderate increase in the second half (d=0.5). There was a moderate decrease in the first half of the IBU protocol (d=0.7), followed by only a small decrease in the second half (d=0.2). However these differences revealed a large ES for PLAC increasing compared to IBU in the second half (d=1.0) (Fig 5-9A&B).

There was also no treatment effect for ΔPC β wave activity (p>0.05). ES revealed only small changes in both the first and second half for the PLAC condition (d=0.2-0.4), while there was a moderate (d=0.7) decrease in the first half of the IBU protocol, followed by a large decrease in the second half (d=1.0) (Fig 5-9C&D).

Finally, there was a significant main effect for time for ΔPC α/β ratio (p=0.05), revealing that α/β ratio significantly increased over the duration of the protocol in both conditions. ES revealed trivial to small changes in the first half of the protocol (d=0.04-0.3) for both conditions, while there was a moderate to large effect for both conditions in the second half of the protocol (d=0.5-0.8) (Fig 5-9E&F).
Figure 5-9. A. Mean ± SEM ∆PC α; B. Mean ± SEM ∆PC α individual responses. C. Mean ± SEM ∆PC β; D. Mean ± SEM ∆PC β individual responses. E. Mean ± SEM ∆PC α/β ratio. F. Mean ± SEM ∆PC α/β ratio individual responses. All figures represent magnitude change between the first half (0-30min) and the second half (0-60min) of cycling with a placebo (PLAC) or ibuprofen (IBU). †Large ES between conditions (d>0.8); §Large ES for IBU (d>0.8).
DISCUSSION

The purpose of this study was to determine whether ingesting an acute dose of 800mg IBU prior to a self-paced cycling task in heat stress would affect perception of effort and regulation of power output through altering physiological feedback. It was hypothesised that IBU would blunt the heart rate and $T_c$ response, while simultaneously exacerbating the release of IL-6. A further hypothesis was that these changes would eliminate performance benefits that might be expected from an anti-inflammatory, and actually reduce power output because the opposing effects of increased IL-6 and reduced thermal strain might alter cortical activity and reduce neuromuscular drive compared to PLAC, while maintaining cerebral haemodynamics. The findings from the present study were minimal in terms of statistical significance. The nature of a clamped RPE protocol enables considerable variability in physiological responses between individuals. We believe the lack of significance is likely due to the large variability in responses. Nevertheless, there are apparent changes within the physiological systems between the first and second half of the protocol and between the interventions that should be considered.

In terms of performance or pacing within the protocols, there were no significant differences between PLAC and IBU, both resulting in an average power output of $124.4 \pm 29.2$ or $123.4 \pm 29.3$ W and decreasing 12.9 and 14.3% over the duration of the protocols, respectively. Likewise, there were no significant differences in reported RPE, thermal sensation or thermal discomfort between either of the trials. Although participants reported an RPE within 15-17 (hard and very hard) at every 5 min time point, we were interested in whether or not the true intensity they maintained did reside within this subjective response with respect to heart rate and power output. We found the average heart rate to be $\sim 80$ and $77\% \text{ HR}_{\text{max}}$ (from peak power output test in normothermic environment) for PLAC and IBU, respectively. Likewise, the average power output for PLAC and IBU was $\sim 42$ and $\sim 43\%$ of the maximal power produced, respectively, which would be expected for
average, non-acclimatised, recreationally active males when cycling at high intensities under environmental heat stress.

The large subjective nature of the protocol and variability in power output might be accounted for in minimising the changes in mean power output and performance. Still, it is interesting to note that the overall decrement in power was markedly less than what was reported in Chapter 4 (Study 2). We can confirm that participant fitness levels were similar, thus the reason for this discrepancy remains unknown. We do acknowledge that two participants in the present study had participated in the earlier study in which they had to complete the same protocol, however, even in the instance that we delete their power output data, we still only see a 4% greater reduction in the PLAC condition in the second half of the protocol, thus this doesn’t seem to have a large impact on the data overall.

**Peripheral Responses**

Generally speaking, the first 30 min of exercise revealed minimal changes in perceptual and peripheral responses between IBU and PLAC. While not statistically significant, there was a large ES revealing a reduction in power output and simultaneous increase in RPE, HR, $T_c$, $T_{sk}$, IL-6, sIL-6R and sgp130, Hb$_{Tot}$, and Hb$_{Diff}$ in the first half. Accordingly, these up-regulated responses would be expected in the clamped high intensity protocol in the heat, at least initially, in an attempt to re-instate homeostatic balance.

In the second half of the protocol, changes in physiological responses, at least according to ES, were slightly more dramatic. Although power output and RPE were generally maintained, there was a moderate increase in heart rate for the PLAC, yet only a small difference between conditions. $T_{sk}$ for both conditions showed a large decrease, with a small increase in $T_c$ for PLAC and a small decrease in $T_c$ for IBU. Hence, there was a moderate difference between the two conditions for $T_c$, revealing that IBU might blunt this response overall. There was a large ES for an increase in IL-6 in both conditions, and a moderate ES between conditions, resulting in an
exacerbated IL-6 response in IBU accompanied by large decreases in sIL-6R and sgp130 in both conditions.

**Central Responses**

Like in the periphery, there were minimal changes in central responses in the first 30 min of exercise. EMG, HbTot and HbDiff showed increases in both conditions according to effect size, suggesting greater motor drive and cerebral oxygenation. There were only very minimal changes for α and β activity across all sites in the first 30 min for both IBU and PLAC. Similar to peripheral responses, central responses in the second half of the protocol resulted in greater changes. Notably, there was a large difference between conditions for EMG in that IBU was attenuated compared to PLAC. HbTot revealed a significant decrease over the second half for both conditions, suggesting greater cerebral oxygenation, while HbDiff only decreased in IBU. The decrease in HbDiff, indicative of less deoxygenation in IBU, might suggest that the drug reduced the need for more oxygen uptake in the brain.

There were also greater changes in EEG in the second half of the protocol compared to the first. Specifically, there was a large increase in the PFC, FC MC and PC for α activity in PLAC, revealing a moderate to large difference in effect size between conditions. This may suggest that there was a greater amount of sensory information (Mima, T., Matsuoka, T., & Hallett, M., 2001) being sent to the brain during the PLAC. There was also a large decrease in β activity in IBU for the PFC, FC, MC and PC with a moderate to large effect between conditions, except in the MC where it was only small.

Studies have shown that information flow is significantly greater in a cortical to muscular (EEG to EMG) direction within high frequency β and gamma (γ) frequencies (Mima, T., Matsuoka, T., & Hallett, M., 2001), while low frequencies such as α and theta (θ) waves are likely to reflect informational processing (analogous to sensory stimuli) in subcortical rhythms in a non-directional manner (Mima, T., Matsuoka, T., & Hallett, M., 2001; Mima, T. et al., 2000). The data here may therefore reflect a
trend towards increased sensory stimuli in the PLAC condition. Nevertheless, most EEG studies in regards to whole body exercise use graded exercise (Bailey, S.P., Hall, E.E., Folger, S.E., & Miller, P.C., 2008; Robertson, C.V. & Marino, F.E., 2015) or fixed intensity protocols (Nielsen, B. et al., 2001; Nybo, L. & Nielsen, B., 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004) as opposed to self-paced protocols. One group did measure EEG during short duration (10 min) self-paced exercise at a clamped RPE of 11 (low) and 15 (high) intensity post performing an exhaustive (90 min) cognitive task (Brownsberger, J., Edwards, A., Crowther, R., & Cottrell, D., 2013). These authors found reductions in power output in both conditions, accompanied by increased β activity in the PFC and sensations of fatigue, although this opposes other research that has reported decreased β activity and increased α activity as a measure of fatigue (Nielsen, B. et al., 2001).

Moreover, it has been reported that IBU reduces cortical excitability through COX-inhibition at the dorsal root ganglion in the spinal tract (Richter, F. et al., 2010), potentially decreasing subcortical informational processing or sensory stimuli. This may explain the blunted α wave activity in IBU compared to PLAC. Although not definitive in this study, IBU may have had a slight inhibitory effect on neural input, which would reduce sensory information and hence, α activity. These changes may, all together, be implicated in the maintenance of power output, despite the reduction in heart rate, thermal strain and EMG in the second half of the protocol.

**SYSTEMIC INTEGRATION**

The most salient result in the present study was the apparent attenuation of $T_c$, EMG and increase of IL-6 in the second half of the protocol for IBU, while power output was maintained. Selective COX-2 inhibitors have been shown to blunt $T_c$ during and after cycling exercise in warm conditions (28°C) (Bradford, C.D. et al., 2007). IBU, conversely, is a non-selective COX inhibitor for which, to our knowledge, the acute effects of ingestion prior to exercise in the heat have not been evaluated. Evidence suggests, however, that when pre-treated with IBU, $T_c$ is blunted during passive
endotoxemia and simultaneously increases circulating IL-6 more than 4-fold (Spinas, G.A. et al., 1991). Exercise in the heat is known to increase intestinal wall permeability and release endotoxins into the circulatory system (Zuhl, M. et al., 2014), stimulating an acute-phase inflammatory response, and hence, the release of IL-6 into the circulation to eliminate the endotoxemic assault (Rhind, S.G. et al., 2004; Starkie, R.L., Hargreaves, M., Rolland, J., & Febbraio, M.A., 2005). Data here show variable, but significant increases in IL-6 in both conditions, although it should be noted that the total increase is remarkably less than other literature, only increasing to ~1.5 pg·ml\(^{-1}\). Nevertheless, there was a slightly greater increase in the second half for IBU, and a near 4-fold change from baseline in IBU (229.4 ±449%) compared to PLAC (62.6 ± 91.2%). Although speculative due to the minimal absolute concentration, this might be explained by an increase in intestinal wall permeability (Bjarnason, I., Williams, P., Smethurst, P., Peters, T., & Levi, A., 1986), release of endotoxins into the circulation and subsequent IL-6 response (Starkie, R.L., Hargreaves, M., Rolland, J., & Febbraio, M.A., 2005). Similar findings have been reported in athletes who completed a 160 km running event (Nieman, D.C. et al., 2006), however measures of endotoxin, LPS or intestinal wall permeability are needed to confirm this hypothesis.

According to the neuroinflammatory model of acute exercise fatigue (Vargas, N.T. & Marino, F., 2014), IL-6 might play a significant role in reducing performance or inducing fatigue. Data from the present study, however, do not support this theory as our power output and RPE were not dramatically altered, despite the increase of IL-6 in both protocols. Again, this may be because the increase was only minimal. The complex integrated systems of fatigue, however, places emphasis on all sensory information having a cumulative effect and so, other systems are likely to contribute to the regulation of perception and power output, simultaneously (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005). Accordingly, it has been shown that \( T_c \) is strongly correlated with reductions in power output during prolonged exercise in the heat (González-Alonso, J. et al., 1999; Nybo, L., Moller, K., et al., 2002). Likewise, \( T_{sk} \) has been implicated as a thermal controller of exercise intensity during self-paced exercise (Schlader, Z.J.,
Simmons, S.E., Stannard, S.R., & Mündel, T., 2011). As present data reveals a large ES for a reduced $T_c$ in IBU compared to PLAC, in addition to a decrease in $T_{sk}$ in the second half of both protocols, the maintenance of power output in IBU, therefore, may have been due to a culminating effect of less afferent input which counter-balanced the greater signalling from IL-6.

Self-paced exercise has shown remarkably similar $T_c$ between HS and NT environments, despite a large increase in mean $T_{sk}$ in the heat (Tatterson, A.J., Hahn, A.G., Martini, D.T., & Febbraio, M.A., 2000). It is plausible, therefore, that during self-paced exercise, power output is regulated by sensitisations to heat stress (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011) and overall perception of effort facilitates self-selecting of a pace that minimises detrimental increases in $T_c$ and discomfort in anticipation of completing the full exercise task (Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006). Still, even the slightest changes in $T_c$, combined with thermal skin stimuli, are likely to provide afferent feedback that modulates central sensitisation to the exercise task at hand. The slightly larger increase in $T_c$ in PLAC may have led to increased sensitivity despite the seemingly reduced IL-6 signalling, further supporting the maintenance in power output between conditions.

Circulating IL-6 primarily exerts its effects at systemic levels through binding with sIL-6R, forming a sIL-6R/IL-6 complex that can bind to the ubiquitous membrane bound gp130 receptor (Rose-John, S. & Neurath, M.F., 2004). However, the antagonistic soluble receptor, sgp130, is known to inhibit the binding of sIL-6R/IL-6 to the membrane bound gp130 receptor (Rose-John, S. & Neurath, M.F., 2004). As inflammation is increased during extreme exertional heat stress, so too are plasma sIL-6R concentrations (Hammami, M.M. et al., 1997). Most studies report linear changes in the receptor concentrations alongside increases in plasma IL-6 during prolonged exercise (Gray, S.R. et al., 2009a; Gray, S.R., Robinson, M., & Nimmo, M.A., 2008), although it has been shown that incremental exercise to exhaustion does not significantly increase sIL-6R, despite an increase in IL-6 and sgp130 (Robinson, M. et al., 2010).
To the best of our knowledge, sIL-6R and sgp130 responses have not been studied during exertional heat stress or self-paced exercise except in Study 2 (Chapter 4) in the present thesis. In comparison to our previous work, the receptor responses in the present study are remarkably different. In the exact same clamped RPE 16 protocol in the heat, we previously found a significant increase in sIL-6R within the second half of the protocol compared to the first, while sgp130 increased, but to a lesser extent. Conversely, in the present study, we saw ~10% decrease in the second half in both IBU and PLAC which were not significant despite large ES for each receptor in both time points ($d=0.9-2.4$). Based on the results, it is suggested that either participants did not work hard enough to stimulate a large increase in receptor response (which could also explain the minimal, yet significant increase in IL-6), or that both receptors were binding to IL-6, thus reducing the circulating receptor concentrations and, potentially, the afferent signalling, at least systemically.

Mauger et al. (2010) reported beneficial effects of ingesting 1500mg of acetaminophen prior to exercise as perceptions of effort and pain were unchanged despite an increase in HR, overall power output and blood lactate during a 16.1 km self-paced time trial in normothermic environments. Although IBU did not lead to increases in power output or a significant change in HR, ES did show a large increase in HR in IBU condition in the first half, while it was attenuated in the second half compared to the PLAC. Still, the apparent blunted HR in IBU suggests a potential overall effect of IBU on sympathetic drive, which may be evident in the central responses.

**CONCLUSIONS**

We acknowledge considerable limitations in the present study, the least of which resides in the piecing together of changes in physiological systems as an overall snap shot of perceptual and subsequent performance regulation during an exercise task with IBU ingestion. Nevertheless, there seems to be evidence that our overall conclusions in respect to the integration of central and peripheral changes warrant further research in order to truly understand
the integration of all systems and their contributions to perception of effort during self-paced exercise.

The complex integrated systems of fatigue draws on theoretical assumptions that perception, at any given moment, leads to performance modifications. Accordingly, an exercise protocol using a perceived rating of exertion requires the physiological systems within the body to be manipulated in order to maintain the level of exertion desired. This can highlight changes within the systems that are likely to be involved in informational processing and behavioural modification. The present study revealed only minimal reductions in power output during self-paced, internally regulated exercise in the heat which was not affected by an acute dose of 800mg IBU taken 1 hour prior to the exercise task. However, there are changes within physiological systems that suggest IBU may have an exacerbated IL-6 response, in addition to reducing thermal afferent feedback, which may have counteracted any performance benefits from ingesting the drug prior to exercise. Further research is warranted to determine whether or not IBU is beneficial or detrimental to performance as it is a commonly used NSAID for reducing inflammation pre and post exercise tasks. Studying the effects during a designated time trial or time to exhaustion protocol may further elucidate physiological effects of the intervention and the interactions within peripheral and central systems.

LIMITATIONS TO THE STUDY

Several limitations to the present study need be acknowledged. First, there were no changes in performance despite a slight increase in IL-6 for both PLAC and IBU conditions. There are two points to note here. Firstly, IL-6 was not released to the extent that was expected and hence, it may be that some individuals were not cycling as hard as they felt they were, or that they were reporting. A second issue here could be that the protocol wasn’t long enough to induce a change in IL-6 to a magnitude great enough to cause performance decrements. It is well known that the IL-6 response to exercise is both intensity and duration dependent, and hence, a longer exercise protocol may in fact provide more insight into the relationship (if any)
between performance and IL-6. A further limitation may be in the NSAID used. Ibuprofen is known to be a fairly ‘light’ NSAID, and hence, may not have had the effect that we expected. However, due to the fact that IL-6 was not released to the expected extent in the first place, this point may not be as relevant. Nevertheless, it would be desirable to identify whether consuming ibuprofen prior to performing a different type of protocol that is known to induce a greater release of IL-6 has any effect on the peripheral response and or central response to exercise.

Another limitation of the study is the use of EEG for determining changes in cortical activity. Although EEG is believed to be indicative of changes in brain activity during exercise, the underlying mechanisms have yet to be elucidated. Furthermore, data collection can be compromised by too much artefact due to movement, muscle contraction, eye blinks and noise, among other issues. In the present study, participants were reminded to maintain the same posture in every EEG recording, and to refrain from moving and clenching their jaw or neck muscles. Data processing involved initial decontamination of the signals by using the company’s proprietary algorithms, followed by manual checking of the data according to methods provided in appendix #10.

**CHAPTER SUMMARY**

- There are no performance benefits during internally regulated self-paced cycling when ingesting 800mg Ibuprofen 1hr prior to the exercise task.
- Although not definitively shown in our data through ‘significance’, Ibuprofen showed trends to decrease the core temperature response and concurrently increase the IL-6 release, the combination which may have prevented any performance benefits.
- While EEG data too showed no significance, data trends suggest a possible increase in α wave activity in the placebo condition which may be related to an increase in afferent feedback from the increased core temperature and other variables. Further research, however is warranted to elucidate this hypothesis.
6. Rinsing Carbohydrates elicits a neuroendocrine mechanism that acts on the peripheral inflammatory response during a 30 km cycling time trial

Study 4
Chapter 6 – Carbohydrate ingestion or rinse, IL-6 and performance during self-paced exercise

**Preface**

The final study in the thesis required participants to perform a 30km cycling time trial. This protocol was performed for two reasons. Firstly, it is documented that ingesting carbohydrates during exercise blunts the release of IL-6 and is believed to help with endurance exercise, especially in prolonged (greater than 1hr exercise). However, it has also been reported recently that simply rinsing carbohydrate solution prior to exercise can benefit performance as well. Hence, we were interested in whether or not the rinsing of carbohydrates might produce a similar mechanism in blunting the IL-6 response despite no increase in energy availability. The protocol we used was a 30km cycling time trial because cycling time trials are often used in performance type of exercise and we believed it would produce the most reliable and ecologically valid results.

**Abstract**

Research supports performance benefits from rinsing a carbohydrate (CHO) solution during exercise ~1hr duration. The purpose of this study was to determine whether or not rinsing or ingesting CHO improves performance during a 30km cycling time trial (TT) and if it is related to the release of IL-6 and its receptors (soluble (s) IL-6 receptor (R) and s glycoprotein (gp) 130). A further aim was to determine if IL-6 alters central measures of cerebral total haemoglobin (HbTot) or haemoglobin difference (HbDiff) in the prefrontal cortex (PFC); or changes in alpha (α) and beta (β) waves in the PFC, frontal (FC), motor (MC) and parietal (PC) cortices. Eight participants completed 3 x 30 km time trials (TT) in a randomised crossover design. Participants either ingested water (WATER) or a CHO (CARB) solution, or rinsed the same CHO solution (RINSE). Blood was sampled pre, 10, 20 and 30km distances. There were no differences in TT completion (p>0.05). There was a main effect for time for ∆PO (p<0.001) showing a decrease after the first 10km in all trials. ∆IL-6 increased significantly in WATER compared to CARB from 10-20 and 20-30km (p=0.01). HbTot and HbDiff only showed an increase in the first 10km for all trials (p=0.01) and decreased thereafter. Only α activity in the PFC was greater in CARB.
compared to RINSE between 0-10km (p=0.05), but was less than rinse at 10-20km (p=0.04). The data suggests that CHO does not increase performance in a 30km TT, however, ingesting CHO attenuates the IL-6 response, with a similar response from rinsing, which also appears to reduce the receptor response. Further research is warranted in regards to the changes in central responses.
INTRODUCTION

Carbohydrate rinsing has generally been shown to have performance benefits for short duration (~1hr) endurance exercise due to central effects on the brain that occur from sensing available energy in the solutions used (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004). The brain therefore believes there is additional energy available and is able to subsequently alter efferent drive (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004). The oral cavity is filled with receptors that send signals to the brain, activating hormonal pathways that modulate the periventricular hypothalamus (PVH) and hypothalamic-pituitary-adrenal axis (HPA-axis), among others (Cai, X.J. et al., 2001). The HPA-axis is an important regulatory centre for maintaining homeostasis of catecholamines and hormones during stress, whether by exercise or disease (Chesnokova, V. & Melmed, S., 2002). Consequently, when the organism is placed under exercise stress, the HPA-axis is believed to be one area that regulates communication between the peripheral and central regions in order to adjust pacing or efforts accordingly.

One biomarker that is largely implicated in periphery to brain communication in exercise and disease is interleukin (IL)-6. IL-6 is time, intensity and environmentally dependent, and may play a role in the complex integrated systems of fatigue, namely by propagating afferent signals to the brain (Robson-Ansley, P. et al., 2010; Vargas, N.T. & Marino, F., 2014). It has previously been shown that IL-6 release is attenuated when carbohydrates (CHO) are ingested during prolonged endurance exercise (Febbraio, M. et al., 2003; Nehlsen-Cannarella, S.L. et al., 1997; Starkie, R. et al., 2001), suggesting a possible mechanism by which IL-6 signalling is decreased and thus leads to improvements in performance. This, however, does not explain the increases in performance that have been reported through simply rinsing, as opposed to ingesting CHO (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004; Chambers, E.S., Bridge, M.W., & Jones, D.A., 2009; Pottier, A. et al., 2010; Sinclair, J. et al., 2014).
IL-6 is released during exercise through both muscular contractions at a peripheral level (Starkie, R. et al., 2001), and neuroendocrine regulatory mechanisms at the central level, specifically through activating the HPA-axis (Rhind, S.G. et al., 2004; Tsigos, C. et al., 1997). Catabolism of intramuscular glycogen to fuel muscle contraction is believed to release IL-6 into circulation, which helps to maintain blood glucose levels (BGL) by stimulating the release of hepatic glucose into the circulation (Febbraio, M.A. et al., 2004). The extent of IL-6 release during exercise also bears a relationship with the magnitude of stress hormones (especially norepinephrine, epinephrine and growth hormone) released (Rhind, S.G. et al., 2004). Steensberg et al. (2001) identified a link between epinephrine and plasma IL-6 after infusing participants with epinephrine in an attempt to match the post-exercise IL-6 values. They didn’t quite reach the same post exercise levels of IL-6, but concluded that epinephrine, and other neuroendocrine mechanisms influence the release of IL-6 during exercise in addition to metabolic regulation (Steensberg, A., Toft, A.D., et al., 2001).

Similarly, when infused with recombinant human (rh) IL-6, there are potent effects on the HPA-axis in the brain, largely stimulating thermogenesis and activity through the release of glucocorticoids, ACTH and cortisol which act as anti-inflammatory agents (Tsigos, C. et al., 1997).

The integration between the periphery and central regions highlights the effect that afferent feedback is likely to have on cortical activity. Specifically, it has been shown that IL-6 can cause central sensitisation, or an altered response of neuronal excitation or inhibition which reduces tolerance to pain and fatigue (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008; Nijs, J. et al., 2012). Cortical activity can be measured through electroencephalography (EEG) and specifically through low frequency alpha (α) and high frequency beta (β) waves that represent calm and rested or active states, respectively (Brownsberger, J., Edwards, A., Crowther, R., & Cottrell, D., 2013). It is also suggested that α waves are likely to represent sensory information that is received in subcortical areas (Mima, T., Matsuoka, T., & Hallett, M., 2001), while β activity may represent efferent drive, or activity in a uni-directional cortical to muscular manner (Mima, T. et al., 2000). Furthermore, cerebral oxygenation is believed to
represent regional changes in blood flow during exercise and can be an indication of neuronal activity at times (Ogoh, S. & Ainslie, P.N., 2009). Accordingly, the measurement of EEG and cerebral oxygenation may reveal a change in central regions from the afferent input of IL-6 if it is indeed altered through CHO ingestion or rinsing.

What remains unclear is whether or not the attenuation of IL-6 upon ingestion of CHO occurs at the muscular level, due to the increase in BG availability, or if a similar response will occur through CHO rinsing, even when there is no direct benefit from increased energy. Furthermore, it is unknown whether changes in IL-6 can stimulate central regions and cause central sensitisation that might be reflected in α and β wave activity. Therefore, the primary purpose of the present study was to determine whether rinsing CHO has the same attenuating effect on the release of IL-6 as ingesting CHO to determine whether the mechanism of release for IL-6 during exercise is primarily located at the level of skeletal muscle, or if it has additional contributions from the neuroendocrine system. The study also aimed to determine if IL-6 can act on central regions and whether there is an effect on cortical activity. It was hypothesised that there would be an improvement in performance during a 30km time trial (TT) when rinsing or ingesting CHO, compared to when water alone was ingested, due to the attenuation of IL-6. A secondary hypothesis was that α activity would be reduced in the CHO trials and α/β ratio would be increased in the water ingestion trial, while cerebral haemodynamics and β activity would be increased in the CHO trials.

**METHODS**

**ETHICAL APPROVAL**

The Institutional Research and Human Ethics Committee approved all methods and procedures and the study conformed to standards set by the latest revision of the Declaration of Helsinki. All participants provided verbal and written consent prior to participating in the study.
SUBJECTS AND STUDY DESIGN

The sample comprised of eight healthy endurance trained participants (6 male and 2 female). Their age, height, body weight and VO$_{2\text{max}}$ (mean ± SD) were 46.75 ± 11.8 years, 174.05 ± 6.8 cm, 77.49 ± 13.94 kg, 46.6 ± 10.4 mL/(kg·min$^{-1}$), respectively. All participants were screened for current and previous exercise and disease history using the Adult pre-screening exercise tool (APSS) (ESSA, 2011). Exclusion criteria included any previous or current history of epilepsy, motor neuron disease, bipolar disorder, dementia, Alzheimer disease, brain damage, and tumour or other injury or medications likely to alter the neurophysiological state of the brain. Further exclusion criteria were any reoccurring or recent (< 3 weeks prior to participation) bout of influenza illness, recent surgical procedures, cholesterol lowering, anti-inflammatory, or any other medications known to interfere with a normal inflammatory response or; those with rheumatoid arthritis, recent and/or current periodontal disease, and any other conditions associated with an altered inflammatory state.

Females were required to complete all trials within the luteal phase (14-28 days pre-menstrual) (Angstwurm, M.W.A., Gärtner, R., & Ziegler-Heitbrock, H.W.L., 1997) of their cycle and thus were asked to refrain from any specific increases in training to prevent adaptations between the first month and second month within which they were tested. Furthermore, in the event that they were on oral contraceptives, they were required to have been on the same oral contraceptive for a minimum of 3 months with no changes to their dosage within this time frame.

Subjects completed 4 sessions consisting of 1 x familiarisation and 3 x experimental trials performed in a randomized crossover design, separated by at least 5-7 days. All trials were completed in a climate chamber at ambient conditions (22°C; 60% relative humidity (Rh)).

FAMILIARISATION TRIAL
Subjects reported to the laboratory for a familiarisation session consisting of an explanation of requirements for the study and equipment used. Subjects also completed baseline testing consisting of body mass, height measures and a graded exercise test to determine VO$_{2\text{max}}$ and peak power output (PO) on a cycle ergometer (Veletron DynaFit Pro, RacerMate Inc., WA, USA). All cycle ergometer measurements were recorded for future use to decrease within-subject variability on equipment. Following 2 min rest, participants were fitted with head gear and were instructed to sit quietly on the cycle ergometer for 1 min while resting metabolic data was recorded. In the final 15 seconds of the rest minute, participants were told they would begin the VO$_{2\text{max}}$ test in shortly and were counted them in from 5 sec. The test commenced at 100W and increased by 20W every minute with cadence maintained at 70 rpms for the whole time. The test ceased when participants voluntarily stopped or when they could no longer maintain a cadence above 70 rpm for at least 5 sec. After a sufficient recovery, participants were asked to perform a 10km TT to ensure understanding of both how to use the cycle ergometer, change gears and to confirm comfort for the 30km TT in the experimental sessions. All participants were familiar with completing a time trial protocol.

**EXERCISE PROTOCOL**

Participants arrived at the laboratory following an overnight fast (8-10hrs), having avoided strenuous physical activity, caffeine and alcohol at least 24 hr prior. The 3 experimental sessions required participants to perform a 30km TT as quickly as possible while ingesting a commercial carbohydrate solution (6.4% CHO; PowerAde, USA), rinsing the same solution, or ingesting water. The trials were randomized and in the first trial, participants were able to ingest or swill the designated solution *ad libitum*. The number of administrations of either CHO or WATER that was ingested, and CHO that was rinsed, was recorded to ensure the availability of CHO matched in each condition, while WATER was also matched and used as a control. The volume of liquid was standardised for the number of swallows taken during each ingestion time point.

**DATA COLLECTION**
Measures pre and post each exercise protocol consisted of nude body weight (NBW), urine specific gravity (USG), resting HR and RPE and a venous blood plasma sample. A 2-minute eyes open snapshot for electroencephalography (EEG) and cerebral oxygenation through near infrared spectroscopy (NIRS) at the prefrontal cortex (PFC) were also taken prior to commencing the exercise protocol. Blood draws were repeated at 10km, 20km and immediately after finishing 30km. During the protocol, HR and RPE were collected after every 5km completed. Snapshots of EEG and NIRS were taken in the last 500m of every 5km completed, beginning at 4.5km. Power output was collected continuously and averaged for the first 500m and subsequently over each 10km completed.

**PERCEPTUAL AND PERIPHERAL MEASURES**

*Power Output and Heart Rate*

Power output was measured continuously through the Veeltron cycle ergometer (RacerMate, Seattle, WA) at a sampling rate of 2,000 Hz, along with heart rate, using a Polar HR Transmitter (Kempele, Finland). Data files were saved as a .csv and transferred to an excel worksheet (Microsoft Office, 2013) for averaging data over the 10km distance completed. Heart rate data were also manually recorded pre and post every snapshot using the accompanying sports watch (RS300X).

*Rating of Perceived Exertion (RPE)*

RPE was manually recorded pre and post every snapshot using the Borg RPE scale (Borg, G.A., 1982).

*Electroencephalography*

A 20-channel wireless EEG system (BAalertX24, San Diego, CA), was fitted to each subject based on the circumference of their head at a level just above their glabella, and the measure of the distance from the glabella to the occipital protuberance and between external acoustic meatus. The impedance of all EEG sites of interest were tested and maintained below 20kΩ as directed by the manufacturer. Raw EEG signals were collected at a sample rate of 256Hz and referenced to the mastoid processes.
Prior to every EEG recording, participants were reminded to refrain from talking or tensing any facial or neck muscles while sitting relaxed on the cycle ergometer with eyes open. For baseline and post cycle measures, 2 min and 30 s, respectively, of EEG of data were collected. Participants were given the same instructions for all SS during the cycling protocol and were required to continue pedalling and keep their eyes open.

Data were quality checked using the manufacturer’s software (BAAlertLab, San Diego, CA) in 4 sites, the PFC, midline frontal (FC), motor (MC), and parietal (PC) cortices. Where <80% good data were reported, data were manually processed to determine if at least 30 epochs of good data were available. If less than 30 epochs of good data were available for analysis, data were rejected. A Kaiser window was applied, and mean power spectral density (PSD) for α (8-12Hz) and β (13-30Hz) waves for each 60s snapshot was computed after automated decontamination of the signals to eliminate known artefacts using in house proprietary algorithms (BAAlertLab, San Diego, CA).

PSD values were copied and pasted into an excel spread sheet (Microsoft Office, 2013) to determine changes in the mean α, β, and α/β ratio at each site during each 60s SS.

**Near Infrared Spectroscopy**

Oxygen delivery to the brain was measured using NIRS (Niro-200x, Hamamatsu Photonics, Hamamatsu, Japan). The optodes were placed on the left prefrontal cortex (PFC), 40 mm apart, using double side adhesive tape. Black rubber was placed over the optodes to shield them from the light. Data were obtained at a frequency of 60Hz during the cycle protocol using wavelengths of 735, 810 and 850nm to calculate the change in oxyhaemoglobin ($\Delta$HbO$_2$) and deoxyhaemoglobin ($\Delta$HHb), total haemoglobin ($\Delta$Hb$_{Tot}$ = HbO$_2$ + HHb) and the difference in haemoglobin concentration ($\Delta$Hb$_{Diff}$ = HbO$_2$ – HHb), as these have been shown to be reliable indicators of tissue de-oxygenation and cerebral blood flow (Billaut, F. et al., 2010; Hoshi, Y., 2007; Rupp, T. & Perrey, S., 2007). NIRS data
were collected in accordance with the same conditions as previously described for EEG recordings. Data were imported into an excel spreadsheet (Microsoft Office, 2013) and manually time-averaged for every SS to determine changes in the above measures over the duration of the exercise protocol.

**Blood Samples**

15mL blood was collected at each time point using a standard 22-gauge cannula (Becton Dickinson, NJ, USA). 9 mL was transferred immediately to an ethylenediaminetetraacetic acid (EDTA) tube and centrifuged at 4°C, 3500 rpm$^{-1}$. After centrifuging for 15 min, 2 mL of plasma was immediately aliquoted into 5 microfuge tubes and stored at -80°C. Blood samples were analysed in duplicate using Merck Millipore Multiplex Assay human cytokine/chemokine magnetic bead panel kit for IL-6 (HCYTMAG-60K, Magpix, Luminex, Austin TX), sgp130 and sIL-6R (HSCRMAG-32K, Magpix, Luminex, Austin TX).

**Statistical Analysis**

Prior to performing parametric analysis, tests of normality and homogeneity were performed on the data. While not every part of the data were completely normal and homogenous, the majority were, and thus parametric tests were considered applicable. Furthermore, the parametric test applied accounted for variability within subjects who repeated the conditions. Data represent the magnitude of change ($\Delta$) between the first 10 (0-10), second 10 (10-20) and third 10 (20-30) km of the TT completed. Time (10, 20, 30), and intervention (WATER, RINSE, CARB) mean effects on the dependent variables (PO, HR, IL-6, sIL-6R, sgp130, Hb$^{Tot}$, Hb$^{Diff}$, $\alpha$ and $\beta$ waves and $\alpha/\beta$ index in PFC, FC, MC, PC) were estimated using a two within-subject linear mixed-model analysis. Mixed modelling was performed using IBM PASW statistical software (SPSS v. 20, Chicago, USA), in which the subject was treated as a correlated random effect, while intervention and time were treated as fixed effects. This was to ensure that the model accounted for subjects repeating each trial. The covariance was unstructured as the nature of the self-paced exercise protocols assumes
variability within each protocol for the respective subject. We felt this model was a more suitable fit for the present data compared to a conventional RM ANOVA, as would otherwise be performed. Pairwise comparisons were identified for main effects and 2-way interactions.

Furthermore, time to completion and average power output were analysed using a one-way ANOVA while nude body weight (NBW) and urine specific gravity (USG) were analysed using a repeated measures ANOVA for comparisons between pre and post measures. All tests were considered significant at an alpha level of $P<0.05$. Results are presented as mean ± SEM (Cumming, G., Fidler, F., & Vaux, D.L., 2007), unless otherwise specified. Furthermore, each figure includes results for corresponding individual data so as to highlight some of the differences in variables amongst individuals. Standardised effect sizes (ES; Cohen’s $d$) analyses were used in interpreting the magnitude of differences between conditions. An ES was designated as trivial ($d<0.20$), small ($d=0.20-0.49$), moderate ($d=0.50-0.79$) or large ($d>0.80$).

**RESULTS**

**TIME TO COMPLETION AND AVERAGE POWER OUTPUT**

There were no significant differences between the three conditions for time to complete the 30km trial ($p=0.959$) or average PO between trials ($p=0.965$) (Table 6-1).

**NBW AND USG**

There was a significant interaction for NBW ($p=0.05$), revealing a decrease from pre to post in RINSE ($p<0.05$). There were no significant differences in USG ($p>0.05$) (Table 6-1).
Table 6-1. Mean ± SD Time to completion (min), Mean Power Output (W), Nude body weight (kg) and Urine Specific Gravity for CARB, RINSE and WATER conditions.

<table>
<thead>
<tr>
<th></th>
<th>CARB</th>
<th>RINSE</th>
<th>WATER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to Completion (min)</td>
<td>61.47 ± 8.8</td>
<td>60.4 ± 7.9</td>
<td>61.48 ± 8.5</td>
</tr>
<tr>
<td>Mean Power Output (Watts)</td>
<td>156.3 ± 45.6</td>
<td>161.2 ± 45.8</td>
<td>155.5 ± 47.0</td>
</tr>
<tr>
<td>Nude Body Weight (kg)</td>
<td>76.1 ± 14.4</td>
<td>75.6 ± 14.2</td>
<td>76.3 ± 14.1</td>
</tr>
<tr>
<td></td>
<td>75.2 ± 13.9*</td>
<td>76.1 ± 14.8</td>
<td>75.3 ± 14.9</td>
</tr>
<tr>
<td>Urine Specific Gravity</td>
<td>1.01 ± 0.009</td>
<td>1.01 ± 0.01</td>
<td>1.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>1.01 ± 0.01</td>
<td>1.01 ± 0.009</td>
<td>1.01 ± 0.009</td>
</tr>
</tbody>
</table>

*Significant condition x time interaction; POST less than PRE for RINSE (p=0.05).
**POWER OUTPUT**

There was only a main effect for distance for ΔPO (p<0.001), revealing that ΔPO in the first 10km was significantly greater than the ΔPO from 10-20km and 20-30km. ES revealed a large decrease in PO between 10 and 20 (d=1.7) and 10 and 30 km (d=1.8).

**RATING OF PERCEIVED EXERTION**

There was only a main effect for distance for ΔRPE, revealing that RPE increased from 10-20km in all conditions (p<0.001), while it decreased from 20-30km (p=0.001). A large ES between 10-20km (d=1.5) and 20-30km (d=1.0) supported these findings.

**PHYSIOLOGICAL MEASURES**

**HEART RATE**

There was a significant condition x time interaction for ΔHR (p=0.01). ΔHR increased for WATER from 10-20km compared to CARB (p=0.05) and RINSE (p=0.04). From 20-30km, ΔHR also increased significantly in CARB compared to WATER (p=0.03) and RINSE (p=0.02) (Fig 6-1A&B).
Figure 6-1. A. Mean ± SEM ∆Power Output; B. Mean ± SEM ∆Power Output individual responses. C. Mean ± SEM ∆Heart Rate. D. Mean ± SEM ∆Heart Rate individual responses. E. Mean ± SEM ∆RPE; D. Mean ± SEM ∆RPE individual responses between the 0-10km, 10-20km and 20-30km of cycling with carbohydrate ingestion (CARB), carbohydrate rinse (RINSE) or water ingestion (WATER). *Significant condition x time interaction; WATER greater than CARB and RINSE (p<0.05); Significant condition x time interaction, CARB greater than WATER and RINSE (p<0.05); #Main effect for distance (p<0.05); a Large ES 10km greater than 20 and 30km (d>0.08). bLarge ES between 10-20 and 20-30km (d>0.08).
BLOOD MEASURES

INTERLEUKIN-6

There was a significant condition x time interaction (p=0.05), revealing that ΔIL-6 increased in WATER compared to CARB from 10-20km (p=0.003) and 20-30km (p=0.004). These findings were further supported by ES which revealed a large effect from 10-20km (d=1.4) and from 20-30km (d=1.5) (Fig 6-2A&B).

SOLUBLE INTERLEUKIN-6 RECEPTOR

There was a significant condition x time interaction (p=0.01) showing that ΔsIL-6R increased from 10-20km for WATER compared to CARB (p=0.003). This was supported by a large ES between CARB and WATER at 10-20km (d=1.3) (Fig 6-2C&D).

SOLUBLE GLYCOPROTEIN 130

There was a significant condition x time interaction (p=0.02), showing that Δsgp130 increased in RINSE compared to WATER (p=0.04) in the first 10km with a large ES (d=1.1). From 10-20km, Δsgp130 increased greater in WATER than both RINSE (p=0.04) and CARB (p=0.002), with large ES for both (d>0.8). Δsgp130 in WATER returned to similar levels as RINSE and CARB from 20-30km (p>0.2) (Fig 6-2E&F).
Figure 6-2. A. Mean ± SEM ∆Interleukin-6; B. Mean ± SEM Absolute Interleukin-6 (pg/mL); C. Mean ± SEM ∆soluble IL-6 Receptor; D. Mean ± SEM Absolute soluble IL-6 Receptor; E. Mean ± SEM ∆soluble glycoprotein130; F. Mean ± SEM Absolute soluble glycoprotein130 between the 0-10km, 10-20km and 20-30km of cycling with carbohydrate ingestion (CARB), carbohydrate rinse (RINSE) or water ingestion (WATER).

*Significant condition x time interaction; WATER greater than CARB (p<0.05); †Significant condition x time interaction, RINSE greater than WATER (p<0.05); ‡Significant condition x time interaction, WATER greater than RINSE (p<0.05); a Large ES WATER greater than CARB (d>0.08); b Large ES RINSE greater than WATER (d>0.08); c Large ES WATER greater than RINSE (d>0.08).
CENTRAL MEASURES

TOTAL HEMAGLOBIN
There was a main effect for distance for $\Delta Hb_{Tot}$ ($p=0.01$), revealing an increase in 0-10km compared to 20-30km ($p=0.004$), although there was only a moderate ES ($d=0.6$) (Fig 6-3A&B).

HEMAGLOBIN DIFFERENCE
There was a main effect for distance for $\Delta Hb_{Diff}$, revealing an increase in the first 10km ($p=0.01$) compared to 10-20 and 20-30km, with a large ES ($d>1.6$) (Fig 6-3C&D).
Figure 6-3.  A. Mean ± SEM \( \Delta \)Total Haemoglobin; B. Mean ± SEM Total Haemoglobin (% Change from baseline); C. Mean ± SEM \( \Delta \)Haemoglobin Difference; D. Mean ± SEM Haemoglobin Difference (% Change from baseline) between the 0-10km, 10-20km and 20-30km of cycling with carbohydrate ingestion (CARB), carbohydrate rinse (RINSE) or water ingestion (WATER).

- Main effect for distance 10km greater than 30km (p<0.05).
- Main effect for distance 10km greater than 20 and 30km (p<0.05).
- Large ES 10km greater than 30km (\( d > 0.8 \)).
- Large ES 10km greater than both 20 and 30km (\( d > 0.8 \)).
CORTICAL ACTIVITY

Prefrontal Cortex (PFC)

There was a significant condition x time interaction for PFC $\alpha$ activity ($p=0.04$), where CARB was less than RINSE at 10km ($p=0.05$) but increased greater than RINSE at 20km ($p=0.04$). ES supported these findings with a large effect between the conditions in the first 10km ($d=1.1$) and 10-20km ($d=1.0$) (Fig 6-4A&B).

PFC $\beta$ activity (Fig 6-4C&D) and $\alpha/\beta$ ratio (Fig 6-4E&F) showed no significant changes in across the TT within and between conditions ($p>0.05$).

Frontal Cortex (FC)

There were no significant differences found for FC $\alpha$ (Fig 6-5A&B), $\beta$ (Fig 6-5C&D) or $\alpha/\beta$ ratio (Fig 6-5E&F) activity across the TT for any distance point or between conditions ($p>0.05$).

Motor Cortex (MC)

There were no significant differences found for MC $\alpha$ (Fig 6-6A&B), $\beta$ (Fig 6-6C&D) or $\alpha/\beta$ ratio (Fig 6-6E&F) activity across the TT for any distance point or between conditions ($p>0.05$).

Parietal Cortex (PC)

There were no significant differences found for PC $\alpha$ (Fig 6-7A&B), $\beta$ (Fig 6-7C&D) or $\alpha/\beta$ ratio (Fig 6-7E&F) activity across the TT for any distance point or between conditions ($p>0.05$) (Figure 6.8).
Figure 6-4.  A. Mean ± SEM ΔPFC α activity. B. Mean ± SEM ΔPFC α activity individual responses. C. Mean ± SEM ΔPFC β activity. D. Mean ± SEM ΔPFC β activity individual responses. E. Mean ± SEM ΔPFC α/β ratio activity. D. Mean ± SEM ΔPFC α/β ratio activity individual responses between the 0-10km, 10-20km and 20-30km of cycling with carbohydrate ingestion (CARB), carbohydrate rinse (RINSE) or water ingestion (WATER). Significant interaction CARB less than RINSE (p<0.05). Significant interaction CARB greater than RINSE (p<0.05). LARGE ES between CARB and RINSE (d>0.08).
Chapter 6 – Carbohydrate ingestion or rinse, IL-6 and performance during self-paced exercise

Figure 6-5.  A. Mean ± SEM ∆FC α activity. B. Mean ± SEM ∆FC α activity individual responses. C. Mean ± SEM ∆FC β activity. D. Mean ± SEM ∆FC β activity individual responses. E. Mean ± SEM ∆FC α/β ratio activity. D. Mean ± SEM ∆FC α/β ratio activity individual responses between the 0-10km, 10-20km and 20-30km of cycling with carbohydrate ingestion (CARB), carbohydrate rinse (RINSE) or water ingestion (WATER). No significance found (p>0.05)
Figure 6-6. A. Mean ± SEM ΔMC α activity. B. Mean ± SEM ΔMC α activity individual responses. C. Mean ± SEM ΔMC β activity. D. Mean ± SEM ΔMC β activity individual responses. E. Mean ± SEM ΔMC α/β ratio activity. D. Mean ± SEM ΔMC α/β ratio activity individual responses between the 0-10km, 10-20km and 20-30km of cycling with carbohydrate ingestion (CARB), carbohydrate rinse (RINSE) or water ingestion (WATER). No significance found (p>0.05).
Figure 6-7. A. Mean ± SEM ΔPC α activity. B. Mean ± SEM ΔPC α activity individual responses. C. Mean ± SEM ΔPC β activity. D. Mean ± SEM ΔPC β activity individual responses. E. Mean ± SEM ΔPC α/β ratio activity. D. Mean ± SEM ΔPC α/β ratio activity individual responses between the 0-10km, 10-20km and 20-30km of cycling with carbohydrate ingestion (CARB), carbohydrate rinse (RINSE) or water ingestion (WATER). No significance found (p>0.05).
DISCUSSION

The purpose of the present study was to determine whether the apparent performance benefits from rinsing CHO can be attributed to neuroendocrine mechanisms that are initiated in response to carbohydrate signalling to the brain, subsequently attenuating the release IL-6. As IL-6 release has been shown to be attenuated when CHO are ingested during endurance exercise, it is plausible that performance benefits may result. If a similar response occurs with CHO rinsing, it is possible that the performance benefits may have a true neuroendocrine mechanism, specifically through CHO inducing changes in the HPA-axis and exerting its effects on the peripheral release of IL-6.

The data revealed that neither CHO rinsing, nor ingestion, improves performance during a 30km cycling TT when rinsed or ingested *ad libitum*. However, the two CHO ingestion interventions did attenuate circulating IL-6 and its receptors compared to the water ingestion trial. When water was ingested, IL-6 accumulation between the 10-20 and 20-30km distances was significantly greater to CHO ingestion, and though not statistically significant, there was also a large effect between rinsing CHO and ingesting water at the same points. These findings highlight a potential centrally driven neuroendocrine mechanism that attenuates the IL-6 response, derived through sensing CHO. Moreover, the central changes seem to have an additive effect of further reducing IL-6 when CHO is ingested.

These findings are novel in that previously, mechanisms of performance benefits have largely been focused on the brain sensing an increase in energy availability (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004) and CHO stimulating reward and motor pathways when rinsed (Chambers, E.S., Bridge, M.W., & Jones, D.A., 2009), while the present data shows an effect on peripheral changes as well. Although we didn’t find an increase in performance, the data do show that inflammatory regulation is likely to be affected by simply rinsing CHO in the oral cavity.
The working mechanism here likely involves both sensory cells in the oral cavity, neural propagation to the brain, and a designated response from the HPA-axis. Notably, the CHO solution used in the present study was made of 6.4% sucrose. Sucrose is known to stimulate orosensory gustatory input from taste receptor cells (TRCs) within the taste buds (Oliveira-Maia, A.J., Roberts, C.D., Simon, S.A., & Nicolelis, M.A.L., 2011). Signals propagate from the TRCs centrally to the solitary tract nucleus (Oliveira-Maia, A.J., Roberts, C.D., Simon, S.A., & Nicolelis, M.A.L., 2011), and are closely acquainted with afferent signals from visceral areas in the periphery (Zheng, H. & Berthoud, H.-R., 2008). Glucose and sucrose sensing neurons are prominent in the ventromedial hypothalamus (VMH), periventricular nucleus (PVN) and the arcuate nucleus (ARC) in the brain, which also react to peripheral changes in circulating blood glucose levels (Cai, X.J. et al., 2001; Figlewicz, D.P., Bennett-Jay, J.L., Kittleson, S., Sipols, A.J., & Zavosh, A., 2011; McCrimmon, R.J. et al., 2006). This means that the brain wants to know about availability of energy long before the energy is metabolised – a potential anticipatory mechanism for self-paced exercise tasks.

The PVN is an important part of the HPA-axis, which stimulates the release of corticotrophin releasing hormone (CRH) (Chesnokova, V. & Melmed, S., 2002). CRH is responsible for maintaining homeostasis during acute and chronic immune challenges and initiates a release of adrenocorticotropic hormone (ACTH) (Chesnokova, V. & Melmed, S., 2002). ACTH activates the adrenal cortex, releasing glucocorticoids, especially cortisol, which have an anti-inflammatory effect in the periphery, ultimately helping to maintain overall inflammatory homeostasis (Chesnokova, V. & Melmed, S., 2002). In the instance that the PVN is activated through signalling through CHO rinsing like in the present study, the anti-inflammatory cascade may ensue and result in reductions of circulating IL-6 in the periphery (Starkie, R. et al., 2001).

IL-6 has previously been shown to have an effect on performance and fatigue (Robson-Ansley, P.J., Milander, L.d., Collins, M., & Noakes, T.D., 2004), is involved in both peripheral metabolic regulation (Febbraio, M. et
al., 2003; Tsigos, C. et al., 1997) and in the regulation of homeostasis through neuroendocrine mechanisms (Chesnokova, V. & Melmed, S., 2002; Tsigos, C. et al., 1997; Venihaki, M., Dikkes, P., Carrigan, A., & Karalis, K.P., 2001). When BG falls into a hypoglycaemic state, the hepatic system releases IL-6, which, when combined with IL-6 released from muscular contractions, can potentiate its own release of glucose from the liver (Jeukendrup, A.E. et al., 1999; McConell, G., Fabris, S., Proietto, J., & Hargreaves, M., 1994). During exercise, CHO ingestion has been shown to attenuate the release of circulating plasma IL-6 concentrations because CHO ingestion replaces BG levels and therefore, hepatic release is down regulated (Febbraio, M. et al., 2003; McConell, G., Fabris, S., Proietto, J., & Hargreaves, M., 1994; Robson-Ansley, P., Barwood, M., Eglin, C., & Ansley, L., 2009). In euglycemia, the requirement for IL-6 and subsequent glucose release from the liver is minimised, which may therefore reduce the amount of circulating IL-6 (Febbraio, M. et al., 2003; Starkie, R. et al., 2001) and, consequently, its ability to provide afferent signalling.

IL-6 can signal tissues through the membrane bound receptors (R), IL-6R and gp130 receptor. IL-6R is only available on certain tissues throughout the body, while gp130 is located ubiquitously and specifically, is found on afferent nerve fibres (Andratsch, M. et al., 2009). Trans-signalling occurs through the gp130 receptor when IL-6 binds to its soluble (s) receptor, sIL-6R, forming a sIL-6R/IL-6 complex (Rose-John, S., 2012). However, signalling is regulated by the antagonist, sgp130, which inhibits sIL-6R/IL-6 from any activity (Rose-John, S., 2012). Previous research has yet to identify how circulating concentrations of sIL-6R and sgp130 interact with IL-6, although it is has been suggested that a greater sIL-6R concentration is needed for systemic signalling as sgp130 has a greater binding affinity to the sIL-6R/IL-6 complex than the membrane bound gp130 receptor (Jostock, T. et al., 2001). Exercise induced changes in sIL-6R and sgp130 is inconsistent, some reporting increases in sIL-6R and/or sgp130 (Gray, S.R. et al., 2009a; Gray, S.R., Robinson, M., & Nimmo, M.A., 2008; Leggate, M., Nowell, M.A., Jones, S.A., & Nimmo, M.A., 2010), while others report no changes (Patterson, S., Reid, S., Gray, S., & Nimmo, M., 2008; Robinson, M. et al., 2010). Overall changes in the present study revealed
that in the water ingestion trial, sIL-6R was significantly increased compared to CHO ingestion at 10-20km, but did not show any differences to CHO rinse. Interestingly, this was accompanied by a significant increase in sgp130 in the water ingestion condition at 20-30km, compared to both CHO trials. These findings might suggest a counter-effect for signalling through sIL-6R/IL-6 complex, however further work is warranted to determine the true signalling responses. Still, the similar receptor responses in both CHO trials supports the hypothesis of a neuroendocrine mechanism that acts on the peripheral inflammatory response through rinsing CHO and, to a greater degree, through ingesting CHO.

The neuroinflammatory model of acute exercise fatigue (Vargas, N.T. & Marino, F., 2014) and the complex integrated systems theory (Lambert, E., St Clair Gibson, A., & Noakes, T., 2005) rely on afferent signalling which the brain processes and uses to regulate overall pace and performance during self-paced exercise tasks. Hence, it is plausible that changes within central brain regions and overall brain state may ensue. For this reason, EEG measures of α, β and the α/β ratio were measured. The low frequency α activity is thought to represent changes in subcortical information from sensory input by peripheral and external factors while β activity is thought to be related to efferent drive from the cortex to the muscle (Mima, T., Matsuoka, T., & Hallett, M., 2001). Furthermore, in fixed intensity exercise, the slowing of β waves with concomitant maintenance or increase in α waves, represented by an increase in α/β ratio, are believed to represent a state of fatigue (Nybo, L & Nielsen, B, 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004), however, in accordance with the previous theory, it could also represent increase in afferent stimuli with a concomitant decrease in efferent drive. Our EEG only revealed changes within the PFC, or the decision making area of the brain. Furthermore, there was a crossover effect of CHO ingestion and rinse, where α activity in the CHO ingestion trial was less than the rinse trial 10km, but increased greater than the rinse trial at 20km. These findings were not accompanied by any changes in β activity, or the α/β ratio, and thus are difficult to interpret. However, it is important to note that EEG is speculative at best, and particularly in this study, several participants’ data were unable to be used
due to large artefact, likely due to muscle activity. Hence, future research could initially test whether there is a change in EEG patterns when CHO is ingested or rinsed, even at rest.

It is important to note that there was no significant change in power output, RPE or HR, which suggests the signalling, if indeed it occurred, did not have a large enough effect on the brain to alter pacing strategies, which is also confirmed by the lack of performance improvement in time to completion in any trial. Furthermore, there was only a significant main effect for changes in CBF and Hb\textsubscript{Diff}, which revealed a decrease in both parameters after 10km. This is also reflected in the large decrease in PO between 10-20km which suggests that individuals down regulated their pacing based on initial responses in the first 10km and in anticipation completing the full 30km in all trials. The changes in plasma concentrations of IL-6 during exercise are entirely dependent on exercise intensity, environment and duration, and thus, it is possible that the stimulus during the present exercise task may not have been great enough to actually see a dramatic change in performance between interventions. However, it is possible that if the TT was extended, we may be able to more reliably conclude that the IL-6 response plays a role in fatigue as the duration increases and the individual continues their best effort in order to complete the TT. Regardless, it is clear that there are both peripheral and neuroendocrine mechanisms at work concerning IL-6 and its receptors during exercise while ingesting or rinsing with a CHO solution and further research is warranted to determine whether or not the capacity to provide afferent signalling through the soluble receptors is a valid theory in terms of exercise induced fatigue.

**Limitations of the Study**

Although novel in its approach to elucidate whether or not a neural mechanism may exist which helps improve performance when rinsing with carbohydrates during a 30km time trial, the present study is not without limitations. One limitation is the lack of blood glucose measures which would provide an indication of internal glucose homeostasis and whether
there was compensation for reduced blood glucose stores in the CHO rinse and water trials compared to the CHO ingestion trial. A further limitation resides in the minimal time that participants were given to familiarise themselves with the exercise protocol. However, all participants were keen cyclists and were aware of how to perform a 30km TT protocol. Hence, a 10km time trial was deemed sufficient, particularly since the study design was randomised among participants, therefore any diminishing any familiarisation effects that may have ensued after the first trial, as they would have been balanced out among trials and participants. The total duration of the protocol may have also limited the response of IL-6. Literature documents that IL-6 is time and intensity dependent and so if the time trial distance was increased, there may have been a greater response in IL-6, and hence an ability to further elucidate mechanisms of glucose ingestion/rinsing on its release. This may be a possible way to test this interaction in future studies.

A final limitation, of course, resides within the use of EEG to measure changes in cortical activity as the underlying mechanisms of EEG brain waves have yet to be understood. Nevertheless, EEG is believed to provide an overall indication of changes in brain activity during exercise and, although signals can be compromised by movement, muscle, and eye blink artefact, reminding participants to refrain from movement and clenching of muscles during the recordings, and proper decontamination of the raw signals eliminates most of these issues. In accordance, we reminded participants of the importance of not moving or clenching and muscles prior to each EEG recording and processed data according to the methods provided in appendix 10 to ensure data quality met criteria for use in analysis.

**CHAPTER SUMMARY**

- A 30km cycling time trial is not affected by either rinsing or ingesting a 6.4% commercially available carbohydrate solution despite them having an attenuating effect on the release of IL-6, however, the study design was not specifically made to identify
changes in performance, but was interested in the underlying mechanisms and hence these findings should be interpreted loosely as a blinded/double blinded trial would better suit a performance based hypothesis.

- Results suggest the possibility of a centrally driven neuroendocrine mechanism derived through sensing carbohydrates in the oral cavity.
- This study does not support the contribution of IL-6 signalling to performance decrements, or changes in EEG, however, we cannot eliminate other physiological contributions to informational processing that are likely to contribute to regulation of performance.
7. **GENERAL DISCUSSION**
OVERVIEW OF THE THESIS

Fatigue is a universal sensation that is felt in chronic and acute situations. In the present thesis, acute exercise fatigue during prolonged exercise of ~60 min duration was studied to evaluate how much the release of the cytokine, IL-6, contributes to the sensation fatigue, changes cortical activity, and thereby modifies the regulation of power output and performance. Additionally, it assessed to what extent other physiological responses may have in overall perception and regulation of power output and performance.

SUMMARY OF THE MAJOR FINDINGS

CHAPTER 3 – CORTICAL ACTIVITY AND SELF-PACED EXERCISE

The aim of this study was to evaluate changes in the PFC, FC, MC and PC regions during a clamped RPE protocol of low or high intensities during self-paced exercise in thermoneutral or heat stress environments on the α and β bandwidth frequencies and the α/β ratio as an indicator of overall brain state and exercise performance.

Chapter 3 (Study 1) highlights the notion of central sensitisation, although more specific criteria need to be determined for future implications in our field. The findings demonstrate an overall change in cortical activity during an internally regulated, high intensity, self-paced exercise protocol. Significant increases in α activity in high intensity compared to low intensity exercise in the respective environments in all sites measured suggest increase afferent signalling to subcortical areas, while increased β wave activity in the PFC, FC, MC and PC in high compared to the respective low intensity condition, suggests an increase in efferent drive in an attempt to maintain the designated high RPE. These changes lead to a significantly greater change in α/β ratio where both high intensity and the heated low intensity conditions tended to have a greater change, indicative of increased sensory and decreased efferent activity in the FC and MC and PFC, while the PC showed an increase in thermal load in heat stress, and hence, a greater change in α/β ratio, compared to thermoneutral conditions.
CHAPTER 4 – IL-6 AND OTHER PHYSIOLOGICAL RESPONSES TO SELF-PACED EXERCISE

The aim of this study was to evaluate the magnitude of change between the first and second half of a clamped RPE protocol of low or high intensity during self-paced exercise in thermoneutral or heat stress environments on the release of IL-6 and its soluble receptors, and associated regulation of power output as an indicator of behavioural modification in an effort to maintain the desired perception of effort; a further aim was to assess peripheral changes in the heart rate, lactate, blood glucose and skin temperature; and central changes in cerebral haemodynamics and neuromuscular activity.

Chapter 4 (Study 2) represents the additional data from Study 1 which provides empirical evidence supporting the complex integrated systems theory of exercise and fatigue during self-paced, internally regulated exercise. The descriptive nature of this study revealed a picture of numerous physiological systems interacting within the body to maintain a perceived effort during internally regulated exercise of different intensities and environmental conditions. In agreement with the EEG data presented in Chapter 3 (Study 1), the reduction in power output in heat stress and high intensity suggests a significant sensitivity to heat in both central and peripheral regions during self-paced exercise. The notion of central sensitisation to the heat is further supported by attenuated neuromuscular drive and cerebral blood flow in the first 30 minutes when exercising at high intensities in heat stress for 60 minutes. Greater metabolic and inflammatory changes likely contributed to the exacerbated reductions in power output in the second 30 min of the exercise task. These findings indicate that perception of effort is influenced by both internal and external factors of which sensitivity to the heat, combined with increased metabolic and inflammatory signalling, leads to greatest reductions in power output, and hence places environment above intensity on the spectrum of responses during self-paced, internally regulated exercise.
CHAPTER 5 – IL-6, IBUPROFEN AND HEAT STRESS

Chapter 5 (Study 3) aimed to examine the effect of manipulating the IL-6 response using Ibuprofen during a clamped RPE protocol of high intensity in heat stress on the regulation of power output as an indicator of behavioural modification in an effort to maintain the desired perception of effort; peripheral changes in heart rate, core temperature and skin temperature, and central changes in cerebral haemodynamics, neuromuscular and cortical activity.

The results from Chapter 5 revealed no performance benefits during internally regulated self-paced cycling from ingesting an acute 800mg dose of Ibuprofen. Furthermore, there were only minimal reductions in PO. Notably, Ibuprofen did seem to have an effect on physiological systems that suggest a counterbalanced reaction through decreased afferent feedback from the blunted core temperature response, with a concurrent increase in IL-6 release when Ibuprofen was ingested, the combination of which may have prevented any performance benefits. Moreover, while there was no statistical significance found in the EEG between Ibuprofen and placebo conditions, there was a trend for increased α wave activity across the frontal, motor and parietal cortices of the placebo condition, while β wave activity was decreased in the frontal region and the α/β ratio increased in the motor cortex. The increase in α activity is made to represent an increase in subcortical activity that is formed in a non-directional manner and is thought to be from informational processing of stimuli from the periphery. In such instances, this would suggest that the placebo condition had greater afferent feedback than the Ibuprofen condition, perhaps stemming from the increased core temperature and other variables that we did not measure.

CHAPTER 6 – CARBOHYDRATE INGESTION OR RINSE, IL-6 AND PERFORMANCE

The aims of Chapter 6 (Study 4) were to determine the effect of ingesting or rinsing a carbohydrate solution during a self-paced 30km cycling time trial on time to complete the trial, the magnitude of change in power output
within each 10km distance completed; and of IL-6 and its soluble receptors; and central changes in cerebral haemodynamics and cortical activity.

The results from Chapter 6 reveal that a 30km time trial performance was not affected by either rinsing or ingesting a 6.4% commercially available carbohydrate solution, although a more scientifically valid study design might further elucidate these findings. Nevertheless, the CHO rinse trial did result in ~1min faster time trial finishing time, which could translate to performance benefits in an actual race. The two carbohydrate interventions did attenuate circulating IL-6, whereby the ingestion trial was significant compared to the water trial, while the rinse trial only showed a trend towards significance. There was, however, a large effect between the carbohydrate rinse trial and water ingestion trial between 10-20km and 20-30km, which may indicate that even with carbohydrate rinsing, IL-6 is attenuated, although further evidence is need to fully establish this. Most importantly, these findings highlight a potential centrally driven neuroendocrine mechanism derived through sensing CHO which is a specific mechanism that may underpin performance benefits through CHO rinse protocols that have been shown recently. Although this study doesn’t support the contribution of IL-6 signalling to performance decrements, we can’t eliminate other physiological contributions to informational processing that are likely to contribute to the performance and power output regulation as well.

**INTERLEUKIN-6 RESPONSE TO SELF-PACED EXERCISE AND EFFECTS OF MANIPULATING IL-6 WITH IBUPROFEN AND CARBOHYDRATES SUPPLEMENTATION**

**EFFECTS OF INTENSITY AND ENVIRONMENT ON IL-6 AND REGULATION OF POWER OUTPUT AND PERFORMANCE DURING PROLONGED CYCLING**
Studies have identified IL-6 as a marker released through muscular contraction (Febbraio, M. et al., 2003; Febbraio, M.A. et al., 2004) and neuroendocrine responses (Rhind, S.G. et al., 2004; Steensberg, A., Toft, A.D., et al., 2001) during exercise, with potential implications of fatigue during prolonged repeated exercise on consecutive days (Robson-Ansley, P. et al., 2009). However, none so far have attempted to identify it as a marker of performance during different environmental conditions and intensities. Furthermore, none have measured it during a perceptually based protocol in which participants employ internal regulation of physiological systems in order to maintain the desired perceptual intensity on the Borg scale (Borg, G.A., 1982).

In analysis of the data from Chapter 4, the difference between the magnitude change in power output and IL-6 during the first and second half of the protocols was determined to look at the trends and possible associations between an increase in IL-6 and a decrease in performance. Unfortunately, the sample size was not large enough to perform correlation or linear regression analysis as initially planned. Furthermore, the release of IL-6 alone, while slightly greater in the heated high intensity condition, did not match the large decrease in power output in this condition compared to the thermoneutral high intensity condition, where IL-6 accumulation was almost the same. It seems clear that, in the second half of the protocol, IL-6 is exacerbated, while performance is decreased, although the strength of this association is indeterminate from the present data. However, the data would suggest that the strength of this association is minimal as the low intensity condition in the heat saw a large decrease in power output in the second half of the protocol, while the IL-6 accumulation actually decreased in this time frame. These results were unexpected as the release of IL-6 should have been duration and heat dependent, and it should have been greater in the heated low intensity compared to the thermoneutral low intensity condition. Nevertheless, they highlight the ability for other physiological systems to contribute to performance decrements, especially in the presence of increased thermal loads.
Effects of acute Ibuprofen Ingestion on IL-6 and Regulation of Power Output During Prolonged High Intensity Exercise in Heat Stress

To further inform the results of the Chapter 4, IL-6 was next manipulated using the same clamped RPE protocol in the heated high intensity condition, with administration of an acute 800mg dose of Ibuprofen or a placebo one hour prior to the performing the exercise protocol. As Ibuprofen is known to cause intestinal permeability, and with previous research highlighting an increase of IL-6 when Ibuprofen is taken prior to performing an ultramarathon (Nieman, D.C. et al., 2006), it was hypothesised that an acute dose of Ibuprofen prior to prolonged exercise during exertional heat stress would exacerbate the IL-6 response. Consequently, it was also expected that the exacerbated IL-6 response would lead to a decrease in power output. In fact, this was not apparent in the results from the present study.

Changes in power output between the two conditions were not different, despite a significant increase in both in the second half of the protocols, and a slightly greater increase in IL-6 in the Ibuprofen condition. The theory that intestinal wall permeability may underpin the increase in IL-6 during exertional heat stress supports our results from the study in terms of the 3-fold increase in the trials. However, the 3-fold increase in our study does not support the proposed neuroinflammatory model for acute fatigue during exercise, as there was not a significant decrease in power output, despite the increase in IL-6. Thus, the findings in this study too suggest that, while the proposed model of IL-6 signalling central regions cannot be eliminated completely in terms of altering perception of effort and regulating power output, there is not a strong association between its release and regulation of power output, and thus, there are clearly other contributing factors that have an additive effect in the overall perception of effort and subsequent behaviour modification.

According the studies in Chapter 4 and 5, IL-6 may still contribute to a decrease in power output, but only in combination with other sources. This
is further supported by work from Robson-Ansley (2009) that determined the attenuation of IL-6 from ingesting a carbohydrate solution did not increase performance in a 90 min running time trial. Still, while IL-6 does not appear to have a dominant role in performance or regulation of power output during exercise, there is not enough evidence to completely eliminate the possibility that signalling from IL-6 to the brain contributes to centrally processed information that has a cumulative effect of regulating performance. Further evidence for instance, has supported a relationship between IL-6 and fatigue after several days of prolonged endurance exercise (Robson-Ansley, P. et al., 2009), which, combined with the findings in the second study specifically, maintains the role of IL-6 as a contributing factor in the complex integrated systems of fatigue.

**EFFECTS OF CARBOHYDRATE INGESTION OR RINSING ON IL-6 AND PERFORMANCE DURING A 30KM CYCLING TIME TRIAL**

The final study employed a 30km cycling time trial as the results are more applicable to sports performance and actual situations that might occur in the real world. As such the carbohydrate solution used in the study was also a commercially available solution to increase ecological validity. Previous research has shown that carbohydrate ingestion attenuates the release of IL-6 during prolonged exercise (Febbraio, M. et al., 2003; Nehlsen-Cannarella, S.L. et al., 1997; Robson-Ansley, P., Barwood, M., Eglin, C., & Ansley, L., 2009). It has been shown that ingestion of carbohydrate during exercise results in performance benefits (Below, P.R., Mora-Rodríguez, R., González-Alonso, J., & Coyle, E.F., 1995; Carter, J., Jeukendrup, A.E., Mundel, T., & Jones, D.A., 2003), but this finding has also been countered (Pottier, A. et al., 2010; Robson-Ansley, P., Barwood, M., Eglin, C., & Ansley, L., 2009). Additionally, it has been shown that simply rinsing the mouth with carbohydrates during exercise results in performance benefits (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004; Pottier, A. et al., 2010; Sinclair, J. et al., 2014), although the mechanism behind this phenomenon is largely unknown with hypotheses suggesting the brain senses an increase in energy availability from glucose receptors within the oral cavity (Pottier, A. et al., 2010) which leads to increased activity in
reward and motivational pathways in the brain (Chambers, E.S., Bridge, M.W., & Jones, D.A., 2009). Taking the previous literature into consideration, the gap between performance benefits of carbohydrate rinsing and attenuation of IL-6 from carbohydrate ingestion, in accordance with the fatigue hypothesis of IL-6 and knowledge of the neuroendocrine regulation of IL-6, it was sought to determine if carbohydrate rinsing had a similar attenuating effect on IL-6 as carbohydrate ingestion, and if this could be a potential mechanism of the increases in performance seen when carbohydrates are rinsed in the oral cavity during exercise.

Data shows that IL-6 was not attributable to changes in performance. IL-6 increased significantly in the water ingestion trial compared to the carbohydrate ingestion trial, but did not result in overall performance benefits of the 30km time trial. Similarly, rinsing the carbohydrate solution in the oral cavity resulted in a non-significant trend towards decreased IL-6 compared to the water trial as well. Again, this result suggests that IL-6 is not a major contributor to the regulation of power output and performance, and that there is a significant contribution from other internal and external factors on the overall performance during self-paced exercise.

In essence, the results of the release of IL-6 in each of the studies in the thesis provides evidence that there are many factors not related to IL-6 that contribute to regulation of exercise tolerance, perception of effort and performance. Hence, the overall findings in this thesis provide further support for the complex integrated systems theory of fatigue which posits a cumulative effect of all sensory, mechanical and chemical stimuli on the overall perception of effort at any given time during an exercise task and the subsequent regulation of power output and performance in self-paced exercise, while not definitively supporting the neuroinflammatory model of acute fatigue during exercise.

**Effects of self-paced exercise and manipulating the release of IL-6 on the soluble IL-6 receptors**
Changes in the soluble receptor response to IL-6 are difficult to interpret due to the highly theoretical aspect in determining whether an increase or decrease in one receptor is due to agonistic or antagonistic binding, and whether signalling afferent nerves is indeed occurring at any given point during the exercise protocol. Still, few studies have looked at the receptor responses in different intensities and environments, and when manipulating the IL-6 response with carbohydrate or Ibuprofen, thus the present results can inform future research. Generally speaking, the receptor concentrations paralleled one another. Furthermore, both high and low intensity conditions in heat stress and thermoneutral environments, along with carbohydrate ingestion and rinsing, and water ingestion, showed a similar pattern as the IL-6 response (Chapter 4 and 6). When in the event that an Ibuprofen or placebo was taken, however, the receptor responses were markedly reduced compared to the IL-6 response (Chapter 5).

It could be concluded that an increase in sIL-6R suggests greater availability of the agonistic binding receptor. Hence, in Chapter 4, the increase in IL-6 and sIL-6R could suggest an increase in the IL-6/sIL-6R binding complex that is able to signal gp130 cells which are located ubiquitously throughout the body, and specifically, on afferent nerve cells. This could have implications for afferent signalling which may alter performance. However, the pattern of the sgp130 antagonistic binding receptor must be accounted for as it binds to the sIL-6R/IL-6 complex with a greater affinity than the membrane bound gp130 receptor on, for instance, afferent nerve cells. Hence, an increase in sgp130 may suggest an increased ability for the signalling complex to be blocked, thereby reducing afferent signalling. Likewise, a maintenance or decrease in sgp130 may suggest a similar event. For these reasons, some research has identified the receptor ratio as an indicator of signalling potential, namely in that a greater increase in sIL-6R compared to sgp130 would increase the probably that the sIL-6R/IL-6 complex can bind with the membrane bound gp130 receptor and propagate the signal (Jostock, T. et al., 2001). Receptor ratio data is presented in Appendix #11as there were no significant increase in sIL-6R compared to sgp130, hence suggesting the potential blocking of afferent signalling. However, IL-6 can signal in many local areas and hence local nerve tissue
may still be sensitive to circulating IL-6 in the presence of sIL-6R. These hypotheses are rudimentary at best and require further research to understand the mechanisms of afferent signalling through sIL-6R/IL-6 complex binding.
<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>Conditions</th>
<th>Power Output or Performance</th>
<th>Cardiovascular</th>
<th>Thermoregulatory</th>
<th>Metabolic</th>
<th>Neuromuscular</th>
<th>Cerebral Blood Flow/Cortical Activity</th>
<th>Inflammatory/Endocrine</th>
<th>Perceptual</th>
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<tbody>
<tr>
<td><strong>Study 1</strong></td>
<td>Cycle - 60min Clamped RPE 16 (HIGH) and 12 (LOW)</td>
<td>HS: 35°C NT: 22°C</td>
<td>PO HS ↓ NT*, PO HS16 ↓ NT16*</td>
<td>HR ↑ HIGH*</td>
<td>Tsk HS16 ↑; NT16*</td>
<td>La: ↓1 HIGH; BG ↑ HS*</td>
<td>NT16 ↑; HS16*</td>
<td>α &amp; β HIGH; ↑ LOW and HS ↑*</td>
<td>IL-6 HIGH ↑; LOW*; sIL-6R HIGH ↑; LOW*; α &amp; β HIGH; ↑ LOW &amp; HS ↑*</td>
<td>RPE HIGH ↑ LOW</td>
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<td><strong>Study 2</strong></td>
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<td><strong>Supporting Literature</strong></td>
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<tr>
<td><strong>Study 3</strong></td>
<td>Cycle - 60min Clamped RPE 16</td>
<td>IBU PLAC</td>
<td>PO ↔ HR ↔ Tsk ↔ Tc ↔</td>
<td>-</td>
<td>-</td>
<td>IBU ↓#</td>
<td>IL-6 ↑#</td>
<td>α/β PFC, FC, MC, PC ↑#</td>
<td>sIL-6R &amp; sgp130 ↔</td>
<td>RPE ↔</td>
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<td><strong>Supporting Literature</strong></td>
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**Note:** Study methods and conditions, power output or performance, cardiovascular, thermoregulatory, metabolic, neuromuscular, cerebral blood flow/cortical activity, inflammatory/endocrine, and perceptual changes are detailed in the table. The table includes studies conducted under various conditions, with changes in power output, cardiovascular parameters, thermoregulatory responses, metabolic status, neuromuscular activity, cerebral blood flow, and inflammatory/endocrine and perceptual changes.
<table>
<thead>
<tr>
<th>Study</th>
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<th>Power Output or Performance</th>
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<th>Neuromuscular</th>
<th>Cerebral Blood Flow/Cortical Activity</th>
<th>Inflammatory/Endocrine</th>
<th>Perceptual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 4</td>
<td>Cycle - 30km TT</td>
<td>WATER CARB RINSE</td>
<td>Performance ↔</td>
<td>HR WATER ↑</td>
<td>CARB &amp; RINSE; HR CARB ↑</td>
<td>WATER*</td>
<td>-</td>
<td>-</td>
<td>α PFC CARB</td>
<td>IL-6 WATER ↑</td>
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<td>Supporting Literature</td>
<td>-</td>
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<td>Pottier et al. (2010)</td>
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<td>HbTot ↑</td>
<td>HbDiff↑</td>
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</table>

#main effect for time (Study 1,2,3) between first (1) or second (2) half or distance (Study 4) between 10 (a), 20 (b) or 30 (c) km; |main effect for intensity; &main effect temperature; *significant interaction

Note: Cortical Activity results have been simplified for general comparisons. Refer to the respective Chapter for exact results; only significant findings are presented in this table, no ES.

Table 7-1. Physiological responses from studies and respective supporting literature.
CORTICAL ACTIVITY DURING SELF-PACED EXERCISE – EVIDENCE OF ACUTE SENSITISATION?

Findings from the present thesis are quite novel in terms of cortical activity and the possibility of acute central sensitisation as an indication of overall brain state and fatigue. Indeed, no previous studies have evaluated the effects of prolonged self-paced exercise on cortical activity measured through EEG and specifically, the dynamic profile across the duration of the exercise task. Data from the present thesis elucidates the effect of self-paced exercise on α and β wave activity, along with the α/β ratio, which has previously been interpreted as a marker of fatigue (Nybo, L. & Nielsen, B., 2001; Rasmussen, P., Stie, H., Nybo, L., & Nielsen, B., 2004). An increase or maintenance of α activity, combined with a decrease in β activity is believed to signify fatigue through increased low frequency or calming activity with a concomitant maintenance or decreased high frequency or active activity (Nybo, L. & Nielsen, B., 2001). Furthermore, some research suggests that it could also reflect increased sensory and decreased efferent motor drive, and therefore acute central sensitisation within the CNS (Mima, T., Matsuoka, T., & Hallett, M., 2001; Mima, T. et al., 2000).

It is interesting that every study in the thesis found the α/β ratio to decrease from baseline, or the respective first snap shot at 5 min (Study 1, and 3) or 500 meters (Study 4) into the designated protocol, whereas previous studies have shown an increase in α/β ratio from baseline. This may have been caused by the fact that participants cycled with their eyes open during the snapshots, while other studies may have used an eyes closed method (not reported). Additionally, it could be due to the nature of the self-paced protocol all together. We err towards the later explanation as self-paced exercise enables internal regulation in a dynamic manner, which is evident across the 60 min profile of EEG in Chapter 3, although further investigation is needed to support this conclusion.
A self-paced exercise protocol enables individuals to take into account how they are feeling and modulate their power output at any given time in response to the perception of effort (Singer, R.N., 2000). It could therefore be postulated that the dynamic workings of informational processing and regulation of power output based on perception, caused the decrease in the α/β ratio. Nevertheless, it remains possible that changes in α/β ratio reflect the overall state of the brain and specifically, a ratio between the sensory information received and efferent signals being sent.

The analysis of all data as presented in chapters 4, 5 and 6, respectively, were performed by averaging the data for each snapshot in the first half and second half of the protocols and identifying the magnitude of change between the protocols. Because each of the studies employed a self-paced exercise protocol, we used the first 5 min, or 500 metre distance snap shot to normalise data. This enabled the data to be compared to a snapshot from when the participants were more or less ‘fresh’ in the beginning of the protocol.

The EEG data in Chapter 3 were normalised to baseline and analysis was performed based on the consecutive snap shots over the duration of the protocol every 5 min to depict the overall profile of the EEG changes. In Chapter 5 and 6, however, normalising data to the baseline snapshots resulted in a biased increase in the first half of the protocol as the baseline snapshot was markedly different at rest compared to exercise. As such, it would be expected that there would be a significant increase in the first 30 minutes. This, however, did not give an accurate depiction of the average increase in the first 30min during exercise compared to second 30min. For these reasons, the first snap shot was used for normalisation of the data with the assumption that the early stages of exercise may reflect minimal changes as individuals were only in the beginning stages of the protocol.

It is important to note, however, that this method could also have its flaws, especially in the 30km time trial protocol in which individuals were not required to cycle at a specific intensity, only to complete the trial as quickly as possible. As such, it is plausible that pacing strategies in some
individuals resulted in altered cortical activity compared to others. This could account for the minimal changes in EEG that were found during the carbohydrate ingestion and carbohydrate swill interventions.

Recently, studies have postulated that the β frequency in an EEG recording is associated with directional flow from the cortical areas to the muscle, hence reflecting efferent drive (Mima, T. et al., 2000). On the contrary, α frequencies are believed to represent non-directional activity, primarily within subcortical regions (Mima, T., Matsuoka, T., & Hallett, M., 2001). It can be assumed therefore, that α waves might reflect informational processing that presents itself at the thalamus and other subcortical regions and branches up into higher cortical areas. Therefore, it can be postulated that cortical changes in an EEG represent overall excitation and central sensitivity during an acute bout of exercise due to the feedback that may be apparent in the α wave activity, and concomitant changes in β activity. Like the α/β ratio theory of fatigue which suggests an increase in α waves represents a calm or resting state, while a maintenance or decrease in β waves represents reduced activity, this hypothesis can be extended to involve increases in sensory information, or central sensitisation seen through an increase in α wave activity, with a concomitant decrease in efferent drive represented through β wave activity.

Central sensitisation can be found at any point along the spinal cord and into the central regions of the brain. It is caused by an increase in excitatory synaptic transmission and a decrease in inhibitory synaptic transmission (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008), thus causing hyperactivity of neuronal cells with a reduction in inhibition. It has been shown that hyperactivity of synaptic transmission is strongly implicated in persistent pain models (Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008) and hence, while central sensitisation is primarily regarded as a mechanism in chronic pain and fatigue, if similar mechanisms occur in an acute sensitising manner during exercise, central sensitisation to sensory feedback may indeed be a plausible hypothesis for the development of fatigue and regulation of power output. While data in the first study,
especially, seem to allude to central sensitisation that can be measured through EEG, further research is necessary to support the theory.

**OTHER CONTRIBUTING FACTORS TO THE REGULATION OF POWER OUTPUT AND PERFORMANCE DURING SELF-PACED EXERCISE**

The overall evidence from the studies in the present thesis suggest that the IL-6 response to exercise is not the only peripheral change that causes signalling and alters overall cortical activity and regulation of power output or performance. Hence, the extent that other physiological systems change during the studies is important to consider. Table 7-1 has been included in order to show the results of the data from the present thesis, and includes supporting literature for some of the responses that were found, however, there are several responses that have not been studied in terms of self-paced exercise, especially in a clamped RPE protocol, or in the heat, and thus, much interpretation of the integrated aspects of the physiological systems are only theoretical. Nevertheless, it is anticipated that data contained within the studies in this thesis will instigate further investigation into these areas.

**CARDIOVASCULAR RESPONSES**

Heart rate responded to self-paced clamped RPE exercise in an intensity dependent fashion in which there was no change in the first 30 min compared to the first 5 min snap shot, although it continued to increase thereafter. Interestingly, despite the RPE being clamped at the 16 for both high intensity conditions, heart rate in heat stress was greater than in thermoneutral in the first 30 min (not significant). Therefore, there seems to be dissociation between heart rate and RPE, potentially owing to the increased thermal load and anticipation of the requirements of the exercise task (Albertus, Y. et al., 2005). Additionally, there was a main effect for time revealing a decrease in heart rate over the second half of the protocol overall, despite the increase in the thermoneutral high intensity condition.
The first have of the protocol reflects the findings by Tucker *et al.* (2006), in which they report an increase in heart rate in the heat compared to cool conditions, however, this was not significant and, they certainly didn’t see a decrease in HR in heat stress. The discrepancy here is likely due to the extended time that the protocol in the present study required. Participants in our study were told to maintain an RPE of 16, but complete a full 60min protocol, with intermittent 30s sprints every 10min (data not shown). The combination of anticipation in completing the full protocol, sensory feedback and physiological stress, would have caused the large decrease in HR in the second half compared to the protocol by Tucker *et al.* (2004), in which the protocol ceased once participants dropped below 70% of their peak power output.

Interestingly, heart rate was not significantly different when either Ibuprofen or a placebo was ingested in the heated high intensity protocol. This is in accordance with the lack of significance found for changes in performance. There was a large effect for Ibuprofen to increase in the first 30 min, however this was blunted in the second half of the protocol, while heart rate in the placebo condition was increased (again, not significant). Still, this finding is in accordance with earlier research that suggests a blunted effect of COX-2 inhibitors on cardiac strain during exercise (Bradford, C.D. *et al.*, 2007).

During the 30km TT with carbohydrate supplementation, heart rate was significantly attenuated in carbohydrate rinse and ingestion conditions between 10-20km, compared to when water was ingested. This, however, was contested in the final 10km where heart rate decreased in the carbohydrate rinse and water conditions, but increased significantly in the carbohydrate ingestion condition. Overall, it can be concluded that the heart rate response to self-paced exercise that is internally regulated during a clamped RPE protocol is dependent on intensity and environment, with environment playing a large role. Furthermore, the heart rate response during a performance time trial can be attenuated, at least initially, with both carbohydrate rinsing and ingestion, without implicating performance. While there were no differences in performance, power output in the
carbohydrate ingestion trial did increase in the final 10km (not significant) in accordance with the significant increase in heart rate, which may indicate a greater availability of energy and hence result in an end spurt that is greater in the carbohydrate ingestion trial compared to water and carbohydrate swill.

**THERMOREGULATORY RESPONSES**

Thermal sensation is suggested to have large implications on both performance and selecting the initial intensity during a self-paced exercise protocol. Indeed, it has been shown that skin temperature alone, or the initial sensation of the external environment, provides sensory information that cannot be discounted in terms of the overall regulation of power output and performance (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011). Indeed, in the clamped RPE protocols, skin temperature nearly mirrored the power output in the first and second half of each condition. Most notably, skin temperature in the heated high intensity condition was significantly increased in the second half compared to the thermoneutral high intensity condition, while power output was significantly decreased in heated conditions compared to thermoneutral conditions. These findings provide further evidence that skin temperature and thermal signals (Schlader, Z.J., Simmons, S.E., Stannard, S.R., & Mündel, T., 2011; Tucker, R., Marle, T., Lambert, E., & Noakes, T., 2006; Tucker, R., Rauch, L., Harley, Y., & Noakes, T., 2004) can be implicated in overall power output during an internally regulated self-paced protocol.

Similar to other variables, the skin temperature was not markedly different during high intensity heated conditions when Ibuprofen or a placebo was ingested. There was not even a main effect for time which would have been expected. Likewise, core temperature was not significantly different between the first and second half of the protocols, although there was a moderate effect for core temperature in the Ibuprofen condition to be attenuated. The only conclusion we can make of the lack of significant findings in changes in thermal strain in Chapter 5 is that, after the familiarisation session, individuals may have entered into the first
experimental session hesitant, and hence there may have been a disconnect between the reported RPE and designated power output. This is especially valid in the fact that participants were only recreationally active and hence the protocol was challenging to complete for all (anecdotally speaking, they each struggled throughout and several were reluctant to come back for a second trial). Nevertheless, the effect of skin and core temperature cannot be discounted in providing afferent signalling to central regions and hence altering the perception at any given moment.

**METABOLIC RESPONSES**

Blood lactate and glucose responses were only measured in Chapter 4 so as to provide an indication of other changes within the peripheral blood response that may have contributed to the overall feelings of perception and cortical activity, or effected the regulation of power output. Results confirm that the high intensity conditions resulted in increased glycolysis and lactate build up in the first half of the protocol. Based on blood glucose responses, it is highly likely that this stemmed from muscle glycogen breakdown, especially in the thermoneutral high intensity condition as the blood glucose levels dropped, hence suggesting an up take of glucose into the muscles. In heated high intensity exercise, blood glucose levels were increased, however it remains plausible that glycogen depletion, the release of IL-6 to signal hepatic glucose release, and the addition of heat stress, caused the increase in blood glucose in the heated conditions. Even low intensity conditions followed the same pattern as the respective high intensity condition and hence the reduction in blood glucose in thermoneutral low intensity is due to glucose initially being utilised from circulating stores wen available, hence sparing glycogen in muscle (Fielding, R. et al., 1985). In contrast, the increase in the low heated condition could indicate upregulated hepatic glucose release from heat stress (Hargreaves, M., Angus, D., Howlett, K., Conus, N.M., & Febbraio, M., 1996).

Interestingly, lactate was removed from the system and returned to near baseline levels in the second half of both high intensity protocols, while power output continued to decrease. Although it seems lactate is unlikely to
be responsible for a large role in performance regulation in the later part of the trials, it is still plausible that the build-up of lactate in the first half of the high intensity protocols provided some of the sensory information that resulted in behaviour modifications reflected in decreased power output, which, in itself, would have reduced the metabolic strain and enabled lactate which had accumulated to be used for energy, hence reducing the circulating levels within the second half of the protocol.

**CEREBRAL HEMODYNAMICS**

During exercise, it is generally found that cerebral blood flow is increased and maintained, however the actual dynamics of haemoglobin concentration can give a better indication as to the oxygen uptake in the designated region of measure. During low intensity exercise, total haemoglobin concentration, increased, though not significant in the first half of the protocol, followed by a slight decrease in the second half in the heated condition, while in the thermoneutral condition, it increased slightly. The difference in haemoglobin concentration, however, provides a better indication of the amount of oxygen that is taken up by the brain. The haemoglobin difference revealed an initial (non-significant) increase, suggesting that there was slightly greater oxygen desaturation in both low intensity conditions and the thermoneutral high intensity condition over the first half of the protocol, while it dropped in the second half of the protocol. In contrast, the heated high intensity condition did not have an increase in desaturation, hence suggesting preservation of oxygen utilisation, perhaps due to the sensation of the heat and anticipation of the high intensity required (Rasmussen, P. et al., 2010). This was also apparent in the total haemoglobin concentration in heated high intensity exercise, whereas that for thermoneutral high intensity exercise showed a large increase in total haemoglobin concentration in the first half of the protocol, followed by marked decrease in the second half of the protocol.

When Ibuprofen or a placebo was ingested prior to high intensity exercise in heat stress, total haemoglobin concentration increased to the same levels in the first 30 min as in the heated high intensity condition in Chapter 4, with a
significant decrease in second half of the protocol, and no differences between conditions. Hence, the ingestion of Ibuprofen didn’t have an overall effect on blood flow to the brain. Interestingly, though not significant, Ibuprofen had a greater increase in haemoglobin difference in the first half of the protocol ($d>0.8$). This might suggest a greater utilisation of oxygen within the first half of the Ibuprofen trial, while it decreased and reached similar levels as the placebo trial in the second half. An increase in oxygen utilisation when Ibuprofen is ingested may indicate greater activity in the PFC, and hence, an ability to maintain PO despite the potential signalling from increases in IL-6.

While no significant differences were seen between conditions in the Chapter 6, there was a main effect for total haemoglobin concentration to increase within the first 10km cycled. Interestingly, compared to clamped RPE low and high intensity thermoneutral conditions, total haemoglobin concentration in the first 10km in all three conditions in Chapter 6 were attenuated. Carbohydrate rinse and ingestion and water ingestion trials resulted in an increase in ~50, 25 and 70% total haemoglobin, respectively, whereas the clamped low intensity exercise in thermoneutral environments increased to ~100% and high intensity increased to ~275% within the first 30 minutes. Although technically these results can’t be compared directly, due to participant variability, it is curious to point out the large differences. This was also seen in the haemoglobin difference data in that carbohydrate rinse and ingestion and water ingestion increased only to ~50% in all trials, while in the clamped low intensity it increased to ~400% in the first half, and in the high intensity, ~250%. The most plausible reason for this is that there is inherent differences in the two protocols, one which requires an individual to internally regulate their pace based on a perceptually based protocol, and the other to perform their best in the overall time trial. Anecdotally speaking, there was a lot of discussion from the participants about how to perform their best in the time trial and it is likely that they employed some anticipatory strategies in which they chose a pace in the beginning that did not require the same amount of oxygen delivery or utilisation to the brain as in the clamped RPE protocol.
**NEUROMUSCULAR DRIVE**

Neuromuscular drive reported in Chapters 4 and 5 provide further indication into central regulation of power output and efferent drive within each condition. Regardless of environment and intensity, EMG increased in the first half of all conditions to ~5% greater than the initial measure. Hence, there is an apparent disconnect between the decrease in power output and neuromuscular recruitment in the first half of the protocol whereby the neuromuscular drive in the first 30 minutes increases and remains stable regardless of power output. Neuromuscular recruitment in the second half of each protocol is curious. In the low intensity conditions, and heated high intensity conditions (including placebo in Chapter 5), EMG was maintained at about the same 5% level despite a decrease in power output in the heat stress conditions. It has been shown that EMG is attenuated in heat stress in anticipation of the exercise task, so this may explain the reduction in the high intensity heat stress environment (Tucker, R., Rauch, L., Harley, Y., & Noakes, T., 2004). Most notable was the increase in EMG in the second half of the thermoneutral high intensity condition, despite a small reduction in power. This finding suggests the ability to maintain central drive throughout the duration of the 60 minute protocol and to contest the reduction in power output with an increase in neuromuscular drive.

**SUMMARY OF THE NEUROINFLAMMATORY MODEL OF ACUTE EXERCISE FATIGUE AND CENTRAL SENSITISATION**

The neuroinflammatory model of acute fatigue during exercise was developed and an attempt made to test the hypothesis in multiple ways in the present thesis. The neuroinflammatory model posits that the release of cytokines, specifically of IL-6 can send sensory information to the brain which contributes to the overall informational processing, conscious or unconscious perception of fatigue and subsequent behaviour modification during exercise. Through the course of testing, however, it became
increasingly obvious that quantifying the amount that IL-6 contributes to the phenomena of fatigue is not an easy task and hence, the notion of central sensitisation was stumbled upon. Central sensitisation is largely implicated in chronic pain and fatigue, however, cytokines are known contributors to central sensitisation and hence can alter the excitatory and inhibitory processes within the brain and reduce the threshold for pain or fatigue. Considering these theories, central sensitisation, albeit in an acute manner, is an appropriate theory for exercise induced transient fatigue. This is a new concept within the field of exercise physiology and should be considered in future research.

The challenge remains to identify a measure of central sensitisation in humans both in exercise and disease. The present thesis likens changes in the cortical activity measured through EEG as an overall indication of central sensitisation, although the exact mechanisms of inhibitory and excitatory pathways remain to be elucidated. Nevertheless, the overall model presented in the thesis may promote further investigation into the likelihood that central sensitisation can be an accurate contributor to acute exercise fatigue, added to by the release of cytokines and ability for them to propagate signals and alter CNS activity.
8. SUMMARY AND CONCLUSIONS
THESIS AIMS AND CONCLUDING STATEMENTS

THESIS AIM #1

To evaluate the exercise response of IL-6, regulation of power output and associated performance during self-paced exercise between different intensities, environments and interventions.

- IL-6 is increased during exercise in an intensity and environment dependent manner, however there are is no conclusive evidence that IL-6 contributes to the concomitant decrease in power output when exercising in the heat.
- IL-6 is exacerbated during exertional heat stress and even slightly more when Ibuprofen is ingested, potentially due to exacerbating the intestinal wall permeability, hence, ingestion of Ibuprofen prior to exercise in heated conditions may be detrimental, although there was no reduction in power output reported in the present study, potentially due to inherent variability in pacing and regulation of power output by participants.
- IL-6 appears to be attenuated during a 30 km cycling time trial when a commercial carbohydrate solution is ingested, with similar trends occurring when with only rinsing the carbohydrate solution. Performance does not appear to be effected, however, a more tightly controlled trial may increase performance benefits in terms of when and how much solution participants ingest/rinse.

Collectively, these studies suggest that IL-6 is not a significant contributing factor to the sensation of fatigue and regulation of power output during prolonged exercise. Instead, it seems likely that, while IL-6 might possess the ability to signal through afferent pathways during exercise, numerous other physiological pathways contribute to the overall sensation of fatigue, regulation of power output and performance.
THESIS AIM #2

Evaluate the changes in cortical activity, regulation of power output and associated performance during self-paced exercise between different intensities, environments and interventions.

- Self-paced exercise of high intensity and in the heat has a tendency to increase α and β wave activity in the FC, MC and PC regions in the brain, suggesting increased sensitisation during exercise tasks of greater internal and external demands.
- Similarly, α/β ratio changes were associated with intensity and thermal loads in the FC, MC and PC, suggesting greater changes during exercise tasks of greater internal and external demands.
- Ingestion of Ibuprofen in the heat changed α/β ratio at the PFC, FC and MC, but not at the PC, with the MC showing a substantial change in the Ibuprofen condition compared to the placebo which may be indicative of increased sensitisation from an increase in sensory feedback.
- Carbohydrate ingestion and rinse only affected α feedback in the PFC, but changes with the water ingestion trial, hence ingesting and rinsing carbohydrates may have different effects on decision making processes.

Collectively, the results from chapters 3, 5 and 6, suggest that there is an increase in cortical activity that may be dependent on increased afferent signalling, and reflected in increases in α activity within the protocols. Furthermore, changes in cortical activity may also be dependent on increases in β activity due to an increase in neuromuscular drive. However, these results are impeded by the fact that there were no significant changes within α and β bandwidth frequencies in the Ibuprofen study, nor in the carbohydrate ingestion and swill study, with the exception of changes in α in the PFC. Importantly, the lack in changes in performance in either of these
studies may be reflected in the lack in changes in cortical activity, compared to the first study. Moreover, it is interesting to note that the change in $\alpha/\beta$ ratio across all studies revealed a decrease during self-paced exercise whereas those in fixed intensity exercise have all shown an increase. This a curious finding, however we suggest it may be due to the nature of self-pacing and the overall state of the brain during a self-paced task, although further research is warranted to interpret this theory.

**Thesis Aim #3**

Evaluate the extent that physiological responses including heart rate, thermal load, plasma glucose and lactate metabolism, cerebral blood haemodynamics and neuromuscular drive might contribute to altered regulation of power output and performance changes during self-paced exercise between different intensities, environments and interventions.

Interleukin-6 is primarily regulated by intensity, duration and environment, with environment having a larger effect than previously suggested. Furthermore, an exacerbation of IL-6 after taking an acute dose of ibuprofen prior to high intensity exercise in environmental heat stress does not affect perceptions of effort, regulation of power output and therefore performance. Similarly, attenuating the release of IL-6 through either rinsing or ingesting a carbohydrate solution during a 30km time trial did not prove to have significant performance benefits, although there were trends towards a reduced time to completion and greater power output. The essence of these results is that exercise tolerance and regulation indeed occurs in response to numerous internal and external factors, perhaps including IL-6, that provide sensory information that is processed at central levels to inform the brain. These other factors include peripheral internal and external thermal stimuli, cardiovascular and thermoregulatory strain, metabolic processes, along with central regulation of efferent drive, cerebral haemodynamics and anticipatory regulation of demands for the required task.

**Recommendations for Future Research**
At present, the complex integrated systems model of fatigue focuses primarily on limitations that exist in regards to metabolic activity and substrate accumulation during exercise. The present thesis highlights a potential role for IL-6 to contribute to feelings of fatigue as one of numerous systems that interact and form a respective perception of effort during exercise at any given moment. Indeed, in their review of the complex integrated systems theory of fatigue, Lambert et al. (2005) suggest the possibility for chemo and mechanoreceptors to signal the brain, of which is possible through signalling of IL-6 at afferent nerves (Andratsch, M. et al., 2009; Kawasaki, Y., Zhang, L., Cheng, J.K., & Ji, R.R., 2008). While evidence in the thesis does not wholly support the neuroinflammatory model (Vargas, N.T. & Marino, F., 2014) presented, further research will help to elucidate the strength of the possible relationship between IL-6 and acute exercise fatigue, and the mechanisms by which the release of IL-6 can alter the associated cortical activity. Future research is also warranted in terms of the neuroendocrine response of IL-6, and whether carbohydrate rinsing effects glucosensing neurons in the brain and activates glucocorticoid release in the HPA-axis, hence attenuating the release of IL-6. Although there were no performance benefits when rinsing carbohydrates, previous literature suggests otherwise (Carter, J.M., Jeukendrup, A.E., & Jones, D.A., 2004; Chambers, E.S., Bridge, M.W., & Jones, D.A., 2009; Sinclair, J. et al., 2014), and hence, it would be advantageous to further elucidate the underpinning mechanisms.

Finally, much research is warranted in terms of cortical activity and measurements through electroencephalography. While it is generally accepted that the changes in α and β waves indicate the relative calm or active state of the brain, respectively, the physiology that underpins cortical activity and changes in brain wave frequencies should be further researched, especially in terms of exercise response. Likewise, a new model of acute central sensitisation measured by cortical activity warrants further investigation. Central sensitisation is an applicable theory in terms of acute changes that may occur in the periphery during exercise, which can alter excitation and inhibition within the CNS. However, whether the relative changes in α activity, which are believed to indicate sensory information
(Mima, T., Matsuoka, T., & Hallett, M., 2001), and β activity, which are believed to represent efferent motor drive (Mima, T. et al., 2000), can accurately reflect central sensitisation in this way remains elusive. Hence, the overall theoretical aspects within the present thesis stimulate much research that can advance our field in terms of acute exercise fatigue induced through a neuroinflammatory response and the overall acute central sensitisation that may or may not be attributed to information processing and the perception of fatigue at any given point during an exercise task.

**LIMITATIONS OF THE THESIS**

There are numerous inherent limitations and assumptions that must be acknowledged in physiological studies of the present kind. This list is by no means exhaustive; however, it is important to acknowledge:

- Physiological responses within the designated participant cohort of healthy, recreationally active, 18-35 (Study 1 and 2) males may not be applicable to other populations, especially those who are not classified as recreationally active, who are not male, and who out of the designated age range. Likewise,

- Physiological responses within the designated participant cohort of healthy, endurance trained 18-60yr old (Study 3) males and females may not be applicable to other populations, especially those who are not classified as endurance trained, or who are outside of the designated age range.

- The collection of EEG is predisposed to artefact that can be compromised by noise, visual stimuli, muscle contraction, among other issues. EEG analysis should be done in a very rigorous manner to ensure quality data for reporting.

- Due to the inherent individual physiological variability and nature of self-paced protocols, the heart rate, electromyography, electroencephalography and near-infrared spectroscopy responses are inherently variable and cannot be assumed to change in the same direction and the same way, hence individual data were presented in graphs alongside mean data for the reader’s information.
It is worth noting that perhaps using the clamped RPE 16 protocol to determine the effects of Ibuprofen on IL-6 and regulation of power output could have been replaced with a time trial type of protocol to determine the performance benefits (if any) as the complex interactions between physiological systems may have attenuated the effects of Ibuprofen.

Likewise, there is a large assumption in that individuals performed the clamped RPE trials as expected.

Study 4 only used water for a placebo condition, which could have affected the results, even though the experimental design was randomised and counter-balanced. Furthermore, individuals ingested or rinsed the respective solution in the first trial ad libitum. They were required to ingest/rinse the same amount at the exact same time in subsequent trials. The variability within this protocol may account for the lack of performance benefits seen. It would be beneficial to perform this study with designated ingestion/rinse times.

**DELIMITATIONS OF THE THESIS**

Participants were requested to refrain from caffeine or alcohol in the 24hrs prior to any experimental testing session and were strictly screened for pre-existing inflammatory or neurological disease or any cold within 3 weeks prior to participating in the trials.

Participants were required to fast for at least 8 hrs prior to the experimental exercise testing sessions except in study 2 (Ibuprofen study), where they were allowed to eat a breakfast consisting of low carbohydrate and high in protein and fat nutrients to reduce effects of taking Ibuprofen on an empty stomach.

Participants were required to maintain a food diary for the 24hr period prior to their first experimental trial and were requested to follow the same diet for the 24hr period prior to each subsequent trial. The CI checked these to ensure they kept the same diet.
EEG data were quality checked and screened very rigorously to ensure data presented do in fact represent changes that occurring due to neural responses and not from other issues artefact.

All data collection procedures were standardised and completed by the chief investigator so as to reduce variation between all testing sessions for every study.

Participants were familiarised with the exercise protocols prior to performing them, and with cycling at variable ratings of perceived exertion to eliminate a learning effect after the first trial.

**Final Remarks**

There remains no question that we humans, and other species, have evolved, and so too has our evolutionary behaviour that enables us to perceive the world around us and adapt accordingly. Moreover, as human beings, we have a unique experience in being able to feel, and communicate how we feel at any given time. The perceptual manifestations of these conscious feelings and subsequent behaviours may never be understood, however, the natural progression of science and knowledge development is that we create new ideas, test the hypotheses and continue to develop evidence for or against a specific theory.

Regardless of whether fatigue is acute or chronic, due to exercise or illness, it is a feeling that everyone can identify with. The phenomena behind why we feel fatigue – the integration of all our different sensory feedback, the anticipation of a large task ahead, the anxiety of something that has happened – can all add to the general fatigue threshold in our everyday lives and in exercise too. It is my belief that this threshold, when reduced, may cause changes in cortical activity and associated central sensitisation. Although the release of cytokines, and specifically IL-6, is clearly not the only contributing factor to the overall sensation, it remains difficult to eliminate it all together considering its wide role in exercise and illness alike.
In the least, let this thesis be a reflection of not only what I have learnt in terms of developing my research skills and knowledge, but also in terms of life over the journey of the PhD. If nothing comes of it, I can attest to the fact that it has increased my skills as a critical thinker in research and beyond, and has encouraged me to continue seeking answers to knowledge of which I am curious, or that remains unknown. My final thought in concluding this thesis is that science is like life. Science is life, but life is also science, both a perpetual cycle of understanding and questioning, but alas, that is the splendour of it all!

“In life, we think that the point is to pass the test or overcome the problem. The real truth is that things don’t really get solved. They come together for a time, and then they fall back apart. Then they come together again and fall apart again...”

- Pema Chodron
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10. APPENDICES
APPENDIX 1: INFORMATION AND INFORMED CONSENT
- STUDY 1 AND 2

INFORMATION SHEET

Changes in corticomotor excitability and central activation following the acute-phase inflammatory response induced by fatiguing aerobic exercise in normothermic and hyperthermic environments

Thank you in advance for your interest in this research project. Please read and understand the information on this page and retain it for your records. If you have questions or concerns regarding this study, please feel free to contact:

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About the Project

The study of fatigue and what makes people cease exercise has been prevalent in the field of exercise science. It is widely thought that there are several physiological regulatory systems that work together in order to communicate how much effort can be put forth, and when we need to slow down. Likewise, it is thought that these systems exist as mechanisms to prevent catastrophic events occurring from over-exertion. In an effort to further study different systems within regulation of exercise exertion, the present study focuses on the role of the inflammatory system, as previous literature has documented an inflammatory response during both intense strength training exercise and prolonged endurance exercise.

Purpose

The present research study aims to determine how the acute-phase inflammatory response to exercise modulates how much muscle recruitment is available and how we perceive our levels of exhaustion or fatigue. Thus, the study protocol will aim to create an inflammatory response from prolonged endurance exercise to see how it affects activity levels within our brain, our ability to voluntarily activate or contract our muscles, and the perceptions of how we feel in both normal and heated environments.

Anticipated Findings

It is anticipated that the study will aide in furthering our understanding of the processes of the acute phase inflammatory response induced through exercise and its effects on the excitability of the motor cortex in the brain, voluntary activation, and the perceived levels of exertion or fatigue. It is expected that there will be an increase in fatigue, a decrease in motor unit recruitment and increased excitability in the motor cortex due to the inflammatory response from exercise.

Study Design
Inclusion criteria for this study requires that participants have no prior sickness or infection whether virus or bacterial and is not presently taking or has taken any anti-inflammatory medications or medication for any type of virus or bacterial infection at least 7 days prior to testing. Likewise, they must currently engage in at least 60 minutes of continuous aerobic exercise, at least 3 times/week. Exclusion criteria include symptoms or risk factors for sedentary lifestyle diseases, musculoskeletal conditions that would constrain the ability to perform prolonged, fatigue cycle ergometry, and conditions that alter the brain activity. Participants must be males between the ages of 18 and 35. **Participants will be required to keep a food diary for the 24 hours prior to each test and they must abstain from caffeine and alcohol, as well as intense exercise for 36-48hrs prior to testing. On the day of the trial, participants must present to the lab in a fasted state, but can drink water ad libitum. Participants must report to the lab wearing workout clothes and closed toe running shoes and bring a water bottle with them.** Participants may be asked to return for further testing in the future as a follow up from this study, but it is not required in order to participate in the present study.

Participant Requirements

The study will involve 5 sessions in which the participant will be required to report for testing. The first session will be a familiarization/screening session. The final 4 sessions will be the experimental sessions in which subjects will either perform non-strenuous exercise, or strenuous exercise in normal or heated environmental temperatures, to determine effects of the exercise and heat on the inflammatory response, and on muscle and motor cortex activity levels as described earlier.

Pre and In-Between Session Procedures

All participants will be required to refrain from any aerobic or strength conditioning exercise for 36-48hrs prior to each session. Participants will be encouraged to follow similar dietary patterns before each testing session. Testing sessions will be completed with at least 3-5 days rest between each.
1. **Familiarisation/screening Session**: This session will involve a test to determine peak power output (PPO) and during a graded exercise cycle test. Participants will be required to perform a self-paced warm up for 5 minutes prior to beginning the test. The resistance on the pedals will begin at 50W and increase every minute by 20W during the test until the participant can no longer continue or the cadence drops below 65revs/min. The participant will be required to remain seated for the duration of the test and maintain a cadence of 70 revs (min). Prior to the test, the participant will become acquainted with the Borg scale (6-20) for measuring ratings of perceived exertion (RPE) (Borg 1982). This introduction and the proceeding exercise test will help to give them a better understanding of rating their own perceptual exertion, which will help to increase reliability in actual experimental trials.

Following the exercise protocol, participants will be introduced to peripheral nerve stimulation (PNS). At this time, we will set up the equipment and document individual settings. Three maximal voluntary contractions (MVC) of the quadriceps will be performed while collecting muscle activity data. The participant will then complete a portion of the PNS protocol that will be used during the actual experimental trials in order to familiarize them with the stimulus. After the PNS familiarization protocol, there will be brief discussion regarding EEG, NIRS and core temperature pills used during the experimental testing.

Finally, participants will perform a brief (~20min) cycling trial at a fixed RPE of 16 (hard-very hard) in ambient temperature (22°C; 60% relative humidity). The participant will be able to self-pace the exercise but will be continually reminded to remain at an RPE of 16 for the whole time to familiarize them with what this means.

2. **Experimental Testing Sessions**: Descriptive measurements of height and weight will be taken. The participant will be required to sit in the normothermic or hyperthermic room (22°C; 60% humidity
or 35°C; 60% humidity, relatively) for 20 minutes (or until their core temperature reaches 39°C for hyperthermic environments) passively. Next they will perform the pretesting PNS protocol. After this, they will perform either the light exercise or strenuous exercise protocol at the respective environmental conditions for the trial, with NIRS, EEG, EMG, RPE (every 5 minutes), temperature and HR will be collected during the exercise phase of the protocol (see details below). Blood draws will be performed at baseline before any testing, immediately pre-exercise, during exercise, immediately post exercise and 1 hr post exercise.

Light Ex: Self-paced at RPE 10-11; 6 intermittent 30s blocks at RPE 14 every 10min (stop = 60min)

Strenuous Ex: Self-paced at RPE 16; 6 intermittent 30s blocks of sprint as hard as possible every 10min (stop = 60min)

Finally, they will repeat the PNS protocol as described below immediately after and 1hr post exercise.

Data Collection

- Blood Samples

A catheter will be placed in the antecubital vein at baseline prior to testing. Six blood samples will be collected throughout the duration of the test. Baseline measures will be drawn prior to testing and post measures following the exercise protocol. 20ml of blood will be collected at each sample time (immediately pre exercise, during exercise, immediately post exercise and 1hr post exercise) to equal a total of 120ml. The catheter will be continually flushed to minimize the need to perform more than one cannulation. Throughout the duration of the protocol, the participant will be encouraged to remain hydrated, but will not be required to drink a specific amount of water after each blood draw.
Blood samples are important in the design of the study because we are interested in determining what kind of inflammatory response occurs during hard exercise, and how this may affect processes that occur in the brain in relation to fatigue and exercise. The Chief Investigator and Supervisor are both trained in performing blood draws and will ensure safety of everyone involved. Should the participant desire the supervisor to be present during data collection, it can be arranged, otherwise, there may be testing sessions in which the supervisor is not present. In the event that the participant has an issue with needles, or the catheter placement, appropriate precautions will be taken. Participants will be asked to lie down on a cot if they are feeling light headed, or the catheter may be inserted with the participant already in this position if they feel more comfortable that way. The investigator and supervisor will do their best to provide and make the participant as comfortable as possible. As always, if the participant cannot handle the blood draws, they are able to remove themselves from the study at any time.

- **Electromyography**
Electromyography is a common way to measure the electrical activity that passes through the vastus lateralis (quadriceps) and biceps femoris (hamstrings) during exercise. Surface EMG will be recorded from these muscles of the right leg during the baseline PNS protocol, throughout the duration of exercise and during the post exercise PNS protocol.

- **Heart Rate**
Heart rate will be collected using a chest transmitter and wrist watch receiver (Polar, Finland) pre and immediately post exercise and continuously throughout the duration of the exercise protocol.

- **Core and Skin Temperature**
Core temperature will be recorded using wireless ingestible core body temperature pills (Thermodata). Participants will be required to ingest a pill 6 hours prior to testing. The pill passes through the stomach into the small
intestine where body temperature is measured during exercise and is later excreted in normal waste.

Skin temperature will be measured using iButtons (Maxim Integrated Products, Sunnyvale, CA). iButtons will be attached to the chest, arm, thigh and lower leg regions. iButtons will be fixed to the skin with adhesive tape to prevent accidental removal during exercise.

- Peripheral Nerve Stimulation (PNS)
PNS will be used to investigate the effects of the exercise inflammatory response on muscle fibre unit recruitment to the right quadriceps muscle pre and post exercise.

PNS involves an electrical stimulus placed at the femoral nerve at the top of the thigh area. The electrical stimulation enables us to determine the maximal amount of muscle fibres that can be recruited from within the muscle. This helps to determine whether there is a decrease in motor unit recruitment, which is thought to be indicative of fatigue. While PNS is not typically described as painful, it can be a strange sensation. Participants are encouraged to tell the investigator if they feel too much discomfort at any time during the PNS portions of the testing.

The PNS protocol is performed pre and post exercise. It consists of 5 MVCs with an electrical stimulus at the peak force and a second stimulus after the muscle is relaxed completely.

- Near Infrared Spectroscopy (NIRS)
Assessment of brain activity of the frontal lobe will be recorded using 2-channel near-infrared spectroscopy (NIRS) of cerebral oxygenation (NIRO, Hamamatsu, Japan). The probes will be placed on the left and right side of the forehead. Lasers are emitted from the probe that measure concentrations of oxygen within the area.
Brain activity, specifically cerebral blood flow and changes in HBO$_2$ and HHb, will be recorded at baseline, during the exercise protocol pre and post exercise.

- **Electroencephalography (EEG)**

EEG is a technique used to measure the brain activity that occurs during exercise. EEG (BAlert Systems, USA) will be collected at baseline and through the duration of the exercise protocol. Small electrodes filled with gel will be applied to the scalp to ensure conductivity through hair and sweat.

**Risks and Benefits**

As in all human research, there are inherent risks involved in participation in exercise type studies. The chief investigator stresses that, to the best of our ability, precautionary steps will be taken to minimize potential risks as much as possible. The methods involved in this study will cause minimal, if any, harm or burden to the research participant. The methods that will be used for all other equipment and procedures, including the blood draws will also be explained in detail to the participant before signing consent forms. When performing blood draws, there is a possibility due to human error that the investigator may miss the first time and have to poke the participant more than once, but the chief investigator will do everything in her power to not let this happen.

While not likely, there is risk that the participants may experience injury, such as a strain or cramp in the muscles used in the PNS protocol or during the cycling protocol. While there may be some discomfort, the participant's status will be evaluated and he will be able to decide whether he is able to continue on with testing.

All participants will be screened to ensure they are healthy and able to participate in the exercise protocol and if the screening tool used (Adult Pre-
Exercise Screening Tool, ESSA 2011), indicates any possible concerns, the participant will need to obtain consent from his doctor prior to participation. All other equipment is very standardized and is not expected to be of risk to the participants. Total time for the research project is anticipated to take about 3 hours per visit, 7 visits total.

Conversely, there are some benefits of the study, which include being involved in research that uses the most advanced technology in our field for studying human physiology. Likewise, the participants will be provided with a peak power index. Should they desire, further reports from individual’s data may be provided to the participants as well. Finally, we hope one day to help determine what makes us feel fatigued and why we stop exercising which may be transferred to fatigue in diseased populations or those with chronic low-grade inflammation. Thus, participation in the study will be a service to not only our field, but to athletes and diseased populations alike.

Data Usage

It is expected that the data collected regarding this research study will be used towards the completion of a PhD Thesis and will be used for publication in a scholarly research article to be published in a prestigious research journal yet to be decided upon.

Confidentiality

The confidentiality of all participants is guaranteed. All data will be kept secure and only the Chief Investigator and Supervisor will have access to the participant’s identities.

Coercion and Withdrawal

It is completely voluntary to participate in this study. Participation will be free of any element of force, deceit, coercion, bribery, or undue influence.
All participants have the right to withdraw from the study at any time, without question, should they desire.

Institutional Review Board

The Charles Sturt University Human Research Ethics Committee has reviewed and approved this project. Should you have any inquiries or complaints about the ethical conduct of the study, you may contact the Executive Officer:

NOTE: Charles Sturt University’s Human Research Ethics Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee through the Executive Officer:

The Executive Officer  
Human Research Ethics Committee  
Office of Academic Governance  
Charles Sturt University  
Panorama Avenue

Tel: (02) 6338 4628  
Fax: (02) 6338 4194

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.

Thank you again for your interest in this research study. If you have decided to agree to participate in the project, please read and sign the consent form attached. Please also keep this information sheet for your records should you have any questions through the duration of your participation in the study!
INFORMED CONSENT

Changes in corticomotor excitability and central activation following the acute-phase inflammatory response induced by fatiguing aerobic exercise in normothermic and hyperthermic environments

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I, ________________________ (print name) consent to participating in the research study titled, “Changes in corticomotor excitability, central activation following an acute-phase inflammatory response at rest in normo and hyperthermic environments”.

By consenting to participate in this study, I acknowledge that I have read and understand the following terms:

1. The purpose of the study has been explained to me and I understand all risks and discomforts that may be involved.

2. I have thoroughly read and retained a copy of the information sheet given to me and understand details of the study.

3. I have had an opportunity to ask questions relating to the study and have been given adequate responses to all questions.
4. I understand all that will be required of me through the duration of the study.

5. I understand that my confidentiality is taken very seriously in this study and that by participating, I have been guaranteed that neither my name nor any other identifying information will be used or published without my written permission.

6. I understand that I can withdraw my consent and cease participation in the study at any time before, during, or after testing, without any penalty.

7. I nominate the person listed below as an emergency contact in an event one is needed:

   Name: ______________________________________________________
   Address: ____________________________________________________
   Phone: ______________________________________________________

8. I am aware that Charles Sturt University’s Human Research Ethics Committee has approved this project has approved this research study has approved this research study. I understand that I may contact the following should I have any complains or concerns about this study:

   Executive Officer
   Ethics in Human Research Committee
   Office of Academic Governance
   Charles Sturt University
   Panorama Ave
   Bathurst NSW 2795

   Phone: (02) 6338 4628
   Fax: (02) 6338 4194

   Participant Signature: __________________________________________
   Date: ________________________________________________________
Chapter 10: Appendices

APPENDIX 2: ETHICS APPROVAL – STUDY 1 AND 2

Ms Nicole Vargas
233 George Street
BATHURST NSW 2795

26 April 2013

Dear Ms Vargas,

Thank you for the additional information forwarded in response to a request from the Human Research Ethics Committee (HREC).

The CSU HREC reviews projects in accordance with the National Health and Medical Research Council’s National Statement on Ethical Conduct in Research Involving Humans.

I am pleased to advise that your project entitled “Cortisolotor excitability and central activation following the acute-phase inflammatory response induced by fatiguing aerobic exercise in normothermic and hyperthermic environments” meets the requirements of the National Statement, and ethical approval for this research is granted for a twelve-month period from 26 April 2013.

The protocol number issued with respect to this project is 2013/049. Please be sure to quote this number when responding to any request made by the Committee.

Please note the following conditions of approval:

- all Consent Forms and Information Sheets are to be printed on Charles Sturt University letterhead. Students should liaise with their Supervisor to arrange to have these documents printed;
- you must notify the Committee immediately in writing should your research differ in any way from that proposed. Forms are available at: [http://www.csu.edu.au/_data/assets/word_doc/0010/17653/ethic_mirrep.doc](http://www.csu.edu.au/_data/assets/word_doc/0010/17653/ethic_mirrep.doc);
- you must notify the Committee immediately if any serious and/or unexpected adverse events or outcomes occur associated with your research, that might affect the participants and therefore ethical acceptability of the project. An Adverse Incident form is available from the website as above;
- amendments to the research design must be reviewed and approved by the Human Research Ethics Committee before commencement. Forms are available at the website above.

Approval after further information.doc

www.csu.edu.au

CRICOS Provider Number for Charles Sturt University, CRICOS PRINCIPAL: 00309A, (NSW) 08473 (ACT) and 02898WC (WAC). ABN: 43 008 786 591
• if an extension of the approval period is required, a request must be submitted to the Human Research Ethics Committee. Forms are available at the website above;
• you are required to complete a Progress Report form, which can be downloaded as above, by 21 March 2014 if your research has not been completed by that date;
• you are required to submit a final report. The form is available from the website above.

YOU ARE REMINDED THAT AN APPROVAL LETTER FROM THE CSU HREC CONSTITUTES ETHICAL APPROVAL ONLY.

If your research involves the use of radiation, biological materials, chemicals or animals a separate approval is required from the appropriate University Committee.

The Committee wishes you well in your research and please do not hesitate to contact the Executive Officer on telephone (02) 6338 4628 or email ethics@csu.edu.au if you have any enquiries.

Yours sincerely

Julie Hicks
Executive Officer
Human Research Ethics Committee
Direct Telephone: (02) 6338 4628
Email: ethics@csu.edu.au
Gr: Professor Frank Maris Professor Robert Robergs

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) National Statement on Ethical Conduct in Human Research (2007)

Approval letter_for_further_information.doc

Last updated: February 2017
Next review: February 2014
APPENDIX 3: INFORMATION AND INFORMED CONSENT
– STUDY 3

INFORMATION SHEET

The effects of an increased cytokine release during strenuous aerobic exercise in the heat on perceptions and physiological measures of fatigue

Thank you in advance for your interest in this research project. Please read and understand the information on this page and retain it for your records. If you have questions or concerns regarding this study, please feel free to contact:

Nicole Vargas (Principal Investigator)  
PhD Student  
School of Human Movement Studies  
Allen House, N1  
Charles Sturt University  
Panorama Ave  
Bathurst, NSW  
2795  
Tel: +61 2 6338 6101  
Email: nvargas@csu.edu.au

Frank Marino (Supervisor)  
Professor  
School of Human Movement Studies  
Allen House, N1  
Charles Sturt University  
Panorama Ave  
Bathurst, NSW  
2795  
Tel: +61 2 6338 4268  
Email: fmarino@csu.edu.au

About the Project
The study of fatigue and what makes people cease exercise has been prevalent in the field of exercise science. It is widely thought that there are several physiological regulatory systems that work together in order to communicate how much effort can be put forth, and when we need to slow down. Likewise, it is thought that these systems exist as mechanisms to prevent catastrophic events occurring from over-exertion. In an effort to further study different systems that aide in regulation of exercise exertion, the present study focuses on the role of the inflammatory system and the effect that the exercise induced acute-phase inflammatory response has on perceptions of fatigue during exercise.

Purpose

The present research study aims to determine how the acute-phase inflammatory response to exercise modulates our ability to voluntarily recruit muscle, changes our brain activity and our perceptions of fatigue during strenuous, prolonged endurance exercise. These will be quantified using measures of neuromuscular recruitment and force production, oxygen concentration in the blood being delivered to the brain, changes in brain waves and through the inflammatory markers in our blood.

Anticipated Findings

It is anticipated that the study will aide in furthering our understanding of the processes of the acute-phase inflammatory response induced through exercise and its effects on the brain, the ability to voluntarily activate the muscle, and the perceived levels of fatigue. It is expected that there will be an increase in fatigue, a decrease in muscle activation and changes in the brain due to the cytokine release from the acute-phase inflammatory response during strenuous aerobic exercise.

Study Design

Inclusion criteria for this study requires that participants have no prior sickness or infection whether virus or bacterial and is not presently taking
nor has taken any anti-inflammatory medications or medication for any type of virus or bacterial infection at least 7 days prior to testing. Likewise, they must currently engage in at least 30-60 minutes of continuous aerobic exercise, at least 3 times/week. Exclusion criteria include symptoms or risk factors for sedentary lifestyle diseases, musculoskeletal conditions that would constrain the ability to perform prolonged, fatiguing cycle ergometry, and conditions that alter the brain activity. Participants should not have any metal implants or pacemakers and must be males between the ages of 18 and 35. **Participants will be required to keep a food diary for the 24 hours prior to each test and they must abstain from caffeine and alcohol, as well as intense exercise for 36-48hrs prior to testing.** Participants must ingest a core temperature pill 6 hours prior to testing and an anti-inflammatory or placebo pill 1.5-2hrs prior to exercise. On the day of the trial, participants must present to the lab having eaten a high protein breakfast (see below for a list of foods) 1 hr prior. They will be able to drink water *ad libitum.* Participants must report to the lab wearing workout clothes and closed toe running shoes and bring a water bottle and towel with them. Participants may be asked to return for further testing in the future as a follow up from this study, but it is not required in order to participate in the present study.

<table>
<thead>
<tr>
<th>Foods to Eat – High Protein, very low Carb</th>
<th>Foods to Avoid – High Carb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs (prepared anyway)</td>
<td>Most Yogurts</td>
</tr>
<tr>
<td>Meats – ham/bacon/ sausage</td>
<td>Bread of any kind</td>
</tr>
<tr>
<td>Protein Shakes – check carbs are ~1% only</td>
<td>Cereals</td>
</tr>
<tr>
<td>Fish</td>
<td>Oats (for porridge)</td>
</tr>
<tr>
<td>Cheese</td>
<td>Most energy bars</td>
</tr>
<tr>
<td>Peanut butter (ONLY organic or no sugar</td>
<td>Fruit (including dried fruit)</td>
</tr>
<tr>
<td>added – limit to 2 tbsp.)</td>
<td>Jams and preservatives</td>
</tr>
<tr>
<td>Tofu</td>
<td>Honey</td>
</tr>
<tr>
<td>Hummus</td>
<td>Sports drinks (i.e. Gatorade).</td>
</tr>
</tbody>
</table>
Participant Requirements

The study will involve 3 sessions. The first session will be a familiarisation/screening session. The final 2 sessions will be the experimental sessions in which subjects will strenuous cycling exercise in a heated environment after taking either a placebo or an anti-inflammatory drug, to determine effects of the anti-inflammatory drug on acute-phase inflammation, excitability in the brain, voluntary muscle activation, perceptions of fatigue and performance during the 60 min cycle test.

Pre and In-Between Session Procedures

All participants will be required to refrain from any aerobic or strength conditioning exercise for 36-48hrs prior to each session. Participants will be encouraged to follow similar dietary patterns before each testing session. Testing sessions will be completed with at least 5-7 days rest between each.

1. Familiarisation/screening session: This session will involve a test to determine peak power output (PPO) and maximal oxygen consumption (VO2max) during an incremental cycling protocol for referencing PPO during the actual experimental trial. Participants will be required to perform a self-paced warm up for 5 minutes prior to beginning the test at a starting power of 100W. At commencement of the test, the participant will be required to remain seated and maintain a cadence of 70 revs (min)$^{-1}$. Workload will be increased by 20 W (min)$^{-1}$ until the participant is unable to match the required cadence. PPO will be determined as the highest mean power output achieved over a 1-min period throughout the test. Prior to the test, the participant will become acquainted with the Borg scale (6-20) for measuring RPE (Borg 1982). This introduction and the exercise test will help to give them a better understanding of rating their own perceptual exertion, which will help to increase reliability in actual trials.
Chapter 10: Appendices

Following the exercise protocol, participants will be introduced to the peripheral stimulation equipment. At this time, we will set up the equipment and document individual settings for the HUMAC machine. Resting twitch amplitude will be established and documented for later reference. The participant will then complete a portion of the neuromuscular testing protocol that will be used during the actual experimental trials in order to familiarize them with peripheral stimulation of the femoral nerve. After this, participants will be shown other equipment used including the electroencephalography (EEG), Near Infra-red Spectroscopy (NIRS), iButtons (skin temperature) and core temperature pills used during the experimental testing.

Finally, participants will perform a brief (~20-30min) cycling trial at a fixed RPE of 16 (hard-very hard) in ambient temperature (35°C; 60% relative humidity). The participant will be able to self-pace the exercise but will be continually reminded to remain at an RPE of 16 for the whole time to familiarize them with what this means.

2. Experimental Testing Sessions x2: Descriptive measurements of height and weight and resting heart rate will be taken. Next, the EEG will be fitted to the participant and a baseline of 2 min with eyes open, followed by 2 min with eyes closed will be performed while sitting quietly in a room. A cannula (20 gauge) will be placed in the antecubital vein of the participant and a sample of venous blood (15ml) will be taken for baseline measures. Participants will perform the pre-cycle neuromuscular testing on the HUMAC and will then be fitted to the cycle ergometer to perform the 60 min cycle test in the 35°C, 60% humidity climate chamber. NIRS, EEG, EMG, RPE (every 5 minutes), temperature and HR will be collected throughout the cycle protocol. Further blood draws will be performed during exercise, immediately post exercise and 1 hr post exercise.

*1 x Cycle at RPE 16 for 60min with placebo** (35°C; 60% humidity)
*1x Cycle at RPE 16 for 60 min with anti-inflammatory** (35˚C; 60% humidity)

**Placebo:** The placebo pill will be 800g of gluten free flour that mimics the look of the anti-inflammatory pill.

**Anti-inflammatory drug:** The anti-inflammatory pill will be 800mg of ibuprofen crushed and placed in an empty capsule for ingestion. 800mg is a safe and commonly used dose for adult individuals to help prevent inflammation and pain as a one-off instance. Participants MUST NOT be allergic to ibuprofen or any medication of similar nature.

Data Collection

- Blood Samples
A catheter will be placed in the antecubital vein at baseline prior to testing. 8 blood samples will be collected for the entire study (4 samples at pre exercise, during exercise, immediately post exercise and 1hr post exercise for each trial). 15ml of blood will be collected at each sample time equating to a total of 60mL per trial (120 for the entire study). The catheter will be continually flushed to minimize the need to perform more than one cannulation. Throughout the duration of the protocol, the participant will be encouraged to remain hydrated, but will not be required to drink a specific amount of water after each blood draw.

Blood samples are important in the design of the study because we are interested in determining what kind of inflammatory response occurs during hard exercise, and how this may affect processes that occur in the brain in relation to fatigue and exercise. The Chief Investigator and Supervisor are both trained in performing blood draws and will ensure safety of everyone involved. Should the participant desire the supervisor to be present during data collection, it can be arranged, otherwise, there may be testing sessions in which the supervisor is not present. In the event that the participant has an issue with needles, or the catheter placement, appropriate precautions will be taken. Participants will be asked to lie down on a cot if they are feeling light headed, or the catheter may be inserted with the participant
already in this position if they feel more comfortable that way. The investigator and supervisor will do their best to provide and make the participant as comfortable as possible. As always, if the participant cannot handle the blood draws, they are able to remove themselves from the study at any time.

15mLs is required for each sample as 10mL will be centrifuged to acquire ~3mL of plasma to be freeze-dried and stored at -80°C for later analysis of cytokines. The other 5mL will be sent through a radiometer for immediate measures of glucose, lactate and other metabolites present.

- **Electromyography**

  Electromyography is a common way to measure the electrical activity that passes through the vastus lateralis (quadriceps) and biceps femoris (hamstrings) muscles during exercise. Surface EMG will be recorded from these muscles of the right leg during the baseline neuromuscular testing, throughout the duration of exercise and during the post exercise neuromuscular testing.

- **Heart Rate**

  Heart rate will be collected using a chest transmitter and wrist watch receiver (Polar, Finland) pre and immediately post exercise and at 5 minute increments throughout the duration of the exercise protocol but not during pre and post testing as it may interfere with other equipment.

- **Core and Skin Temperature**

  Core temperature will be recorded using wireless ingestible core body temperature pills (Thermodata). Participants will be required to ingest a pill 6 hours prior to testing. The pill passes through the stomach into the small intestine where body temperature is measured during exercise and is later excreted in normal waste.

  A warning bracelet stating that the participant cannot have an MRI during the time the capsule is still in the system will be given to the participant.
Skin temperature will be measured using iButtons (Maxim Integrated Products, Sunnyvale, CA). iButtons will be attached to the chest, arm, thigh and lower leg regions. iButtons will be fixed to the skin with adhesive tape to prevent accidental removal during exercise.

- Neuromuscular Testing - Peripheral Nerve Stimulation (PNS)
PNS will be used to investigate the effects of the exercise inflammatory response on muscle fibre unit recruitment to the right quadriceps muscle pre and post exercise.

PNS involves an electrical stimulus placed at the femoral nerve at the top of the thigh area. The electrical stimulation enables us to determine the maximal amount of muscle fibres that can be recruited from within the muscle. This helps to determine whether there is a decrease in motor unit recruitment, which is thought to be indicative of fatigue. While PNS is not typically described as painful, it can be a strange sensation. Participants are encouraged to tell the investigator if they feel too much discomfort at any time during the PNS portions of the testing.

The PNS protocol is performed pre and post exercise. It consists of 5 MVCs with an electrical stimulus at the peak force and a second stimulus after the muscle is relaxed completely.

- Near Infrared Spectroscopy (NIRS)
Assessment of brain activity of the frontal lobe will be recorded using 2-channel near-infrared spectroscopy (NIRS) of cerebral oxygenation (NIRO, Hamamatsu, Japan). The probes will be placed on the left and right side of the forehead. Lasers are emitted from the probe that measure concentrations of oxygen within the area.

Brain activity, specifically cerebral blood flow and changes in HBO$_2$ and HHb, will be recorded at baseline, during the exercise protocol pre and post exercise.
Electroencephalography (EEG)

EEG is a technique used to measure the brain activity that occurs during exercise. EEG (BAlert Systems, USA) will be collected at baseline and through the duration of the exercise protocol. Small electrodes filled with gel will be applied to the scalp to ensure conductivity through hair and sweat.

Risks and Considerations

As in all human research, there are inherent risks involved in participation in exercise type studies. The chief investigator stresses that, to the best of our ability, precautionary steps will be taken to minimize potential risks as much as possible. The methods involved in this study will cause minimal, if any, harm or burden to the research participant. The methods that will be used for all other equipment and procedures, including the blood draws will also be explained in detail to the participant before signing consent forms. When performing blood draws, there is a possibility due to human error that the investigator may miss the first time and have to poke the participant more than once, but the chief investigator will do everything in her power to not let this happen.

While not likely, there is risk that the participants may experience injury, such as a strain or cramp in the muscles used in the PNS protocol or during the cycling protocol. While there may be some discomfort, the participant's status will be evaluated and he will be able to decide whether to continue on with testing.

All participants will be screened to ensure they are healthy and able to participate in the exercise protocol and if the screening tool used (Adult Pre-Exercise Screening Tool, ESSA 2011), indicates any possible concerns, the participant will need to obtain consent from his doctor prior to participation.
All other equipment is very standardized and is not expected to be of risk to the participants. Total time for the research project is anticipated to take about **3.5 hours per visit, 3 visits total.**

Conversely, there are some benefits of the study, which include being involved in research that uses the most advanced technology in our field for studying human physiology. Likewise, the participants will be provided with a peak power index. Should they desire, further reports from individual’s data may be provided to the participants as well. Finally, we hope one day to help determine what makes us feel fatigued and why we stop exercising which may be transferred to fatigue in diseased populations or those with chronic low-grade inflammation. Thus, participation in the study will be a service to not only our field, but to athletes and diseased populations alike.

**Data Usage**

It is expected that the data collected regarding this research study will be used towards the completion of a PhD Thesis and will be used for publication in a scholarly research article to be published in a prestigious research journal yet to be decided upon.

**Confidentiality**

The confidentiality of all participants is guaranteed. All data will be kept secure and only the Chief Investigator and Supervisor will have access to the participant’s identities.

**Coercion and Withdrawal**

It is completely voluntary to participate in this study. Participation will be free of any element of force, deceit, coercion, bribery, or undue influence. All participants have the right to withdraw from the study at any time, without question, should they desire.
Institutional Review Board

The Charles Sturt University Human Research Ethics Committee has reviewed and approved this project. Should you have any inquiries or complaints about the ethical conduct of the study, you may contact the Executive Officer:

**NOTE:** Charles Sturt University’s Human Research Ethics Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee through the Executive Officer:

The Executive Officer
Human Research Ethics Committee
Office of Academic Governance
Charles Sturt University
Panorama Avenue

Tel: (02) 6338 4628
Fax: (02) 6338 4194

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.

Thank you again for your interest in this research study. If you have decided to agree to participate in the project, please read and sign the consent form attached. Please also keep this information sheet for your records should you have any questions through the duration of your participation in the study!
INFORMED CONSENT

The effects of an increased cytokine release during strenuous aerobic exercise in the heat on perceptions and physiological measures of fatigue

Nicole Vargas (Principal Investigator)  Frank Marino (Supervisor)
PhD Student  Head of School
School of Human Movement Studies  School of Human Movement Studies
Charles Sturt University  Charles Sturt University
Panorama Ave  Panorama Ave
Bathurst, NSW  Bathurst, NSW
2795  2795

Tel: +61 2 6338 6101  Tel: +61 2 6338 4268
Email: nvargas@csu.edu.au  Email: fmarino@csu.edu.au

I, ______________________________ (print name) consent to participating in the research study titled, ‘The effects of an increased cytokine release during strenuous aerobic exercise in the heat on perceptions and physiological measures of fatigue’

By consenting to participate in this study, I acknowledge that I have read and understand the following terms:

3. The purpose of the study has been explained to me and I understand all risks and discomforts that may be involved.
4. I have thoroughly read and retained a copy of the information sheets given to me and understand details of the study.
5. I have thoroughly read through and understand the risks of the exercise protocol and taking the anti-inflammatory drug required in this study.
6. I understand the risks of taking the core temperature capsule and have been given a bracelet to ensure I do not have an MRI before the core temperature capsule has been excreted.

7. I have had an opportunity to ask questions relating to the study and have been given adequate responses to all questions.

8. I understand all that will be required of me through the duration of the study.

9. I understand that my confidentiality is taken very seriously in this study and that by participating, I have been guaranteed that neither my name nor any other identifying information will be used or published without my written permission.

10. I understand that there is a possibility that data collected during this study may be used for comparison in future studies.

11. I understand that I can withdraw my consent and cease participation in the study at any time before, during, or after testing, without any penalty.

12. I nominate the person listed below as an emergency contact in an event one is needed:

   Name: _____________________________________________________
   Address: _____________________________________________________
   Phone: ________________________________

13. I am aware that the Charles Sturt University Ethical Review Committee has approved the study. I understand that I may contact the following should I have any complains or concerns about this study:

   Executive Officer
   Ethics in Human Research Committee
   Office of Academic Governance
   Charles Sturt University
   Panorama Ave
   Bathurst NSW 2795

   Phone: (02) 6338 4628
   Fax: (02) 6338 4194
Participant Signature: ________________________________
Date: ________________________________
APPENDIX 4: ETHICS APPROVAL – STUDY 3

27 June 2014

Ms Nicole Vargus
School of Human Movement Sciences
BATHURST CAMPUS

Dear Ms Vargus,

Thank you for the additional information forwarded in response to a request from the Human Research Ethics Committee (HREC).

The CSU HREC reviews projects in accordance with the National Health and Medical Research Council’s National Statement on Ethical Conduct in Research Involving Human.

I am pleased to advise that your project entitled “The effects of an increased cytokine release at rest and during strenuous aerobic exercise in the heat on perceptions and physiological measures of fatigue” meets the requirements of the National Statement; and ethical approval for this research is granted for a twelve-month period from 27 June 2014.

The protocol number issued with respect to this project is 2014/115. Please be sure to quote this number when responding to any request made by the Committee.

Please note the following conditions of approval:

- all Consent Forms and Information Sheets are to be printed on Charles Sturt University letterhead. Students should liaise with their Supervisor to arrange to have these documents printed;
- you must notify the Committee immediately in writing should your research differ in any way from that proposed. Forms are available at: [http://www.csu.edu.au/...](http://www.csu.edu.au/...)
- you must notify the Committee immediately if any serious and/or unexpected adverse events or outcomes occur associated with your research, that might affect the participants and therefore ethical acceptability of the project. An Adverse Incident form is available from the website above;
- amendments to the research design must be reviewed and approved by the Human Research Ethics Committee before commencement. Forms are available at the website above;
- [Approval letter further information doc](http://www.csu.edu.au/...)

Last updated: February 2014
Next review: February 2015

www.csu.edu.au

OXCODE: Provider Numbers for Charles Sturt University are OX1001 (NSW), OX1002 (VIC) or OX0001 (ACT). AHRN: 83 674 786 641
• if an extension of the approval period is required, a request must be submitted to the Human Research Ethics Committee. Forms are available at the website above;
• you are required to complete a Progress Report form, which can be downloaded as above, by 17 April 2015 if your research has not been completed by that date;
• you are required to submit a final report, the form is available from the website above.

YOU ARE REMINDED THAT AN APPROVAL LETTER FROM THE CSU HRRC CONSTITUTES ETHICAL APPROVAL ONLY.

If your research involves the use of radiation, biological materials, chemicals or animals a separate approval is required from the appropriate University Committee.

The Committee wishes you well in your research and please do not hesitate to contact the Executive Officer on telephone (02) 6545 4628 or email ethics@csu.edu.au if you have any enquiries.

Yours sincerely

Julie Hirta
Executive Officer
Human Research Ethics Committee
Direct Telephone: (02) 6545 4628
Email: ethics@csu.edu.au

This HRDC is committed and operates in accordance with the National Health and Medical Research Council’s (NHMRC) National Statement on Ethical Conduct in Human Research (2007).

Approval after further information.doc

Last updated: February 2014
Next review: February 2015
APPENDIX 5: INFORMATION AND INFORMED CONSENT
– STUDY 4

INFORMATION SHEET

Effects of Carbohydrate Ingestion and Swilling on Cytokine Release and Performance during Cycling

Thank you in advance for your interest in this research project. Please read and understand the information on this page and retain it for your records. If you have questions or concerns regarding this study, please feel free to contact:

Nicole Vargas (Principal Investigator)
PhD Student
School of Human Movement Studies
Allen House, N1
Charles Sturt University
Panorama Ave
Bathurst, NSW
2795
Tel: +61 2 6338 6101
Email: nvargas@csu.edu.au

Frank Marino (Supervisor)
Professor
School of Human Movement Studies
Allen House, N1
Charles Sturt University
Panorama Ave
Bathurst, NSW
2795
Tel: +61 2 6338 4268
Email: fmarino@csu.edu.au

About the Project
Current research reveals an increase in performance during a cycling task both when carbohydrates are ingested (Starkie et al., 2001) and simply swilled in the mouth, without swallowing (Chambers et al., 2009). Thus, it has been hypothesized that the metabolic effects of carbohydrate breakdown during endurance exercise may not be what increases performance, but rather, that performance can be enhanced simply by the brain sensing that there is carbohydrate available through sensitizing neural feedback from swilling a carbohydrate type of drink in the mouth. The purpose of the research project is to determine the mechanism behind the increase in performance when carbohydrates are either ingested or swilled in the mouth during exercise. A second purpose for the study is to see whether there is either an exacerbating or a blunting effect of the carbohydrate treatment on the release of cytokines (namely IL-6) that is seen in endurance type exercise.

Purpose

The principle aims of the research study are to determine whether performance is increased when carbohydrates are ingested vs. when they are only swilled in the mouth, and whether either carbohydrate treatment method has an effect on the release of cytokines and performance. It is hypothesized that the carbohydrate ingestion and swilling will not change performance, but that the carbohydrate ingestion will blunt cytokine release compared to carbohydrate swilling, which may indicate an interaction between the metabolism of glucose and release of IL-6.

Anticipated Findings

It is anticipated that the study will aide in furthering our understanding of the processes involved in performance and energy availability during an endurance cycling task. While prior research suggests there is a blunting effect of carbohydrate ingestion on the release of cytokines, there is no literature which looks at the neural effects of carbohydrate swilling on the release of cytokines. Furthermore, the mechanism of cytokine release remains largely elusive in literature, and so looking at ingestion or swilling
of carbohydrates can help determine if the release of cytokines does in fact stem from the periphery, or if there is a neural component involving the hormone response at the hypothalamic pituitary axis. It is expected that energy availability in through carbohydrate ingestion will improve performance and blunt IL-6 response compared to carbohydrate swilling. However, it is also expected that carbohydrate swilling with provide similar performance as carbohydrate ingestion simply through the brain sensing energy availability. Water ingestion is expected to have decreased performance compared to carbohydrate trials.

Study Design

Inclusion criteria for this study requires that participants have no prior sickness or infection whether virus or bacterial and is not presently taking nor has taken any anti-inflammatory medications or medication for any type of virus or bacterial infection at least 7 days prior to testing. Likewise, they must currently engage in at least 30-60 minutes of continuous aerobic exercise, at least 3times/week. Exclusion criteria include symptoms or risk factors for sedentary lifestyle diseases, musculoskeletal conditions that would constrain the ability to perform prolonged, fatiguing cycle ergometry, and conditions that alter the brain activity. Participants must be males or females between the ages of 18 and 55. Females will only be tested during specific times of their menstrual cycle. An appropriate schedule will be discussed with the chief investigator prior to commencing the study. Participants will be required to keep a food diary for the 24 hours prior to each test and they must abstain from caffeine and alcohol, as well as intense exercise for 36-48hrs prior to testing. On the day of the trial, participants must present to the lab in a fasted state, but can drink water ad libitum. Participants must report to the lab wearing workout clothes and closed toe running shoes and bring a water bottle and towel with them.

Participant Requirements
The study will involve 4 sessions. The first session will be a familiarisation/screening session. The final 3 sessions will be the experimental sessions in which subjects will perform a 30k time trial on a cycle ergometer in an environmental chamber controlled at 22°C, 60% humidity.

Pre and In-Between Session Procedures

All participants will be required to refrain from any aerobic or strength conditioning exercise for 36-48hrs prior to each session. Participants will be encouraged to follow similar dietary patterns before each testing session. Testing sessions will be completed with at least 5-7 days rest between each.

1. Familiarisation/screening session: This session will involve a test to determine peak power output (PPO) and aerobic fitness via a maximal oxygen consumption (VO2max) during an incremental cycling protocol for referencing PPO during the actual experimental trial. Participants will be required to perform a self-paced warm up for 5 minutes prior to beginning the test at a starting power of 100W. At commencement of the test, the participant will be required to remain seated and maintain a cadence of 70 revs (min)^{-1}. Workload will be increased by 20 W (min)^{-1} until the participant is unable to match the required cadence. PPO will be determined as the highest mean power output achieved over a 1-min period throughout the test. Prior to the test, the participant will become acquainted with the Borg scale (6-20) for measuring RPE (Borg 1982). This introduction and the exercise test will help to give them a better understanding of rating their own perceptual exertion, which will help to increase reliability in actual trials.

After this, participants will be shown other equipment used including cannulas, electroencephalography (EEG), Near Infra-red Spectroscopy (NIRS), iButtons (skin temperature), and a urine specific gravity (USG) test used during and/or pre and post the experimental testing.
Finally, participants will perform a 10k time trial to familiarize them with the cycle ergometer and changing gears in the environmental chamber used for testing.

2. Experimental Testing Sessions x3*: Descriptive measurements of weight and resting heart rate, rating of perceived exertion and USG will be taken upon entering the lab. Next, the EEG will be fitted to the participant and a baseline of 2 min with eyes open, followed by 2 min with eyes closed will be performed while sitting quietly in a room. A cannula (22 gauge) will be placed in the antecubital vein of the participant and a sample of venous blood (25ml) will be taken for baseline measures. iButtons, EMG and NIRS leads will be attached to the participant and they will be fitted to the cycle ergometer to begin testing. A 3-5 min warm up will be performed prior to commencing the test. During the 30k time trial, NIRS, EEG, EMG, RPE (every 5 minutes), temperature and HR will be collected. Blood draws will be performed at baseline, during exercise (10 and 20k) and immediately post exercise.

*Water: 30k TT ingesting water ad libitum.
*Carbohydrate ingestion: 30k TT ingesting 6.4% carbohydrate solution ad libitum.
*Carbohydrate swilling: 30k TT swilling the same 6.4% carbohydrate solution ad libitum.

**Note, experimental sessions will be randomized. Participants will be allowed to drink or swill ad libitum in the initial session. The chief investigator will document the time and amount of fluid ingested or swilled. The documented times and amounts will be mimicked in the following two trials in order to standardize the amount and times at which fluids were swilled/ingested.
• Blood Samples
A catheter will be placed in the antecubital vein at baseline prior to testing. 12 blood samples will be collected for the entire study (4 samples per experimental trial). A total of 400ml blood will be collected for the entire study (25ml of blood at each sample time - baseline, during exercise, immediately post exercise and 1hr post exercise) to equal a total of 100ml/trial. The cannula will be continually flushed to minimize the need to perform more than one cannulation. Throughout the duration of the protocol, the participant will be encouraged to remain hydrated, but will not be required to drink a specific amount of water after each blood draw.

25mLs is required for each sample for centrifugation to acquire ~10mL of plasma to be freezeed and stored at -80°C for later analysis of IL-6 and its receptors (sgp130 and sIL-6R), glucose and lactate. Blood samples are important in the design of the study because we are interested in determining what kind of inflammatory response occurs during hard exercise, and how this may affect processes that occur in the brain in relation to fatigue and exercise. The Chief Investigator and Supervisor are both trained in performing blood draws and will ensure safety of everyone involved. Should the participant desire the supervisor to be present during data collection, it can be arranged, otherwise, there may be testing sessions in which the supervisor is not present. In the event that the participant has an issue with needles, or the catheter placement, appropriate precautions will be taken. Participants will be asked to lie down on a cot if they are feeling light headed, or the catheter may be inserted with the participant already in this position if they feel more comfortable that way. The investigator and supervisor will do their best to provide and make the participant as comfortable as possible. As always, if the participant cannot handle the blood draws, they are able to remove themselves from the study at any time.

• Electromyography

Electromyography is a common way to measure the electrical activity that passes through the vastus lateralis (quadriceps) and biceps femoris (hamstrings) muscles during exercise. Surface EMG will be recorded from these muscles of the right leg during the baseline neuromuscular testing, throughout the duration of exercise and during the post exercise neuromuscular testing.

- **Heart Rate**
Heart rate will be collected using a chest transmitter and wrist watch receiver (Polar, Finland) pre and immediately post exercise and at 5 minute increments throughout the duration of the exercise protocol but not during pre and post testing as it may interfere with other equipment.

- **Skin Temperature**
Skin temperature will be measured using iButtons (Maxim Integrated Products, Sunnyvale, CA). iButtons will be attached to the chest, arm, thigh and lower leg regions. iButtons will be fixed to the skin with adhesive tape to prevent accidental removal during exercise.

- **Near Infrared Spectroscopy (NIRS)**
Assessment of brain activity of the frontal lobe will be recorded using 2-channel near-infrared spectroscopy (NIRS) of cerebral oxygenation (NIRO, Hamamatsu, Japan). The probes will be placed on the left and right side of the forehead. Lasers are emitted from the probe that measure concentrations of oxygen within the area. Brain activity, specifically cerebral blood flow and changes in HBO$_2$ and HHb, will be recorded at baseline, during the exercise protocol pre and post exercise.

- **Electroencephalography (EEG)**
EEG is a technique used to measure the brain activity that occurs during exercise. EEG (BAlert Systems, USA) will be collected at baseline and through the duration of the exercise protocol. Small electrodes filled with
gel will be applied to the scalp to ensure conductivity through hair and sweat.

Risks and Considerations

As in all human research, there are inherent risks involved in participation in exercise type studies. The chief investigator stresses that, to the best of our ability, precautionary steps will be taken to minimize potential risks as much as possible. The methods involved in this study will cause minimal, if any, harm or burden to the research participant. The methods that will be used for all other equipment and procedures, including the blood draws will also be explained in detail to the participant before signing consent forms. When performing blood draws, there is a possibility due to human error that the investigator may miss the first time and have to poke the participant more than once, but the chief investigator will do everything in her power to not let this happen.

All participants will be screened to ensure they are healthy and able to participate in the exercise protocol and if the screening tool used (Adult Pre-Exercise Screening Tool, ESSA 2011), indicates any possible concerns, the participant will need to obtain consent from his doctor prior to participation.

All other equipment is very standardized and is not expected to be of risk to the participants. Total time for the research project is anticipated to take about 2 hours per visit, 4 visits total.

Conversely, there are some benefits of the study, which include being involved in research that uses the most advanced technology in our field for studying human physiology. Likewise, the participants will be provided with a peak power index. Should they desire, further reports from individual’s data may be provided to the participants as well. Finally, we hope the study will be an important addition to literature in the area of sports performance and peripheral vs. central regulation of exercise. Thus, participation in the study will be a service to not only our field, but to athletes and diseased populations alike.
Data Usage

It is expected that the data collected regarding this research study will be used towards the completion of a PhD Thesis and will be used for publication in a scholarly research article to be published in a prestigious research journal yet to be decided upon.

Confidentiality

The confidentiality of all participants is guaranteed. All data will be kept secure and only the Chief Investigator and Supervisor will have access to the participant’s identities.

Coercion and Withdrawal

It is completely voluntary to participate in this study. Participation will be free of any element of force, deceit, coercion, bribery, or undue influence. All participants have the right to withdraw from the study at any time, without question, should they desire.

Institutional Review Board

The Charles Sturt University Human Research Ethics Committee has reviewed and approved this project. Should you have any inquiries or complaints about the ethical conduct of the study, you may contact the Executive Officer:

**NOTE:** Charles Sturt University’s Human Research Ethics Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee through the Executive Officer:

The Executive Officer
Human Research Ethics Committee
Office of Academic Governance
Charles Sturt University
Panorama Avenue

Tel: (02) 6338 4628
Fax: (02) 6338 4194

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.

Thank you again for your interest in this research study. If you have decided to agree to participate in the project, please read and sign the consent form attached. Please also keep this information sheet for your records should you have any questions through the duration of your participation in the study!
INFORMED CONSENT

Effects of Carbohydrate Ingestion and Swilling on Cytokine Release and Performance during Cycling

Nicole Vargas and Caroline Robertson
PhD Students
School of Human Movement Studies
Allen House, N1
Charles Sturt University
Panorama Ave
Bathurst, NSW
2795
Tel: +61 2 6338 6101
Email: nvargas@csu.edu.au

Frank Marino (Supervisor)
School of Human Movement Studies
Allen House, N1
Charles Sturt University
Panorama Ave
Bathurst, NSW
2795
Tel: +61 2 6338 4268
Email: fmarino@csu.edu.au

I, ________________________ (print name) consent to participating in the research study titled, ‘Effects of Carbohydrate Ingestion and Swilling on Cytokine Release and Performance during Cycling’.

By consenting to participate in this study, I acknowledge that I have read and understand the following terms:

1. The purpose of the study has been explained to me and I understand all risks and discomforts that may be involved.

2. I have thoroughly read and retained a copy of the information sheet given to me and understand details of the study.
3. I have had an opportunity to ask questions relating to the study and have been given adequate responses to all questions.

4. I understand all that will be required of me through the duration of the study.

5. I understand that my confidentiality is taken very seriously in this study and that by participating, I have been guaranteed that neither my name nor any other identifying information will be used or published without my written permission.

6. I understand that I can withdraw my consent and cease participation in the study at any time before, during, or after testing, without any penalty.

7. I nominate the person listed below as an emergency contact in an event one is needed:

   Name: _______________________________ __________________________
   Address: ________________________________________________________
   Phone: __________________________________________________________

8. I am aware that Charles Sturt University’s Human Research Ethics Committee has approved this project. I understand that I may contact the following should I have any complaints or concerns about this study:

   Executive Officer
   Ethics in Human Research Committee
   Office of Academic Governance
   Charles Sturt University
   Panorama Ave
   Bathurst NSW 2795

   Phone: (02) 6338 4628
   Fax: (02) 6338 4194

   Participant Signature: __________________________________________
   Date: ____________________________________
APPENDIX 6: ETHICS APPROVAL – STUDY 4

14 November 2014

Miss Nicole Vargas
Charles Sturt University
Panorama Avenue
BATHURST NSW 2795

Dear Miss Vargas,

Thank you for submitting your research proposal entitled “Effects of Carbohydrate Ingestion and Swallowing on Cytokine Release and Performance during Cycling” for ethical review. The Human Research Ethics Committee (HREC) considered this proposal at the 10 November 2014 meeting.

In order to determine the ethical and scientific acceptability of your project please provide the additional information, clarification or modification as described below:

Risk and Benefits: Section 2 Chapter 2.1 pp. 15-18
- the Committee recommends the chief researcher include participants 18 years or older in the research project. If it is necessary, to include younger participants please justify this;
- the chief researcher clarifying how many sessions will be held and amending, the Information Sheet, if necessary;
- the chief researcher providing more detail in section 5.3.5 of the application form to clarify the screening or inclusion and exclusion criteria; and
- the chief researcher providing an explanation about the risk of a vasovagal response and syncope with venous puncture, along with how this risk will be minimized for the participants if this does occur.

Consent: Section 2 Chapter 2.2 & 2.3 pp. 19-24
- the Committee acknowledges that a detailed Information Sheet is required, but due to the technical nature of the research the Committee is seeking clarification as to whether the chief researcher will conduct an Information session for potential participants in order to explain what is required of participants, prior to the distribution of the Information Sheet and subsequent consent to participate in the project.

www.curstu.edu.au further information.doc
CRQOS Provider Numbers for Charles Sturt University are 000009 (HREC), 015475 (OHC) and 000600 (ICT). Last updated: September 2014

Last updated: September 2015

www.Denmark.dk
In order to facilitate the HREC’s consideration of your project, please provide the requested information as soon as possible and in accordance with the following instructions:

- highlight the changes made
- only provide documentation that is requested DO NOT forward the whole application again
- forward your response to the Executive Officer, Human Research Ethics Committee, by email ethics@csu.edu.au

Please note:
- your response will be reviewed by two Committee members
- you must not commence your research until notification is do so has been received and you have been issued with a Protocol Number
- that if the requested information is not received within 3 months, or 3 HREC meetings (whichever comes first) from the date of this letter, the proposal will be considered withdrawn and you will be required to resubmit the proposal with full documentation.

Please don’t hesitate to contact the Executive Officer on telephone (02) 6318 4624 or email ethics@csu.edu.au if you have any enquiries.

Yours sincerely,

Jalie Hicks
Executive Officer
Human Research Ethics Committee

To: Professor Frank Martin

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) National Statement on Ethical Conduct in Human Research (2007).

Request_for_further_information.doc Last updated: September 2014
Next review: September 2015
APPENDIX 7: ADULT PRE-SCREENING EXERCISE TOOL

This screening tool does not provide advice on a particular matter, nor does it substitute for advice from an appropriately qualified medical professional. No warranty of safety should result from its use. The screening system in no way guarantees against injury or death. No responsibility or liability whatsoever can be accepted by Exercise and Sports Science Australia, Fitness Australia or Sports Medicine Australia for any loss, damage or injury that may arise from any person acting on any statement or information contained in this tool.

Name: 
Date of Birth: ___________________ Male ☐ Female ☐ Date: ___________________

STAGE 1 (COMPULSORY)

AIM: to identify those individuals with a known disease, or signs or symptoms of disease, who may be at a higher risk of an adverse event during physical activity/exercise. This stage is self-administered and self-evaluated.

Please circle response

1. Has your doctor ever told you that you have a heart condition or have you ever suffered a stroke?
   - Yes ☐ No ☐

2. Do you ever experience unexplained pains in your chest at rest or during physical activity/exercise?
   - Yes ☐ No ☐

3. Do you ever feel faint or have spells of dizziness during physical activity/exercise that causes you to lose balance?
   - Yes ☐ No ☐

4. Have you had an asthma attack requiring immediate medical attention at any time over the last 12 months?
   - Yes ☐ No ☐

5. If you have diabetes (type 1 or type 2) have you had trouble controlling your blood glucose in the last 3 months?
   - Yes ☐ No ☐

6. Do you have any diagnosed muscle, bone or joint problems that you have been told could be made worse by participating in physical activity/exercise?
   - Yes ☐ No ☐

7. Do you have any other medical condition(s) that may make it dangerous for you to participate in physical activity/exercise?
   - Yes ☐ No ☐

IF YOU ANSWERED ‘YES’ to any of the 7 questions, please seek guidance from your GP or appropriate allied health professional prior to undertaking physical activity/exercise.

IF YOU ANSWERED ‘NO’ to all of the 7 questions, and you have no other concerns about your health, you may proceed to undertake light-moderate intensity physical activity/exercise.

I believe that to the best of my knowledge, all of the information I have supplied within this tool is correct.

Signature ___________________________ Date __________________

V1 (2011)
APPENDIX 8: HEALTH, ALLERGY AND MEDICATION QUESTIONNAIRE

Health, Allergy and Medication Questionnaire

To approve participation in the study, we need to know if you have any medication allergies or medical conditions. We also need to know what non-prescription medications you take regularly. Your honest answers to the following questions will help protect you against any allergic reactions to ibuprofen and interactions with other medications that you may take regularly.

Your privacy is important to us. We take all confidentiality regulations in regards to participation in the study very serious and will protect this information.

Please complete all sections below using blue or black ink. Please print and sign at the bottom of the page stating that all information is true to the best of your knowledge.

Section 1: Participant Information

Participant Name: _______________ Gender: __________________

Date of Birth: ________________Contact Phone: ______________
Section 2: Participant medication allergies

Check the box if you have had an allergy or serious reaction to any of the following medications:

- Aspirin and salicylates
- Codeine (i.e. Tylenol #3)
- Erythromycin, Biaxin, Zithromax
- Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) (i.e. ibuprofen, Advil, Motrin)
- Penicillins (i.e. Amoxil, amoxicillin, cephalexin)
- Sulfur Drugs (i.e. Septra, Bactrum)
- Tetracycline antibiotics

If you have an allergy for a drug that is not listed above, print the name below:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Section 3: Medical Conditions

Have you ever been diagnosed with any of the conditions listed below? Please check the box next to if you have.

<table>
<thead>
<tr>
<th>Allergies (hay fever)</th>
<th>Haemophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>High Blood Pressure</td>
</tr>
<tr>
<td>Asthma</td>
<td>High Blood Sugar (Diabetes type I or II)</td>
</tr>
<tr>
<td>Chest Pain (angina)</td>
<td>High Cholesterol</td>
</tr>
<tr>
<td>Crohn’s Disease</td>
<td>Inflammatory Bowel Disease</td>
</tr>
<tr>
<td>Chronic</td>
<td>Peptic, stomach or duodenal ulcer</td>
</tr>
<tr>
<td>Bronchitis/Emphysema</td>
<td>Heart Attack</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Stroke</td>
<td>Over/under active Thyroid</td>
</tr>
<tr>
<td>Neurological Disorders</td>
<td>Other:_________________________</td>
</tr>
<tr>
<td>(MS/Parkinson’s)</td>
<td></td>
</tr>
</tbody>
</table>

Please check the appropriate box if you have any of the following:

<table>
<thead>
<tr>
<th>Metal (or any type) of implants in your head/body</th>
<th>A pacemaker</th>
</tr>
</thead>
</table>

Section 4: Non-prescription Medications
Please check the box if you take any of the following over the counter medications regularly:

<table>
<thead>
<tr>
<th>Paracetamol</th>
<th>Ibuprofen/acetaminophen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naproxen</td>
<td>Aspirin</td>
</tr>
<tr>
<td>Benadryl/diphenhydramine</td>
<td>Other:_________________________</td>
</tr>
</tbody>
</table>

I agree that the above answers are truthful to the best of my knowledge.

Printed Name:____________________
Signature: ________________________
Date: ____________________________
APPENDIX 9: PRE-EXERCISE QUESTIONNAIRE AND FOOD DIARIES

Trial Pre Test Questionnaire

Name___________________Session________________Date___________

1. If you could use one word to describe how you feel today, what would it be?

2. When was the last time you had a cold or felt overly tired?

3. Have you taken any of the following medications in the past week?
   a. Aspirin
   b. Ibuprofen
   c. Naproxen
d. Celecoxib

e. Indomethacin

f. Diclofenac

4. When did you last exercise? Briefly describe the activity you did.
### Study 1 and 2

**Important Reminders:**
- Begin fasting from 11:00pm the night before your trial (You may only drink water after this time and in the morning).
- No strenuous exercise 36hrs prior to your trial.
- Please refrain from taking any anti-inflammatory medications at least 3-4days before your trial. If you do take some, please let me know what it is so I can ensure it will not have an effect on the results of the blood draw.
- If you forget to engage in any of the above activities, it’s okay! Just let me know so that we can reschedule as these things can seriously impair the test design.

Your next appointment is:_____________ at _______________. Please contact me as soon as possible if you will not be able to make this time.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Task 1</th>
<th>Task 2</th>
<th>Task 3</th>
<th>Task 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Lunch</td>
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<td></td>
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</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
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<td></td>
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<tr>
<td>Time</td>
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<td></td>
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<td></td>
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</tbody>
</table>

Important Reminders:

- Begin fasting from 11:00pm the night before your trial (You may only drink water after this time and in the morning).
- No strenuous exercise 36hrs prior to your trial.
- Please refrain from taking any anti-inflammatory medications at least 3-4days before your trial. If you do take some, please let me know what it is so I can ensure it will not have an effect on the results of the blood draw.
- If you forget to engage in any of the above activities, it’s okay! Just let me know so that we can reschedule as these things can seriously impair the test design.

Your next appointment is:_____________ at _______________. Please contact me as soon as possible if you will not be able to make this time.
### Study 3

#### Important Reminders:
- Begin fasting from ~10:00pm the night before your trial (You may only drink water after this time and in the morning)
- No strenuous exercise ~3hrs prior to your trial
- Please refrain from taking any anti-inflammatory medications at least 3 days before your trial. If you do take some, please let me know what it is so I can ensure it will not have an effect on the results of the blood draw
- If you forget/engage in any of the above activities, it’s okay! Just let me know so that we can reschedule as these things can seriously impair the test design!

**Your next appointment is: ______________________ at ______________________. Please contact me as soon as possible if you will not be able to make this time.**

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day</strong></td>
<td><strong>Time</strong></td>
<td><strong>Time</strong></td>
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<table>
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<tr>
<th>Lunch</th>
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<tbody>
<tr>
<td><strong>Day</strong></td>
<td><strong>Time</strong></td>
<td><strong>Time</strong></td>
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<table>
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<tr>
<th>Dinner</th>
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<tbody>
<tr>
<td><strong>Day</strong></td>
<td><strong>Time</strong></td>
<td><strong>Time</strong></td>
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<tr>
<th>Activity/Exercise</th>
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<tbody>
<tr>
<td><strong>Day</strong></td>
<td><strong>Time</strong></td>
<td><strong>Time</strong></td>
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</table>
## Study 4

### Important Reminders:

- Begin fasting from 3:00 pm the night before your trial (You may only drink water after this time and in the morning)
- No strenuous exercise 12 hrs prior to your trial
- Please refrain from taking any anti-inflammatory medications at least 5 days before your trial. If you do take some, please let me know what it is so I can ensure it will not have an effect on the results of the blood draw
- If you forget/engage in any of the above activities, it’s okay! Just let me know so that we can reschedule as these things can seriously impair the test design!

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snack</td>
<td></td>
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<tr>
<td>Time</td>
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<td>Lunch</td>
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<td>Dinner</td>
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<td>Time</td>
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<tr>
<td>Activity/Exercise</td>
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<td>Time</td>
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Your next appointment is: ______________________ at ___________________. Please contact me as soon as possible if you will not be able to make this time.
APENDIX 10: EEG DATA COLLECTION, PROCESSING AND ANALYSIS

EEG ELECTRODE LOCATION

All EEG sites for the 24 channel EEG were identified using the international 10-20 system as designated in Fig 1. Headgear was automatically sized to an individual and placed ensuring the placement of the strip rested just on top of the glabella (G) and centred to the midline of the forehead so that Fp1 and Fp2 were located on the left and right side of the prefrontal cortex, respectively, and FZ, CZ and PZ were centred along the midline in the frontal, motor and parietal cortices, respectively.

![Fig 1 Placement of EEG electrodes according to the international 10-20 system. Sites used for data analysis included: Fp2, FZ, CZ and PZ.](image)

Signals were grounded to the mastoid process and prior to data collection, impedance analyses were run in order to check that all sites were <20KΩ as designated by the manufacturer. In the event that a site did not reach the
necessary impedance, it was cleaned, hair was moved out of the way and more gel was applied.

**EEG Data Collection**

Prior to commencing exercise, a baseline with 2 min eyes open and 2 min eyes closed was collected. During the exercise testing, participants were instructed to remain still in their upper body, looking straight ahead whilst continuing to pedal for the required time (1 min, 1.5 min or 500 metres) based on the time point and study. Furthermore, prior to beginning each snapshot of data, participants were reminded to refrain from clenching their jaw, speaking and to relax their upper neck muscles as much as possible. Each snapshot was taken with their head in the same, neutral, forward looking position when on the bike.

In order to eliminate visual and audio stimulus, participants were faced starting at a blank white wall and inside a climate chamber which minimised any outside noise. Additionally, signs were placed informing individuals in the lab, outside of the climate chamber, that EEG testing was in place and to refrain from making noise that could be heard inside the chamber.

**EEG Data Processing**

The system used to collect, process and analyse EEG data with was BAlert x 24 and the software was BAlert Lab. BAlert Lab comes with proprietary algorithms that enable you to check data quality, remove artefact and obtain power spectral densities, among other data processing techniques as desired. Data in each study in the thesis were processed using BAlert Lab in the following way:

1. Each recording site of each snapshot was first visually spot-checked using an EDF viewer (Polyman v1.9) to ensure no data were missing.
2. Data were then quality checked to ensure that at least 80% of data were considered ‘good data’. If there was not 80% of good data in any individual site.

3. In the event that not 80% of data was good in an individual site, that site was manually checked to see if at least 30 epochs (seconds) of data were good. If at least 30 seconds of data were good, that 30 seconds was averaged and included in later analysis. If there was not at least 30 s of ‘good data’, data for the relative site were not used for later analysis.

4. Power spectral densities (PSD) were then acquired for each snapshot at each site.
   o Obtaining PSD’s involved automatic ‘cleaning’ of eye blinks, muscle and/or movement artefact from within the snapshots.
   o Data was then manually checked to ensure at least 30 seconds of data were available for analysis.

5. PSD for each individual subject, each individual site and each individual time point were collated and mean data were calculated for later analysis using SPSS.
# Appendix 11: Receptor Ratio Data for Chapters 4, 5 and 6

## Chapter 4: sIL-6R/SGP130

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre</th>
<th>30min</th>
<th>Post</th>
<th>1Hr Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>$HS_{16}$</td>
<td>0.17 ± 0.17</td>
<td>± 0.17</td>
<td>± 0.17</td>
<td>± 0.17</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.15</td>
<td>0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>$NT_{16}$</td>
<td>0.16 ± 0.15</td>
<td>± 0.17</td>
<td>± 0.16</td>
<td>± 0.15</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.11</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>$HS_{12}$</td>
<td>0.16 ± 0.18</td>
<td>± 0.17</td>
<td>± 0.16</td>
<td>± 0.15</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.16</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>$NT_{12}$</td>
<td>0.17 ± 0.18</td>
<td>± 0.17</td>
<td>± 0.16</td>
<td>± 0.15</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.17</td>
<td>0.15</td>
<td>0.13</td>
</tr>
</tbody>
</table>

## Chapter 5: sIL-6R/SGP130

<table>
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<tr>
<th>Condition</th>
<th>Pre</th>
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<th>Post</th>
<th>1Hr Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>0.17 ± 0.12</td>
<td>0.16 ± 0.07</td>
<td>0.16 ± 0.08</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>Placebo</td>
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<td>0.16 ± 0.08</td>
<td>0.16 ± 0.08</td>
<td>0.17 ± 0.10</td>
</tr>
<tr>
<td>Condition</td>
<td>Pre</td>
<td>10km</td>
<td>20km</td>
<td>30km</td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>CHO Rinse</strong></td>
<td>0.14 ± 0.05</td>
<td>0.14 ± 0.04</td>
<td>0.14 ± 0.05</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td><strong>CHO Ingest</strong></td>
<td>0.15 ± 0.05</td>
<td>0.15 ± 0.04</td>
<td>0.15 ± 0.05</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td><strong>WATER</strong></td>
<td>0.14 ± 0.05</td>
<td>0.14 ± 0.04</td>
<td>0.15 ± 0.04</td>
<td>0.14 ± 0.04</td>
</tr>
</tbody>
</table>