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The effects of a sport drink containing carbohydrates and electrolytes with or without
caffeine on 20km cycling time trial performance in men and women

by
Svein Piene

A Thesis
Submitted to the Faculty of Graduate Studies
through the Faculty of Human Kinetics
in Partial Fulfillment of the Requirements for
the Degree of Masters of Human Kinetics at the
University of Windsor

Windsor, Ontario, Canada

2012

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The effects of a sport drink containing carbohydrates and electrolytes with or without caffeine on 20km cycling time trial performance in men and women.

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Abstract

Caffeine is a commonly used substance by athletes and has become more widespread since its legalization by the World Anti Doping Association (WADA) and the International Olympic Committee (IOC). Extensive research has been done on the effect of caffeine on exercise performance, however, most studies were done at low intensity, and none compared differences in performance between men and women. Thus, 18 university aged (9 men and 9 women) individuals were recruited to perform three 20km cycling time trials including a familiarization trial (FAM), a carbohydrate plus placebo condition (CHO+P) and carbohydrate plus caffeine (5mg/kg) condition (CHO+C). Time to complete the 20km distance was significantly decreased in the CHO+C trial compared to the CHO+P trial in both men and women. This was concomitant with an increase in post-exercise blood glucose levels in the CHO+C trial but not in the CHO+P trial. However, ratings of perceived exertion (RPE), respiratory exchange ratio (RER), tympanic temperature and a novel measure of arousal (long term excitement, LTE) were not different between trials. It is concluded that caffeine can improve performance when combined carbohydrate over carbohydrate alone. Moreover, these performance improvements are not different between sexes. Of the subjective (RPE), physiological (HR, RER) and excitement (LTE) variables that were measured, it is unclear why caffeine had this effect. Nonetheless, these findings are intriguing, especially for those athletes looking to gain an advantage in activity that requires intense work over the span of 20-35min, as well as WADA in their decisions to examine the use of caffeine during competition. Caffeine is currently a legal ergogenic aid and these data add to the significant body of literature indicating its powerful ergogenic properties in many activities.

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Table of Contents

Author's Declaration of Originality	iii
Abstract	iv
Acknowledgements	v
List of Tables	viii
List of Figures	ix
List of Appendices	x
List of Abbreviations	xi
1. LITERATURE REVIEW	1
1.1 Physiological Effects of Caffeine.....	1
1.2 Caffeine and Exercise Performance	3
1.3 Sex Differences in Performance	5
1.4 Caffeine Dose and Sensitivity.....	7
1.5 Effects of Caffeine on Ratings of Perceived Exertion (RPE)	9
1.6 Effects of Caffeine on Blood Glucose	10
1.7 Effects of Caffeine on the Respiratory Exchange Ratio (RER).....	12
1.8 Effects of Caffeine on Heart Rate	14
1.9 Central Nervous System Fatigue.....	15
1.10 The Effects of CHO on Exercise Performance	18
1.11 The Effects of Caffeine and CHO on Exercise Performance.....	22
1.12 Summary	24
1.13 Reference List	26
2. INTRODUCTION	35
3. METHODS	37
3.1 Participants.....	37
3.2 Prior Caffeine Consumption	39
3.3 VO_{2peak} Test.....	39
3.4 Cycling Time Trials	42
3.5 Expired Gas.....	48
3.6 Blood Glucose.....	48
3.7 Tympanic Temperature.....	49
3.8 Ratings of perceived Exertion (RPE).....	49

3.9 Central nervous system arousal or long term excitement (LTE)	49
3.10 Statistics	50
4. RESULTS	52
4.1 Nutritional composition of pre-exercise meal.....	52
4.2 Performance Measures.....	52
4.3 Ratings of Perceived Exertion.....	56
4.4 Physiological Measures.....	56
Blood Glucose.....	56
Respiratory Exchange Ratio (RER)	60
Heart Rate	63
Tympanic Temperature	63
Long term excitement	68
4.5 Pearson Correlations	68
5. DISCUSSION	71
5.1 Performance Measures.....	71
6. LIMITATIONS	79
7. FUTURE DIRECTIONS	81
8. CONCLUSION.....	82
8.1 Reference List	83
Appendices.....	91
Vita Auctoris.....	95

List of Tables

Table 3.1. Participant characteristics	38
Table 4.1. Caloric composition of pre-exercise meals.....	53
Table 4.2. Mean time trial performance (seconds)	54

List of Figures

Figure 3.1. Cycling ramp test.....	41
Figure 3.2. Nutritional facts labels for Powerade™ and Powerade™ Zero.	45
Figure 3.3. Study design.	46
Figure 4.1. Performance time.....	55
Figure 4.2. RPE values in each exercise trial.....	57
Figure 4.3. Blood glucose values in each exercise trial.	59
Figure 4.4. RER values in each exercise trial.	61
Figure 4.5. Heart rate values in each exercise trial.	64
Figure 4.6. Tympanic temperature values in each exercise trial.....	66
Figure 4.7. LTE values in each exercise trial.....	70

List of Appendices

Appendix A. Caffeine Consumption Questionnaire. 91
Appendix B. Par Q form..... 93
Appendix C. VO2 max vs. performance time..... 94

List of Abbreviations

µl (microliters)
AT (Anaerobic Threshold)
BPM (Beats Per Minute)
BG (Blood Glucose)
CAF (Caffeine)
cAMP (Cyclic Adenosine Monophosphate)
CCQ (Caffeine Consumption Questionnaire)
CHO (Carbohydrate)
EEG (Electroencephalography)
FAM (Familiarization Trial)
FFA (Free Fatty Acid)
g (grams)
GLUT 1 (Glucose Transporter 1)
h (hour)
HSL (Hormone Sensitive Lipase)
HZ (Hertz)
IOC (International Olympic Committee)
km (kilometer)
LTE (Long Term Excitment)
min (minute)
ml (millilitres)
mm/L (millimoles/litre)
Na⁺ (Sodium)
PRK (Protein Kinase)
RER (Respiratory Exchange Ratio)
RPE (Ratings of Perceived Exertion)
RPM (Rotations Per Minute)
SGLT1 (Sodium Glucose Transporter 1)
VO₂ (Volume of Oxygen Consumed)
W (Watts)
WADA (World Anti Doping Agency)
α/β index (Alpha/Beta Index)

1. LITERATURE REVIEW

1.1 Physiological Effects of Caffeine

Caffeine (trimethylxanthine) is a naturally occurring substance found in approximately 60 different plants, and in North America is often ingested through coffee consumption (Graham et al., 2008). Coffee is the most commonly used drug worldwide, and in terms of international trade, is second only to oil in dollar amounts traded (Keisler & Armsey, 2006). Scandinavian countries account for the highest average intakes per person at nearly 400mg of caffeine/person/day compared to 238mg for those in the United States (Cauli & Morelli, 2005). Further, caffeine ingestion through other sources such as energy drinks is becoming increasingly prevalent in youth and, in particular, athletes who take caffeine supplements prior to competition in order to improve performance.

Once ingested, caffeine is absorbed through the gastrointestinal tract, metabolized by the liver, and has a half-life of approximately 5.7 h, indicating that caffeine levels in the blood are reduced by half within this timeframe (Statland & Demas, 1980). Elevated levels appear in the blood stream 15-45 min after consumption, and peak one hour post ingestion (Harland, 2000). Caffeine is lipid soluble, and therefore crosses the blood brain barrier with relative ease. This is an important property since many of the effects of caffeine are due to central nervous system modulation (Goldstein et al., 2010). Caffeine is an adenosine receptor inhibitor, and caffeine consumption is related to the up-regulation of adenosine receptors in the neural tissues of the brain. Adenosine receptor blockade results in a cascade of cellular events which include the increased release of

dopamine and noradrenaline (norepinephrine), both of which are used to explain the ergogenic effects of caffeine (Fredholm et al., 1999). Adenosine receptors (both A1 and A2) regulate oxygen consumption and blood flow through the coronary arteries, and regulate the release of dopamine and glutamate in the brain. Caffeine may also act to increase descending drive from the motor cortex by blocking the effects of adenosine acting to increase the excitation of motor units (Kalmar & Cafarelli, 1999). This increases synaptic drive to the cell body of an alpha motor neuron bringing it closer to threshold, thus facilitating maximal activation for muscle recruitment (Kalmar & Cafarelli, 1999).

Caffeine also inhibits the enzyme phosphodiesterase, a key regulatory enzyme of cyclic adenosine monophosphate (cAMP) signalling, thereby enabling cAMP to remain active at the cell membrane for a longer period of time (Yeo, et al., 2005). cAMP is a ubiquitous and important second messenger responsible for increasing cAMP-dependent protein kinase (protein kinase A, PKA) and a multitude of different downstream (i.e. subsequent signaling molecule activation) events. Interestingly, glucose absorption across the intestinal border through the Na⁺/glucose cotransporter (SGLT1) in the small intestine is increased by cAMP and consequently, higher levels of CHO are made available for oxidation (Hulston & Jeukendrup, 2008). Further, caffeine may mobilize free fatty acids from adipose tissue, increasing fat oxidation rates, thereby sparing muscle glycogen given that cAMP signaling is important to the activation of the rate-limiting enzyme, hormone sensitive lipase (HSL) in lipolysis. This metabolic theory, pioneered by Costill et al. (1978) suggests that when individuals spare muscle glycogen in favour of blood borne fuels (i.e. plasma glucose and free fatty acids), they have the ability to exercise harder for a longer duration. However, there is considerable evidence that the

ergogenic benefits of caffeine are not always explained by the metabolic theory (Cox et al., 2002). For example, Ivy et al. (1979) fed trained cyclists 250 mg of caffeine prior to 2 h of cycling exercise, and again at 15 min intervals for the first 90 min. Total work performance was increased by 7.4%, however, fat and carbohydrate (CHO) oxidation rates were similar to that of the placebo trial, and therefore did not explain the performance benefits. Further, Laurent et al. (2000) looked at the effect of ingesting 6 mg/kg of caffeine 90 min before exercise on the neuroendocrine axis during 2 h of cycling at 65% VO_2 max. They observed that epinephrine levels (an important hormone/neurotransmitter of the sympathoadrenal system) were significantly increased and plasma β -endorphin, (an opiate hormone associated with analgesia), levels almost doubled in the caffeine-treated groups (Laurent et al., 2000). Together, these results suggest that caffeine has the ability to influence performance through activation of the central nervous system.

1.2 Caffeine and Exercise Performance

The knowledge of caffeine's ergogenic effects on the body are important to athletes whose margin between winning and losing is becoming considerably less in competitive sport. Caffeine is the most commonly used ergogenic substance taken by competitive athletes prior to competition in both short and long duration exercise (Burke, 2008). Its widespread use is due not only to its ease of availability and social acceptance, but also to the fact that caffeine is considered legal regardless of its dosage. Initially, caffeine was banned in high doses by the World Anti-Doping Agency (WADA), stating that caffeine levels of over 12 $\mu\text{g/ml}$ in the urine were considered illegal, however, this

decision was reversed in 2004, leading to widespread use by athletes and extensive research done on the effect of caffeine on exercise performance (Burke, 2008). Prior to that point, however, the pioneering research in this field was conducted by Costill and colleagues in 1978, who evaluated the effect of caffeine (330 mg) on cycling to exhaustion at 80% of maximal oxygen consumption (VO_{2max}) in competitive cyclists. Subjects were able to cycle for 90.2 min compared to only 75.5 min in the placebo trial, with the performance increase being attributed to a significantly higher fat oxidation during the caffeine compared to the placebo trial (Costill et al., 1978). This increase in lipid metabolism was measured by an increase in plasma free fatty acid levels, plasma glycerol, and a decrease in the respiratory exchange ratio, used to measure the proportion of fat to CHO burned during exercise (Costill et al., 1978). Finally, the participants' ratings of perceived exertion (RPE) were significantly less in the caffeine trial compared to the placebo, indicating that even though the participants cycled significantly further, their perceived exertion levels in the caffeine trial were significantly less (Costill et al., 1978). The authors concluded that the performance increase was due to the combined effects on metabolism and central nervous system activation (Costill et al., 1978). One year later, a study conducted by Ivy et al. (1979) revealed similar results. The authors showed that the ingestion of caffeine before prolonged (2 h) cycling exercise significantly increased work production and plasma free fatty acid levels, indicating greater fat oxidation. However, the authors also noted that caffeine exerted an effect on the central nervous system (Ivy et al., 1979). When the participants consumed caffeine before exercise, they began the exercise bout at a much higher intensity, yet perceived the same exertion level compared to the placebo and glucose only trials (Ivy et al., 1979).

Additionally, caffeine mobilized free fatty acids from adipose tissue, producing higher rates of fat oxidation and hence sparing muscle glycogen which is important during prolonged exhaustive exercise (Ivy et al., 1979).

As suggested in the early research, caffeine has the ability to increase performance in prolonged cycling (over 1h) through changes in fuel utilization and a possible activation of the central nervous system. These results were confirmed in later research, extending the literature to shorter duration exercise. For example, it is clear that moderate amounts of caffeine (4-6mg/kg) have the ability to increase performance during a trial to exhaustion at 85% VO₂ max (Graham & Spriet, 1991; Graham et al., 1998). It is also evident that similar dosages of caffeine can improve performance in time trial tests lasting less than an hour (Bridge & Jones, 2006; McNaughton et al., 2008). Studies which employ a time trial test are much more practical as they simulate a real life racing scenario. This study design is less common throughout the literature, however, should be employed in order to more closely replicate competition.

Thus, it appears that caffeine ingestion has the ability to significantly improve prolonged and high intensity endurance performance, and these performance effects are typically observed when caffeine is ingested 1 h prior to exercise. The performance improvements during short duration exercise (< 1 h) are likely due to changes in neural activation (Jackman et al., 1996), and not the sparing of muscle glycogen, as glycogen depletion is not a limiting factor during this period.

1.3 Sex Differences in Performance

In a recent review by Goldstein et al. (2010) it was noted that there have been few studies done on the effect of caffeine on intense aerobic exercise in woman. Further, none of these studies evaluated sex differences in terms of performance (time to exhaustion) or substrate utilization during exercise. In one study that examined women, the effects of varying doses of caffeine on fuel metabolism and short term (10 min), high intensity aerobic exercise performance was studied in well trained female rowers (Anderson et al., 2000). Participants ingested 3 ml/kg body mass of water with capsules containing either 6 mg/kg caffeine, 9 mg/kg caffeine or a placebo containing 500 mg of glucose (Anderson et al., 2000). Performance time improved significantly after ingestion of the high caffeine dose, although the lower dose did not significantly improve performance compared to the placebo (Anderson et al., 2000). Further, MacLeod (2004) tested only female participants and found a 13% increase in time to exhaustion (68.4 to 77.2 min) at 80% VO_2 max after ingesting 5 mg/kg of caffeine compared to a placebo. These studies suggest that caffeine can produce a significant performance improvement in females, however, this does not give insight into performance differences between men and women. In another study, the effect of caffeine ingestion on substrate metabolism and glucose production was evaluated in trained endurance athletes who performed 60 min of cycling exercise at 65% VO_2 max (Roy et al., 2001). Participants included both men and women, but once again, sex differences and performance measures were not evaluated (Roy et al., 2001). This was also the case in a study examining the effect of caffeine ingestion at different time points prior to exercise at 80% VO_2 max in males and females (Bell & McLellan, 2002). While caffeine significantly improved time to exhaustion compared to the placebo, with the improvement being greater in non-users, sex

differences were not reported (Bell & McLellan, 2002). These studies indicate that while caffeine may be effective for female exercisers, sex differences in its effectiveness are unclear. However, given that caffeine can produce greater arousing affects in males compared to females at rest (Adan et al., 2008), it could be that females do not respond as well to caffeine during exercise. Interestingly, after caffeine was removed from the WADA banned list, the prevalence of caffeine use by male and female athletes (measured as that excreted in the urine) was similar, however the authors noted that this data should be viewed cautiously given that there were 4 times as many male samples as females (Del Coso et al., 2012).

1.4 Caffeine Dose and Sensitivity

While it appears that caffeine can increase performance through stimulation of the central nervous system and possible modifications in fuel utilization, there are other factors which may influence the effectiveness of the drug. For example, there is evidence that caffeine dosage and sensitivity may play an important role. In a simple dose response study, the effect of various caffeine doses (3, 6 or 9 mg/kg) on metabolism and endurance performance was investigated in well trained endurance athletes (Graham & Spreit, 1995). Participants ingested caffeine an hour before exercise performed at 85% VO_{2max} until exhaustion. Endurance was increased with both 3 and 6 mg/kg of caffeine ingested prior to exercise compared to the placebo, however, there was no significant performance increase when ingesting 9 mg/kg of caffeine (Graham & Spreit, 1995). This is in agreement with a recent position stand by Goldstein et al. (2010), who indicated that there are no further increases in performance when ingesting 9 mg/kg of caffeine prior to

exercise compared with 3 and 6 mg/kg. However, Graham & Spriet (1995) indicated that in their previous work, significant increases in endurance performance were found after ingesting 9 mg/kg of caffeine. They speculated that the differences may lie in the participants' ingestion of caffeine on a regular basis, as the previous study had fewer light and/or caffeine non-users than the latter study, and generally, the light caffeine users showed no response or negative responses, and complained of mental confusion after ingesting the high dosage of caffeine (Graham & Spreit, 1995).

However, there is research indicating that caffeine may have a greater effect on caffeine non-users during exercise. For example, time to exhaustion during a trial at 80% VO_{2max} was significantly longer (28.8 min compared to 24 min average for all subjects) when subjects ingested 5 mg/kg of caffeine 1,3 and 6 h prior to exercise compared to a placebo (Bell & McLellan, 2002). The performance improvement was significantly greater in non-caffeine users (less than 50 mg/day) compared to users (greater than 300 mg/day). As previously described, caffeine acts on the central nervous system to antagonize adenosine receptors resulting in increased release of dopamine. It is suggested that the same concentration of caffeine could block a higher percentage of adenosine receptors for non-users compared to caffeine users (Bell & McLellan, 2002). Therefore, a higher blood concentration of caffeine would be needed to block the same percentage of receptors for caffeine users. The authors further suggest that a dose of 5 mg/kg would be closer to optimal for caffeine non-users. It is important to note that while higher caffeine doses (>5 mg/kg) may be effective for caffeine users, these doses may produce negative effects such as nausea, anxiety and confusion in non-users of the drug (Graham & Spriet, 1995). It becomes even more complicated considering that individual differences in

sensitivity to the drug may also create large variability in its effectiveness (Bell & McLellan, 2002).

1.5 Effects of Caffeine on Ratings of Perceived Exertion (RPE)

Regardless of caffeine's mechanism of action and variability between individuals, another possible outcome of caffeine ingestion throughout the literature is the change in participants' perceptual response, evaluated by RPE a measure of how hard the participant is exercising (Doherty & Smith, 2005). Bell & McLellan (2002), found that the RPE was reduced when caffeine was ingested compared to a placebo in caffeine users and non-users. Further, there was a trend for lower RPE values during an 8 km time trial when trained male distance runners ingested 3 mg/kg of caffeine before exercise, however, it did not reach statistical significance (Bridge & Jones, 2006). The authors suggested that the trend for reduced RPE with caffeine ingestion, along with an increased heart rate during exercise, indicated that caffeine may have an effect on the central nervous system by reducing the perception of effort and fatigue (Bridge & Jones, 2006). In contrast, the ingestion of 6 mg/kg or 9 mg/kg of caffeine prior to a 2000 m rowing time trial had no effect on RPE taken after the trial in elite female rowers (Anderson et al., 2000). The authors suggested that the mechanism behind the performance improvement was associated with an effect on skeletal muscle and subtle changes in neuromuscular recruitment (Anderson et al., 2000). These results are not in agreement with those generally found throughout the literature. A meta-analysis revealed that there is a 6% reduction in RPE when caffeine is ingested compared to a placebo, and that the mean change in RPE is significantly related to mean change in performance (Doherty & Smith,

2005). The authors, however, indicated that there is no difference in RPE at the conclusion of exhaustive exercise done at a constant load, suggesting that fatigue and sense of effort experienced at the end of exhaustive exercise (e.g. a time trial) is the same regardless if caffeine is ingested or not (Doherty & Smith, 2005). Therefore, it is clear that caffeine can decrease the perceptual response during exercise, possibly allowing participants to centrally recruit more motor units, thereby increasing power output (Doherty & Smith, 2004). Variables such as subject withdrawal from caffeine and caffeine dosage did not have any influence on the effects of caffeine on RPE, although individuals with the highest VO_{2max} had the largest reduction in RPE during exercise (Doherty & Smith, 2005). Finally, one study found that the RPE was dependent on exercise intensity, concluding that the RPE was lower during the caffeine condition (5 mg/kg) during exercise 10% below the anaerobic threshold and not exercise 10% above the anaerobic threshold (Denadai & Denadai, 1998). The authors, however, did not offer any insight as to why caffeine reduced the RPE during low intensity and not high intensity exercise

1.6 Effects of Caffeine on Blood Glucose

Apart from an influence on performance time and RPE, caffeine can influence other physiological factors that are important during maximal aerobic exercise. For example, it has been suggested that caffeine can increase blood glucose levels during exercise (Bell & McLellan, 2002; Graham & Spriet, 1995; Desbrow et al., 2012). Caffeine ingestion (5 mg/kg) significantly increased blood glucose levels during exercise (Bell & McLellan, 2002). Further, blood glucose levels were significantly increased at 15 and 30

min when subjects ingested 6 mg/kg of caffeine prior to exercise compared to a placebo during exercise at 85% VO_{2max} (Graham & Spriet, 1995). Trice and Haymes (1995) indicated that the decline in blood glucose levels were significantly less in the caffeine compared to the placebo trial when subjects ingested 5 mg/kg of caffeine before exercise at 85-90% maximum workload. Finally, a lower dose of 3 mg/kg of caffeine had no effect on plasma glucose levels after exercise, however, a higher dose of 6 mg/kg significantly increased plasma glucose levels after exercise compared to the placebo trial (Desbrow et al., 2012). It is difficult to speculate how caffeine can significantly increase blood glucose levels post exercise in these studies, especially since none of these investigations provided explanation as to why this occurred. One possibility is that individuals may exercise at a higher intensity after ingesting caffeine which may promote increased liver glucose output into the blood stream. A more likely mechanism is caffeine's ability to increase intestinal glucose absorption during exercise. This has been attributed to a direct action of caffeine on the GLUT1 transporter which is responsible for bringing glucose from the enterocyte (an intestinal cell responsible for absorption) to the blood (Van Nieuwenhoven et al., 2000). It has been suggested that a significant increase in blood glucose levels after acute administration of 200 mg of caffeine at rest is independent of an increase in insulin levels and may be the result of a caffeine induced catecholamine release or adenosine receptor antagonism which would inhibit glucose uptake into the muscle (Pizziol et al., 1998).

In contrast to these findings, there is research showing that caffeine has no effect on blood glucose levels during exercise. For example, caffeine (4.45mg/kg) had no effect on blood glucose levels in treadmill running to exhaustion at 85% VO_{2max} (Graham et al.,

1998). Further, no differences in blood glucose levels were found when subjects ingested 6 mg/kg of caffeine 90 min before exercise to exhaustion at 80% $\text{VO}_{2\text{max}}$ (Greer et al., 2000). Finally, in a review article by Graham (2001), the author indicates that although some studies suggest that caffeine increases blood glucose levels during exercise, most find no change.

1.7 Effects of Caffeine on the Respiratory Exchange Ratio (RER)

Early research suggests that the ergogenic properties of caffeine are mediated by enhanced fat oxidation and a decreased reliance on CHO during exercise (Costill et al., 1978; Ivy et al., 1979), however, this has been challenged in recent research (Graham et al., 2000; Bell & McLellan, 2002; Laurant et al., 2000). Although caffeine may have the ability to influence fuel utilization during exercise, the RER, which determines the proportion of CHO and fats burned, has for the most part failed to represent this increase in fat utilization. For example, Bell & McLellan (2002), found that the RER was similar between trials and not influenced by 5 mg/kg of caffeine ingested 1, 3 and 6 h before exercise in a total of six trials. Further, the RER was not significantly different during 105 min of steady state exercise followed by a 45 min time trial when subjects ingested either CHO or CHO + caffeine (5.3 mg/kg) prior to exercise (Hulson & Jeukendrup, 2008). Finally, the RER was unaffected by a high caffeine dose during a 20 km time trial which followed 2 h of cycling, however, when caffeine was taken with CHO, the RER was lower than with CHO alone (Slivka, 2008). The authors indicated that this finding, in contrast to other investigations which found no change in RER between CHO and caffeine combined with CHO (Jacobson et al., 2001, Hulson & Jeukendrup, 2008), may

be the result of the participants being in a negative energy balance achieved with dietary restriction 2 days prior to the exercise trial (Slivka, 2008)

The general finding that caffeine is unable to influence the RER during exercise suggests that muscle glycogen and blood glucose oxidized along with total fat oxidation is unaltered (Graham et al., 2000). Further, muscle biopsy analysis reveals no sign of glycogen sparing during exercise. The authors suggest that while caffeine was unable to affect CHO and fat metabolism in the active muscle, other effects on the liver, resting muscle tissue, and tissues of the nervous system were significantly affected by caffeine ingestion (Graham et al., 2000). To confirm this finding, data was pooled from several studies which looked at the effect of caffeine on key metabolic intermediates which are proposed to be increased by fat oxidation and inhibited by CHO metabolism during exercise (Graham et al., 2008). The results showed that caffeine had no effect on glycogen, acetyl-coA, citrate, and glucose-6-phosphate during exercise, providing evidence that caffeine is unable to affect fuel makeup during exercise (Graham et al., 2008). The authors did find that caffeine significantly increased cAMP concentrations during exercise, which they suggest is compatible with caffeine's inhibition of adenosine receptors (Graham et al., 2008). Further, Roy and colleagues (2001) examined the effect of caffeine on serum FFA concentrations and glucose rate of appearance and disappearance and found that these variables were increased at the onset of exercise, however, were unaffected by caffeine ingestion (6 mg/kg) (Roy et al., 2001). It was concluded that caffeine does not influence glucose kinetics or energy metabolism during exercise at 65% VO_{2max} in trained athletes (Roy et al., 2001). It is also plausible that higher levels of catecholamines produced during exercise may negate the glycogen

sparing effect of caffeine (Laurant et al., 2000). Thus, there is ample evidence indicating that the performance enhancing effects of caffeine are not due to changes in fat and CHO metabolism during exercise (Graham et al., 2008).

1.8 Effects of Caffeine on Heart Rate

There is a common statement made throughout the literature that caffeine can increase exercising heart rate through stimulation of the central nervous system (Jacobson et al., 2001). Though this is often the case during high intensity exercise, there is evidence that caffeine can decrease heart rate when exercising at low intensity. For example, low dose caffeine (3 mg/kg) resulted in significantly lower heart rates in caffeine non-users during an incremental test to exhaustion, however, this finding only appeared during low-moderate intensity exercise and not when the subjects reached exhaustion (McClarran & Wetter, 2007). This finding is in agreement with Gaesser and Rich (1985), who found that the administration of 5 mg/kg of caffeine resulted in lower heart rates during exercise at 30-70% $\text{VO}_{2\text{max}}$, but no difference in heart rate when participants exercised above 75% $\text{VO}_{2\text{max}}$. At a higher intensity, 5 mg/kg taken prior to exercise significantly increased heart rate at 85% $\text{VO}_{2\text{max}}$ in habitual and non-caffeine users, yet had no effect on exercise at 50% $\text{VO}_{2\text{max}}$ (Bell & McLellan, 2002).

The ability of caffeine to decrease heart rate at low-moderate exercise intensities may be attributed to increased or optimized heart stroke volume due to enhanced contractility or higher preload with caffeine ingestion (Gould, 1973). For example, caffeine can increase stroke volume as a result of enhanced left ventricular end-diastolic volume (Gould, 1973). An increase in calcium efflux affecting the contractile state of the

heart combined with caffeine's ability to dilate blood vessels may also be a plausible mechanism (Gould, 1973). Nonetheless, it is not entirely clear why caffeine can decrease the heart rate at submaximal intensities yet not at higher intensities. It may be that contractility (stroke volume) cannot be further improved at a higher intensity (McClarran & Wetter, 2007), however, further research is warranted to determine this mechanism.

Although caffeine may decrease exercising heart rate at low intensities, it has the opposite effect at high intensity. For example, caffeine ingestion (3 mg/kg) resulted in a significantly higher mean heart rate during an 8 km time trial compared with the control and placebo trials in trained male distance runners who were caffeine users (Bridge & Jones, 2006). The authors suggested that the participants were able to exercise at a higher percentage of their maximal heart rate during the caffeine trial (Bridge & Jones, 2006). Kovacs et al. (1998) also found that heart rate was significantly higher when subjects ingested caffeine and CHO before and during a 1 h cycling time trial. They suggested that the higher heart rate with caffeine ingestion was not a direct effect of caffeine, yet a result of the higher work output when subjects ingested caffeine before and during exercise (Kovacs et al. 1998). Thus, caffeine can increase heart rate at high exercise intensities, yet produces the opposite effect at submaximal intensity.

1.9 Central Nervous System Fatigue

Although the exact mechanisms by which caffeine exerts its ergogenic effects are still unknown, it has been shown that caffeine affects neuromuscular function through stimulation of the central nervous system (Goldstein et al., 2010). If caffeine improves performance, yet fuel utilization is unchanged, then a central component can be suggested

as the reason for performance improvement. The central governor model suggests that exercise is regulated by complex mechanisms in the central nervous system (Noakes, 2011). This model proposes that there is continuous feedback from various organs in the body which send information on physiological factors, such as fuel reserves and hydration state, directly to the central nervous system, thereby allowing the brain to regulate exercise by modifying the number of motor units recruited. In order to measure changes in central regulation during exercise, EEG can be used to monitor changes in brain cortical function. The alpha to beta ratio (α/β index) can be used as an index of fatigue, where an increase in alpha activity combined with a decrease in beta activity can represent decreased arousal (Nielsen & Nybo, 2003). Alpha waves are in the frequency range of 8-13 Hz and originate from the occipital lobe during wakeful relaxation and may play an active role in network communication (Palva & Palva, 2007). Beta waves are in the frequency range of 12-30Hz, and in the motor cortex they are associated with muscle contractions (Baker, 2007). Although it is unknown how caffeine will influence alpha and beta waves, it is clear that exercise has the ability to modulate brain wave activity. For example, Ftaiti et al. (2010) showed that the α/β index increased significantly from the onset of exercise to exhaustion when exercising in the heat. Further, the changes in α/β ratio at the end of exercise were due to significant decreases in beta activity before exhaustion. These results are in agreement with those of Nybo and Nielson (2001), who reported that the increase in the α/β index was due to decreases in beta activity, while alpha activity was unchanged. It is suggested that the increase in alpha activity is the greatest 5-10 min after exercise and is the strongest in the frontal brain regions (Boutcher

& Landers, 1988; Shneider et al., 2010). Further, it is clear that the increase is specifically related to emotional effects resulting from exercise as the frontal cortex is associated with emotional processing (Coan & Allen, 2004). It is suggested that alpha activity is inversely related to cerebral activity where an increase in alpha activity would represent a decrease in cortical activation (Shneider, 2010).

Decreased cortical activation or arousal level corresponding to fatigue was named the inverted “U” hypothesis over 50 years ago (Hebb, 1955), and is still an excepted model today (Kamijo et al., 2004). As exercise intensity increases, arousal level also increases reaching an optimal level during medium intensity exercise and then dropping as an individual approaches fatigue (Kamijo et al., 2004). Decreased activation may be the result of neuroregulatory feedback from the heart to the brain during exhaustive exercise (Lacey & Lacey, 1978). In this model, baroreceptors in the carotid arteries are stimulated by blood from the heart contraction phase, resulting in feedback that causes decreases in brain activation.

This change in arousal level is related to alterations in central nervous system functioning and may also be related to temperature increases during exhaustive exercise. Central nervous system command is the primary system that causes sympathetic activation of the skin during static and dynamic exercise in humans (Vissing et al., 1991). Further, the increase in the α/β index during exercise is related to increases in core temperature as exercise intensity increases (Nielson et al., 2001). In this case, central nervous system fatigue appears to be related to inhibitory signals sent from the hypothalamus which result from increase in brain temperature (Nybo, 2008). Thus, it is

clear that high intensity exercise to exhaustion results in a reduction in arousal levels (Kamijo et al., 2004). The reduced arousal levels which accompany fatigue are represented by an increase in the α/β ratio and may be associated with increases in temperature as exercise intensity increases.

Changes in arousal levels during exhaustive exercise represent central fatigue, however, it is unclear how the ingestion of caffeine influences arousal levels during exercise, and how these cortical changes are different between males and females.

1.10 The Effects of CHO on Exercise Performance

Fatigue during endurance exercise is multifaceted, but is augmented by reduced blood glucose and muscle glycogen concentrations, both prior to and during exercise (Green, 1991). Not surprisingly then, the design and sale of sports drinks containing CHO is a major component of the sports supplement industry. CHO (typically 6-8% glucose) oral fluid replacement drinks such as Gatorade™ or Powerade™, ensure adequate hydration during exercise and provide a supplementary exogenous glucose source. Since the 1980s, it has been commonly agreed that exogenous CHO are beneficial during prolonged exercise due to the maintenance of plasma glucose concentrations and high rates of CHO oxidation. Until recently, it was thought that the ergogenic effects of CHO ingestion would only appear in endurance exercise lasting 2 h or longer (Jeukendrup et al., 2008). It is now apparent that CHO can improve shorter duration exercise through central mechanisms in addition to sparing muscle glycogen during prolonged exercise.

Many studies have been done investigating the effect of CHO on long duration low intensity and short duration high intensity exercise (Khanna & Manna, 2005; Rollo & Wasiams, 2009; El Sayed et al., 1996). These studies have shown that CHO ingestion during endurance exercise improves performance when given periodically. For example, total endurance time at 70% VO_{2max} was significantly higher after ingestion of a 5% CHO drink at 15 min intervals during exercise compared to a placebo in endurance trained individuals (Khanna & Manna, 2005). Further, distance covered during a 1 h treadmill run (approximately 80% VO_{2max}) was significantly longer and running speed was significantly faster when a 6.4% CHO solution (8 ml/kg, 30 min before and 2 ml/kg at 15 min intervals throughout the 1 h run) was ingested compared with two placebo trials (Rollo & Wasiams, 2009). Finally, the ingestion of an 8% CHO solution 25 min prior to exercise significantly increased mean power output and improved distance covered in comparison to a placebo solution over a 1 h time trial in elite cyclists (El Sayed et al., 1996). El Sayed et al., (1996) found that plasma glucose concentrations were significantly higher 30 min post exercise in the CHO trial compared to the placebo, however, the RER was not appreciably different between trials. The authors suggested that CHO ingestion may have an effect on central fatigue, and cannot be entirely dismissed as a reason for enhanced performance from pre-exercise CHO feeding in this study (El Sayed et al., 1996).

In contrast, other studies have found no performance improvement when ingesting CHO during exercise compared to a control or placebo. For example, the exercise time to exhaustion was not significantly different in well-trained men who ingested a 6% glucose solution or a placebo during exercise at 83% VO_{2max} lasting approximately an hour

(McConnell et al., 2000). In a shorter duration test, there were no differences in the time to complete a 25 min time trial after ingesting a 6% CHO solution 5 min before exercise and at 25, 50 and 75% completion compared to the placebo with either treatment (Jeukendrup et al., 2008). In fact, the times were remarkably similar, and it was suggested that CHO intake prior to and during short term high intensity cycling has no benefit to performance (Jeukendrup et al., 2008). This study is in contrast to several investigations which have found improvements when ingesting CHO during exercise lasting approximately an hour. However, most of these studies were performed in a fasted state, while this particular study was done in the fed state, which represents a more realistic situation (Jeukendrup et al., 2008). It is possible that the pre-exercise feeding may have masked the effect of CHO feeding during exercise. However, since the mechanism of improved performance is unlikely related to fuel stores, it is difficult to speculate how pre-exercise feeding would influence performance effects (Jeukendrup et al., 2008).

There have been fewer studies done on the effects of liquid CHO ingestion before exercise, without ingestion during exercise. In a practical sense, additional CHO are not ingested during running races less than 10km, while they would be during races of longer duration. It is also worth noting that many studies done on the effect of liquid CHO ingestion before exercise focused on low intensity exercise (below 75% VO_{2max}) rather than higher intensity exercise that would simulate a 10 km race. Athletes exercising for longer periods (90 min or longer) would surely ingest CHO during exercise, making the following studies impractical. For example, performance was improved by 15% after ingestion of 312 g of CHO in solution, 4 h before 95 min of cycling exercise followed by

a time trial (Sherman, 1989). In a different study, the same authors again found a significant improvement in cycling exercise at 70% VO_{2max} after ingestions of 1.1 and 2.2 g/kg of liquid CHO 1 h before exercise (Sherman et al., 1991). Total CHO oxidation was also greater in the CHO trials compared to the placebo trial (Sherman et al., 1991). In contrast, no differences in exercise time to exhaustion were observed compared to a placebo when ingesting 75 g of glucose in 350 ml of water prior to exercise to exhaustion at 75% VO_{2max} (Hargreaves et al., 1987). The lack of performance improvement in this study may be due to the fact that less CHO was ingested before exercise compared to the first two investigations. Regardless, the ingestion of CHO before exercise is important during shorter duration exercise, as those competing in longer duration races will most likely be ingesting CHO during the competition.

The improvements in performance as a result of CHO ingestion before a short (1 h) high intensity (80-85% VO_{2max}) bout of exercise cannot be explained by the same mechanisms which determine improvements in prolonged exercise lasting 2 h or more (Jeukendrup et al., 2008). During high intensity (85% VO_{2max}), short duration exercise plasma glucose levels do not normally drop significantly, and are sometimes increased as a result of glucose output by the liver (Romijn et al., 1993). When looking at the effect of CHO ingestion from a substrate availability standpoint, it is unlikely that CHO influence short duration high intensity exercise as muscle glycogen depletion is not a limiting factor (Jeukendrup et al., 2008). Therefore, the method in which CHO improves performance during short duration, high intensity aerobic exercise has been suggested to be through central nervous system modulation (Jeukendrup et al., 1997). A possible mechanism by which this may occur is that when CHO are not ingested before and during exercise,

plasma insulin levels are reduced and higher rates of fat oxidation occur. As a consequence, high levels of free fatty acids bound to albumin may result in higher levels of free tryptophan, an amino acid normally bound to albumin when free fatty acid levels are lower (Curzon et al., 1973). This causes an increase in the ratio of tryptophan to branch chain amino acids, allowing higher levels of tryptophan to diffuse across the blood-brain barrier, possibly causing central fatigue (Curzon et al., 1973). In addition to the increases in tryptophan, branched chain amino acids are taken up by the muscle during exercise, further increasing this ratio (Blomstrand, 2006). Davis & Bailey (1997) proposed that higher levels of tryptophan cause increased levels of brain serotonin, which affects arousal and mood by increasing the perception of effort and muscle fatigue.

1.11 The Effects of Caffeine and CHO on Exercise Performance

Many studies have looked at the effect of caffeine combined with CHO on exercise performance (Kovacs et al., 1998; Cureton et al., 2007; Hulston & Jeukendrup, 2008; Yeo et al., 2005), however, few studies were found investigating the effect of caffeine combined with CHO ingestion on high intensity aerobic exercise (~85-90% VO_{2max}) lasting between 20-35 min. Moreover, the majority of these studies did not include female subjects, and none evaluated differences in response between males and females. Nonetheless, several of these investigations found performance improvements when ingesting caffeine combined with CHO compared with CHO alone, but were done at low intensity or were followed by a time trial. For example, Kovacs et al. (1998) evaluated performance and found that participants completed a 1 h time trial significantly faster and with higher mean work output after ingesting a CHO electrolyte solution in

addition to 225 mg of caffeine compared with placebo water, placebo CHO electrolyte solution or CHO electrolyte solution plus 150 mg of caffeine. The greatest performance increase was observed after ingestion of 225 mg of caffeine, while no further performance improvements were seen after ingestion of 320 mg (Kovacs et al., 1998). Further, the work completed during an all-out 15 min performance ride which followed 135 min at 60-75% VO_{2max} , was 15-23% higher when ingesting CHO combined with caffeine compared to CHO alone (Cureton et al., 2007). The combination of CHO (6.4%) and caffeine ingestion (5.3 mg/kg) increased time trial performance by 4.6% compared to a CHO trial, and 9.0% compared to a placebo after 105 min of steady state cycling, followed by a 45 min time trial (Hulston & Jeukendrup, 2008). Finally, the effect of a CHO beverage (5.8% glucose solution), a caffeine + CHO beverage and water alone was examined on 2 h cycling performance at 55% maximum wattage (Yeo et al., 2005). In the final 30 min of exercise, exogenous CHO oxidation rates were 26% higher in the CHO plus caffeine trial compared to the CHO only trial (Yeo et al., 2005). Further, the RER was significantly higher in the caffeine plus CHO trial compared to CHO alone, however, it is important to note that CHO and caffeine (total 5 mg/kg) were distributed in increments every 15 min throughout the exercise bout.

One study found no performance improvement when caffeine was added to CHO compared to CHO alone. Jacobson et al. (2001) found that caffeine (6 mg/kg) co-ingested with CHO (2.6 g/kg) 1 h before exercise had little effect on metabolism or exercise performance compared to CHO ingestion only when exercising for 2 h at 70% VO_{2max} . A similar study was performed which found a performance increase with the addition of caffeine to a CHO drink (Kovacs et al., 1998). Metabolic measures were not

taken during this study, and it is unlikely that glycogen depletion was limiting during 1 h of exercise in this investigation. Therefore, the data from Jacobson et al. (2001) and that of Kovacs et al. (1998), suggest that caffeine doesn't affect substrate metabolism when ingested with CHO, however, does decrease RPE compared to CHO alone (Jacobson et al., 2001). This suggests that a central mechanism is enhanced when caffeine is combined with CHO compared to CHO alone.

In summary, the intake of CHO prior to and during exercise in combination with caffeine has the ability to improve performance to a greater extent than CHO ingestion alone. In a meta-analysis done by Conger et al. (2011), the average performance difference reported in various studies suggested that CHO combined with caffeine might result in a 6% performance improvement compared to CHO alone. Eighteen of the twenty one studies in this analysis yielded a positive effect size favoring CHO combined with caffeine with an overall effect size of 0.26 (95% CI 0.15-0.38) (Conger et al., 2011).

1.12 Summary

In summary, it is clear that caffeine has the ability to improve performance in both low intensity and high intensity exercise and may affect other variables such as heart rate, blood glucose and RPE. It is interesting that the WADA has removed caffeine from the banned substance list given the numerous reports that it can improve exercise performance. It may be that caffeine use is so widespread, mainly through coffee consumption, energy drinks and sports bars that it would cause too much controversy among the competing athletes. Further, caffeine is a drug easily available to most athletes worldwide, which gives each competitor an equal opportunity to take this

supplement prior to competition. It is clear that there are gaps in the research on caffeine and exercise performance. Few studies have looked at caffeine combined with carbohydrates on high intensity aerobic exercise. Most studies have used a time to exhaustion protocol, which is not a good simulator of a competition. Further, to our knowledge, there are no studies which evaluated the effect of caffeine ingestion on exercise performance differences between men and women. Although there has been extensive research in this field, it is clear that further studies should be conducted to bridge the gaps in the research.

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2. INTRODUCTION

While the supplementation with exogenous carbohydrate (CHO) primarily relies on the principal of increased substrate availability and/or a sparing of endogenous CHO stores during exercise, the ergogenic effects of caffeine, a known stimulant that is not metabolized for energy, are less well understood. Some suggest that caffeine can improve performance in exercise greater than an hour by increasing the use of fat stores as fuel and thus sparing muscle glycogen (Costil et al., 1978; Ivy et al., 1979). There is an increasing amount of research, however, that suggests that caffeine may improve performance in shorter duration exercise (less than 1h) by having an effect on the central nervous system (Goldstein et al., 2010). Despite numerous reports on the performance improving effects of caffeine, the World Anti-Doping Agency (WADA) and International Olympic Committee (IOC) have removed caffeine from its banned list and placed it on the monitoring list to examine how common its use is among elite athletes (The World Anti-Doping Code, 2010). Interestingly, in a recent study, it was reported that 3 out of 4 athletes in a multitude of sports had detectable levels of caffeine in their urine after competition (Del Coso et al., 2011).

Several studies have examined the effect of caffeine, and caffeine combined with CHO on aerobic performance lasting longer than 45 min (Cureton et al., 2007; Graham & Spreit, 1991; Graham & Spreit, 1995; Hulston & Jeukendrup, 2008; Jacobson et al., 2001; Kovacs et al., 1998; Yeo et al., 2005) or on anaerobic sprint and weight lifting performance (Davis & Green, 2009). Very few studies were found investigating the effects of caffeine combined with CHO ingestion on high intensity aerobic exercise (~85% maximal oxygen consumption (VO_{2max})) lasting between 20 and 35 min where a

time trial performance was used. There is a smaller coefficient of variation when subjects perform a time trial test compared to an exercise test to exhaustion (Laursen et al., 2007), and a time trial more closely simulates an actual racing situation (for example, a cross country run for male and female collegiate athletes lasts ~20-35 min and requires that they sustain a high intensity close to the anaerobic threshold for the entire race). Caffeine use among these athletes is increasing, as one study reported that 32% of track and field athletes use caffeine supplements prior to competition in order to improve performance (Chester & Wojek, 2008).

No studies were found examining sex differences in performance in response to combined caffeine and CHO ingestion prior to high intensity aerobic exercise (~85-90% VO_{2max}). In a recent meta-analysis done by Conger et al. (2011), the authors reported that 93% of participants tested in this field were male. Further, these studies did not look at sex differences and other important variables that may be influenced by caffeine ingestion such as the respiratory exchange ratio (RER), blood glucose (BG), RPE and heart rate.

While both CHO and caffeine drinks are commonly used by athletes to hydrate and improve performance before and during aerobic activity, many athletes are unsure of the proper combination of caffeine and/or CHO to ingest before their specific event. Energy drinks such as Red Bull™ and Rockstar™, which contain both CHO and caffeine are increasing in popularity, but are often used recreationally, and are not targeted toward athletes. Further, practical evidence in this field is lacking, as many investigations do not adequately simulate a race or competition and any sex related differences are underreported or ignored. Therefore, a novel study was designed to

evaluate whether caffeine combined with CHO can be beneficial during a high intensity aerobic exercise time trial, and whether these responses are different between males and females. Further, this would be the first complete study to examine the usefulness of a portable brain activity monitor, the Emotiv Epoc, to study arousal in a sporting situation.

It was hypothesized that caffeine combined with CHO would improve performance over CHO alone. Further, given that males tend to be more responsive to caffeine, it was also hypothesized that males would show a greater performance improvement than females. The secondary hypotheses include that blood glucose levels will be elevated greater post exercise in the caffeine trial, RER will be unchanged with caffeine ingestion, heart rate will be elevated in the caffeine trial, and that there will be a decrease in LTE with fatigue that will be less pronounced in the caffeine trial.

3. METHODS

3.1 Participants

Participants were recruited from the University of Windsor using a Faculty email recruitment form and by word of mouth. Participant characteristics are presented in table 3.1.

Table 3.1. Participant characteristics

	n	Age	% Caffeine High- Users	Weight (kg)	Height (cm)	BMI	VO₂max (ml/kg/min)
Males	9	22.7 ±2.3	67	78.6 ±13.9	180.3 ±8.0	24.0 ±2.9	38.8 ±7.6
Females	9	21.3 ±2.4	33	63.5 ±5.5	168.2 ±7.0	22.4 ±1.8	28.0 ±4.2
All participants	18	22 ±2.4	50	71.1 ±12.9	174.3 ±9.6	23.2 ±2.5	33.4 ±8.1

Table 3.1. All values are presented as means ± standard deviations (SD). Males had a higher mean weight, height and VO₂ max than females (p<0.05).

This study consisted of a double-blind placebo controlled design. All methods were cleared by the University of Windsor Research Ethics Board prior to study initiation.

3.2 Prior Caffeine Consumption

Prior caffeine use was established by having participants indicate their daily and weekly intakes of caffeinated drinks and foods using a Caffeine Consumption Questionnaire (CCQ) adapted from R.E. Landrum (1988) (Appendix A). The participants were asked verbally about their intakes of caffeinated substances within the past week, and these were subsequently recorded on the questionnaire. Popular drinks such as Red Bull™ and Rockstar™, as well as a section for other energy drinks were added to the questionnaire developed by R.E. Landrum (1988). The questionnaire was completed once, at the beginning of the study prior to exercise testing. This information was then entered into a food processor program (ESHA food processor program) by the researcher in order to determine daily caffeine use. Participants were considered caffeine high-users if they ingested more than 1.1mg/kg of caffeine per day as has previously been described (Frary et al., 2005).

3.3 VO_{2peak} Test

Participants were asked to report to the PACR lab in the Human Kinetics building along with their exercise attire (e.g. shorts, gym shoes, t-shirt, etc.), where they were required to read and sign a written consent form and Par-Q (Appendix B). After the written consent, height and weight were recorded using a standard weigh scale and a measurement chart mounted to the wall. The participants were then shown how to

position and secure a standard remote heart rate monitor (Polar, Canada) before being given a thorough walk through (verbal and visual description) of the testing protocol for that day.

Before starting, participants were again informed that their participation in the test was entirely voluntary and at any time throughout the test they could choose to discontinue testing and the procedure would be terminated. The participants were also informed that if they exhibited a drop in heart rate with an increase in exercise intensity, an inability to measure heart rate, signs of poor perfusion such as pale skin, confusion, or dizziness, or any signs that they were under duress, the experimenter would end the test early. Participants were asked to sit on the stationary bike in order to determine the optimal seat height given their leg length. The seat height was adjusted as needed to ensure the participant was comfortable with legs slightly bent during pedal extension. Subsequently, the head strap used to secure a mouthpiece for gas (expired oxygen and carbon dioxide) analysis was fitted over their head. The participants were asked to remain seated on the bike for a period of 2 min while pre-exercise physiological data was recorded (i.e. volume of oxygen consumed (VO_2), volume of carbon dioxide produced (VCO_2), the respiratory exchange ratio (RER), tympanic temperature (TT), and heart rate (HR)).

The first test performed was a ramp test to exhaustion in order to determine peak recorded oxygen consumption. This test is detailed in figure 3.1.

Figure 3.1. Cycling ramp test.

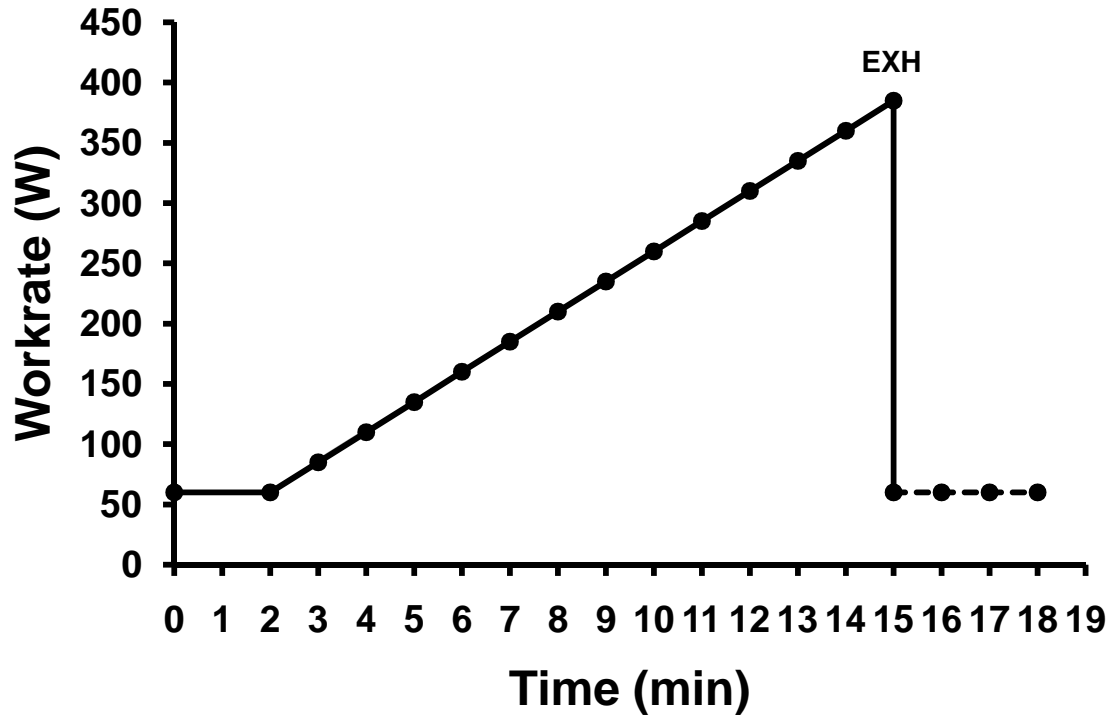


Figure 3.1. Exhaustive cycling ramp test. Workload (watts) was increased 25 watts per min and stopped when the participant could no longer maintain the pedal rate. Exhaustion (EXH) represented the termination point for each test at which point the participant was then allowed an active cool down. The cool down period consisted of a self-selected intensity (hence dotted line on the graph). The time of each test was different for each participant, but typically lasted between 8 and 15 min.

After the 2 minute resting period, the participant was asked to start cycling. The electronically braked bicycle ergometer (Ergoline), provides a constant wattage independent of the pedal rate. The participant began cycling at a resistance of 60 W while maintaining a constant pedal rate of 90 rpm for 2 min. During the test, the participant breathed into a mouthpiece that allowed a metabolic cart (Ergocard, Medisoft) to measure gas (O_2 and CO_2) exchange. Heart rate was also recorded for the duration of the test using a Polar telemetric heart rate monitor (Polar, Canada). After the warm-up period, the resistance on the bike was increased in an incremental fashion until volitional exhaustion (typically 8-15 min). Verbal encouragement was provided by those administering the test in an attempt to ensure the participant produced a maximal effort, and this maximal effort was confirmed against at least 2 of the following: heart rate within 10 beats of predicted maximum, an inability to maintain a pedal rate of at least 70 rpm, a respiratory exchange ratio (RER) greater than 1.1 and/or volitional fatigue. After the test was completed, the participant was required to remain pedalling on the bike at a self-designated intensity until their heart rate returned to within 10 beats of the initial starting heart rate (typically ~5 min). Water bottles were provided as needed. At this time, the initial testing procedures were completed and the performance tests were scheduled. Participants were asked to refrain from caffeine use for 24 h prior to the testing procedures.

3.4 Cycling Time Trials

The participants returned to the laboratory 3-4 days after the VO_{2peak} test for the first of 3 performance test. Upon arrival, a 24 hour dietary recall was administered in order to determine dietary intake over the past 24 h. The participants were questioned

about food eaten at each meal within the past 24 h (i.e. from the time of testing counting 24 h backwards). All food was written down on a recording sheet in order to ensure the participant had not ingested caffeine within the past 24 h. This food diary was then copied and handed to the individual and they were asked to replicate the same dietary intake 24 h before each performance trial. This process was repeated for the subsequent performance trials.

Female participants were asked to perform the first performance test 2-3 days after the end of menses in order to ensure that tests took place within the follicular phase of the menstrual cycle. After completion of the first performance test (the placebo trial), they were asked to return to the lab 2-3 days after their next menses (~28 days later) for the second and third performance trials, which were separated by one week (consequently, within 14 days after menses which is consistent with the average follicular phase length in females). This was an attempt to ensure that the female participants were in the same cycle phase (follicular) for each performance test, reducing the chance of performance/metabolic changes due to cycle phase. The males performed the first performance test 3-4 days after the VO_{2peak} test and to keep testing procedures similar between sexes, and were asked to return one month later to perform the second and third performance trials. The participants were asked to fast for 3 h prior to each performance trial. This was designed to simulate a normal racing condition where athletes eat a normal meal 3-4 h before competition. Three time trials were completed: a familiarization ride in which participants were given a CHO free drink only (FAM), a CHO plus placebo condition (CHO+P) and a CHO plus caffeine condition (CHO+C). Except for the FAM trial, the CHO+P and CHO+C trials were performed in randomized

order to eliminate order bias. For the FAM trial, the participants ingested either Mixed Berry or Grape Powerade™ Zero (7 ml/kg) prior to exercise in order to serve as a placebo, and to allow them to become familiar with the 20 km time trial. Powerade™ Zero contains water, electrolytes and a sugar substitute that is metabolically inert. Aside from the lack of carbohydrates, the nutritional composition of the drinks is nearly identical. Nutrition facts labels for Powerade™ Zero and regular Powerade™ are presented in figure 3.2. The FAM trial was included in the design as a familiarization trial and was not intended to be factored into the final analysis. For the CHO+P and CHO+C tests (approximately 1 month after the FAM trial), participants ingested a commercially available (14 g/240 ml) CHO solution (either Mixed Berry or Grape Powerade™) in combination with placebo or commercially available caffeine pill (Life™)(5 mg/kgBM), respectively, one hour prior to the time trial. Figure 3.3 outlines the timeline of events.

Figure 3.2. Nutritional facts labels for Powerade™ and Powerade™ Zero.

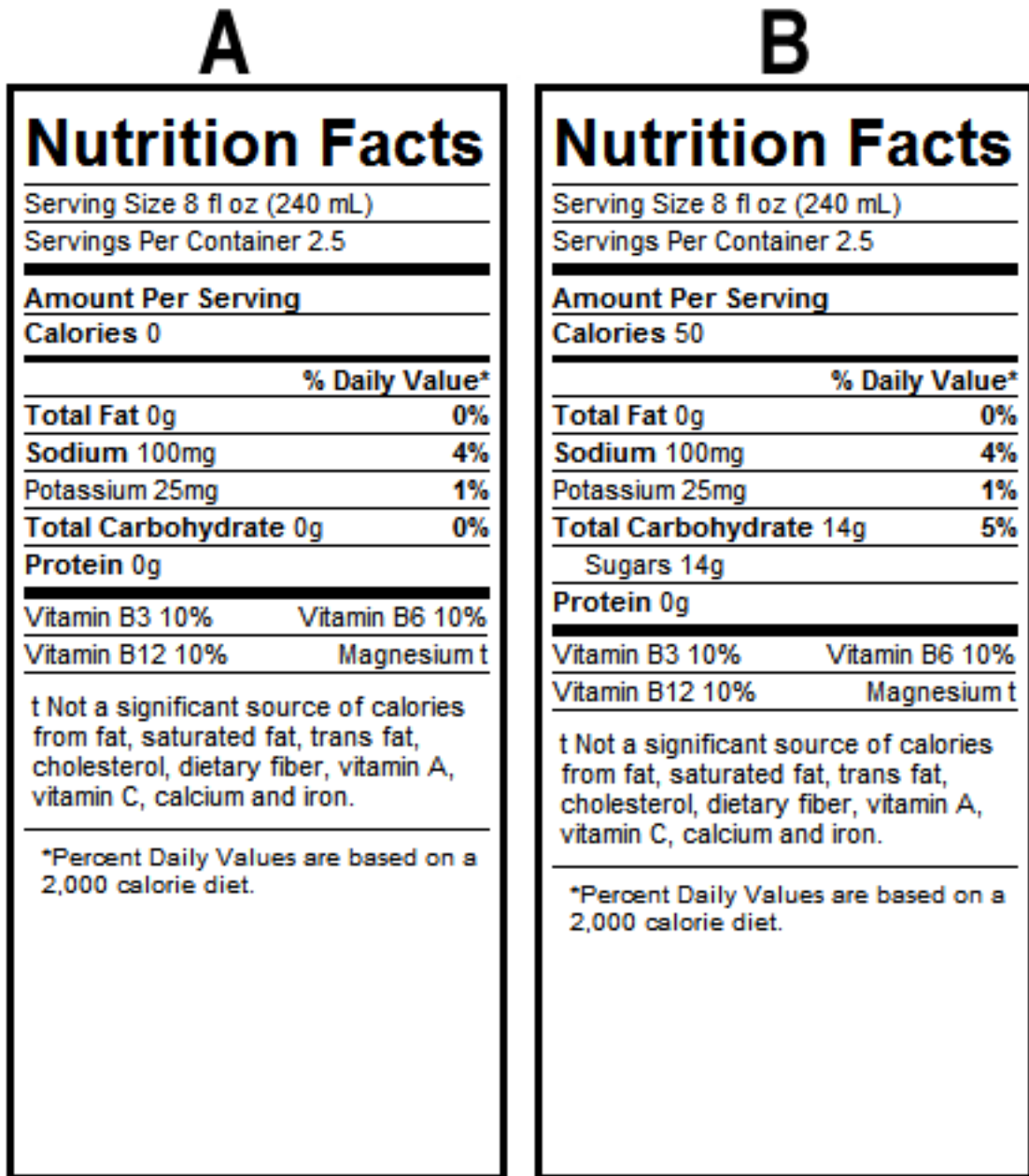


Figure 3.2. Nutrition facts labels for A) Powerade™ Zero and B) Powerade™ taken from the Coca-Cola™ Company nutrition website (<http://productnutrition.thecoca-colacompany.com/welcome>). These labels are the same for both the Mixed Berry and Grape flavours.

Figure 3.3. Study design.

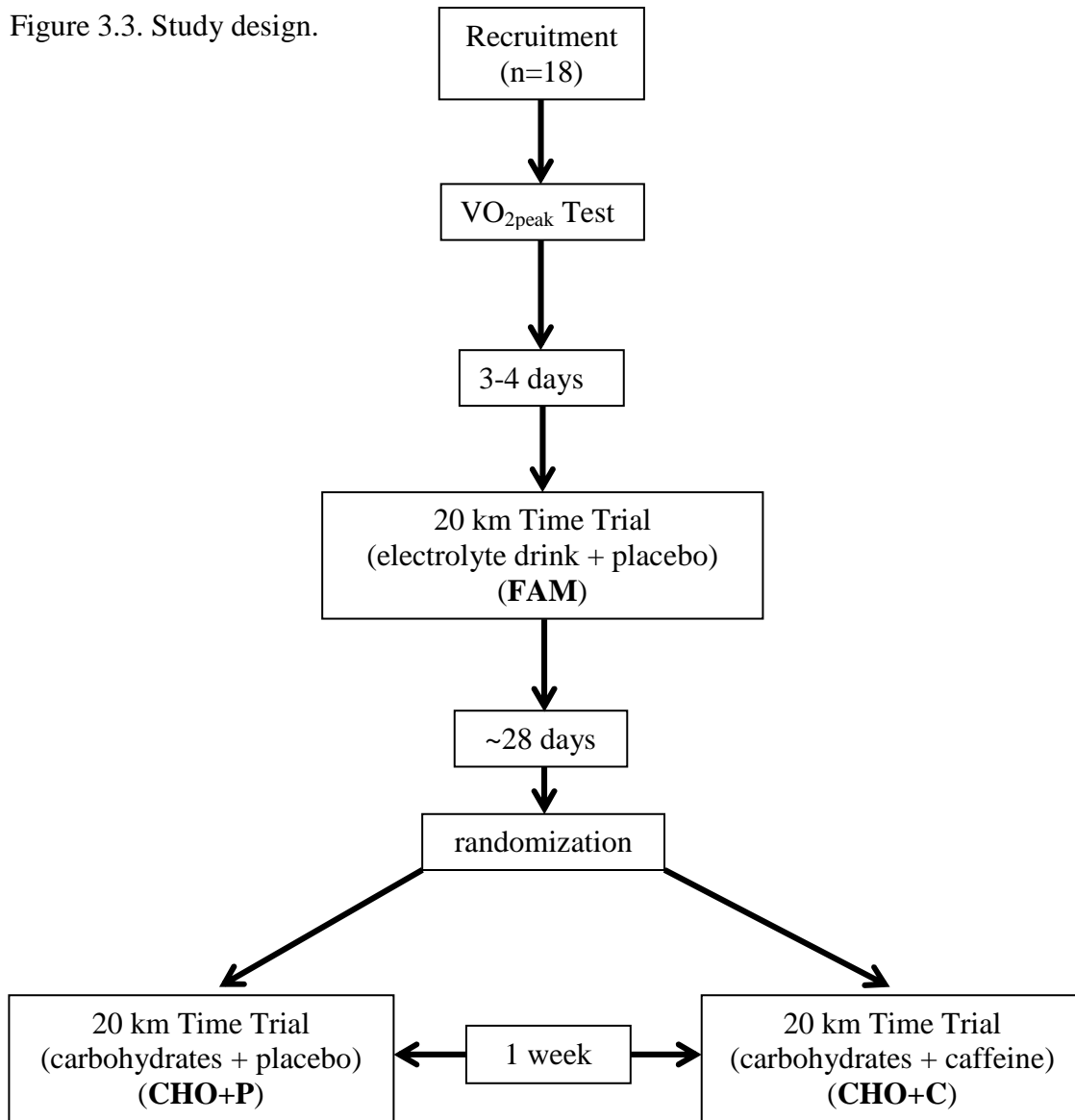


Figure 3.3. Flow diagram of study procedures. All participants performed all of the trials. Trial order after the FAM trial was randomized. The period between the FAM trial and subsequent tests was ~28 days for males and after a complete menstrual cycle for females.

For comparison, a cup of brewed coffee can contain up to 120 mg of caffeine in a 250 mL solution which would amount to a dosage of 1.7 mg/kg for a 70 kg individual. The dosage of 5mg/kg of caffeine was chosen because it is similar to the levels used in previous investigations (Graham & Spreit, 1995; Goldstein et al. 2010). The participants ingested the same flavor of Powerade™ prior to each performance trial, and volume was adjusted for body size (7 mL solution/kg body mass; i.e. ~ 500 ml or 2 cups for a 70kg person). In the CHO+P trial, the carbohydrate solution was taken with a placebo pill made from cellulose fiber (100 mg/pill, Jamieson Vitamins™). In the CHO+C trial, the carbohydrate solution was taken with caffeine pills (100 mg/pill, Life Brand). Both the experimenter and participant were blind to pill constituency at the time of testing, making this a double blind placebo controlled study. One hour after ingesting the drink and pills, the participants performed a timed 20 km performance test. The actual time trials consisted of the participant cycling on a stationary Monark bike at a resistance of 1.5 kg for males and 1.2 kg for females at a self-selected pace. The resistance remained constant throughout the trial. In order to complete the 20 km distance in the shortest amount of time, participants were informed that they could cycle at the highest frequency possible and should attempt to complete the 20 km in the fastest time possible. The test was terminated prematurely if the participant indicated he/she could no longer continue or exhibited signs that contraindicated the continuation of the test (i.e. a drop in heart rate with an increase in exercise intensity, an inability to measure heart rate, signs of poor perfusion such as pale skin, confusion, or dizziness). Arousal was recorded using the EmotivEPOC headset 10 min prior to exercise, while at 5 min prior to exercise, resting measures for heart rate, VO₂, RER, tympanic temperature, and blood glucose were taken

to determine baseline values. During the test, physiological variables including heart rate, VO₂, RER, tympanic temperature, long term excitement, and RPE were taken and recorded every 5 min. Participants were continuously aware of the distance remaining and could alter their speed to reach the distance in the quickest time. The time trial was completed once the 20 km distance was reached. At the completion of exercise, a post exercise blood glucose sample was taken from the participant (within 1 minute of completion of the test). Further, the participants were asked to sit on a chair in order to record post exercise brain activity in a non-exercising state. Subsequently, participants were allowed to cool down on the stationary bike at their desired speed and resistance. At the conclusion of this performance test, they were asked to return again in 1 week to perform the time trial a second time with the different condition.

After completion of the study, the participants were given a Human Kinetics t-shirt and were thanked for their participation in the study.

3.5 Expired Gas

Expired gas was analyzed for the duration of exercise using the methods described above in the VO_{2peak} test, and was used to measure VO₂ and RER throughout exercise.

3.6 Blood Glucose

Pre- and post-exercise blood glucose was assessed using the finger prick technique and a standard glucose monitor (Freestyle Freedom Lite™). The Freestyle Lite system has a measuring range of 20-500 mg/dL (Lock et al., 2011). It has been shown that 98% and 78.7% of samples fall within 10% and 5% of reference values respectively

(Lock et al., 2011). Further analysis revealed that 99.4% of results taken were clinically accurate with an overall variability of 2% at glucose levels <75mg/dL, and 3% at glucose levels >75mg/dL (Lock et al., 2011). The finger prick site was wiped with sterile alcohol swabs prior to and after testing. The volume of blood needed for this test was ~10 micro litres (or 1 small drop).

3.7 Tympanic Temperature

Tympanic temperature was measured throughout the time trial using a Genius™ 2 Tympanic Thermometer. This is a hand held device used with disposable caps, and measures temperature in the ear. Tympanic temperature gives a very close approximation of the temperature of the blood passing by the hypothalamus (i.e. the primary regulator of core temperature in the body).

3.8 Ratings of perceived Exertion (RPE)

RPE was taken using the Borg scale which is a scale from 6 to 20 with 6 being no exertion at all to 20 being maximal exertion (Borg, 1970). In order to determine RPE during exercise, the participant was asked to point to a number on the scale rather than verbalize it in order to not affect continuous expired gas monitoring.

3.9 Central nervous system arousal or long term excitement (LTE)

Brain activity was recorded using the Emotiv EPOC neuroheadset for 10 min prior to exercise with the subject sitting relaxed on a chair, throughout the entire exercise trial, and for 5 min after the time trial was completed. This system has been developed along with proprietary software to determine acute arousal that when averaged over a

period of 5 min, gives a measure designated as long term excitement (LTE). Long term excitement has been reported to be consistent with, and in some cases better than, the galvanic skin response and other measures of arousal. Preliminary data from our lab has shown real-time changes in long term excitement with intense exercise over a 20 km cycling time trial with reward (Rama Mustafa and Kevin Milne, unpublished observations).

3.10 Statistics

This study used a randomized double blind placebo controlled procedure. The FAM trial was not randomized within the study design, and was the first exercise trial for all individuals. Thus, the FAM trial was not used in subsequent analyses unless specifically noted.

All statistical analyses were performed using IBM SPSS Statistics for Windows version 2.0. The primary outcome variable of this study was time to complete the 20km distance. A 2-way repeated measures (RM) analysis of variance (ANOVA) was used to determine significant differences in the primary outcome as well as heart rate, RER, RPE, LTE, tympanic temperature and blood glucose values. The repeated measure of trial (CHO+P or CHO+C) and between group measure of sex (male versus female) were used for ANOVA tests. The variables of VO_{2peak} , weight, caffeine use and drink color were put in as covariates in the first ANOVAs. Significance was determined at $p < 0.05$. Significant interactions (i.e. trial x sex) were subsequently analyzed by 1 way ANOVA after regrouping of variables. A Tukey's post hoc analysis was used to determine significant differences between groups in this subsequent analysis. For the analysis of

BG, a RM ANOVA was performed, using the repeated measure of trial (CHO+P or CHO+C), the repeated measure of time (pre-and post-time trial) and between group measure of sex (male versus female). Significant interactions were analyzed in the same manner as described above. For the analysis of continuously monitored variables, 2 forms of analysis were used. In the first case, the mean value of each variable across the time trial was calculated and used in a 2 way RM ANOVA as described above. A 3-way RM ANOVA was used incorporating the repeated measure of trial (CHO+P or CHO+C), the repeated measure of time (5 min, 10 min, 15 min, 20 min, 25 min or 25%, 50%, 75%, 100%) and between group measure of sex (male versus female). A Pearson correlation was performed between pre-exercise fitness (VO_{2peak}) and difference between CHO+C and CHO+P trials. Further, Pearson correlations were determined for each trial between the within trial variables of heart rate, RPE, LTE, blood glucose, RER, and tympanic temperature. This was done to examine potential relationships between physiological variables, arousal (LTE) and performance. Pearson products (r^2) and significance (p-value) are reported.

All data are reported as means, standard deviations (SD) and effect size unless otherwise noted.

4. RESULTS

4.1 Nutritional composition of pre-exercise meal

There were no differences in caloric composition of meals taken 24 hours before each trial ($F(2,16)=0.246$, $p>0.05$). There were no differences in CHO ($F(2,16)=0.091$, $p>0.05$), fat ($F(2,16)=1.094$, $p>0.05$) or protein ($F(2,16)=1.667$, $p>0.05$) ingested between trials at any meal. There were no differences in calories ($F(1,16)=0.070$, $p>0.05$), CHO ($F(1,16)=0.006$, $p>0.05$), fat ($F(1,16)=0.172$, $p>0.05$), or protein ($F(1,16)=2.884$, $p>0.05$) ingested between sexes prior to each exercise trial. There were significant differences in protein ingested between sexes, as males ingested more protein prior to each exercise bout than females ($F(1,16)=5.876$, $p<0.05$). This data is presented in Table 4.1.

4.2 Performance Measures

Performance times for the 3 trials were 1799 ± 246 , 1724 ± 216 and 1694 ± 241 seconds in the FAM, CHO+P and CHO+C trials respectively. There were significant main effects for performance time when all three trials were included in the analysis ($F(2,15)=24.770$, $p<0.05$, $ES=0.608$). The improvement in time trial performance was 4.2% between the FAM and CHO+P trial, 5.9% between the FAM and CHO+C trial and 2% between the CHO+P trial and the CHO+C trial. The FAM trial was significantly slower than both the CHO+P trial ($p<0.05$) and the CHO+C trial ($p<0.05$) (Table 4.2). Males were faster than females for all trials ($F(1,16)=4.692$, $p<0.05$, $ES=0.227$) (Figure 4.1). Since the FAM trial was used as a familiarization trial, it was removed from further analysis. There were significant main effects for performance time, where CHO+C improved performance over CHO+P ($F(1,16)=4.807$, $p<0.05$, $ES=0.231$) (Figure 4.1).

Table 4.1. Caloric composition of pre-exercise meals.

	Meal/Sex	Trial		
		FAM	CHO+P	CHO+C
Calories(kcal)	Breakfast	311.2±156.6	328.3±176.7	315.8±159.4
	Lunch	540.0±511.6	514.4±468.5	500.3±474.9
	Dinner	887.0±618.3	862.0±379.6	844.7±362.9
	Total	1738.3±851.2	1704.8±541.8	1660.9±478.3
<i>Mean Calories</i>	<i>Males</i>	<i>1828.1±1123.6</i>	<i>1751.3±690.2</i>	<i>1634.7±394.4</i>
	<i>Females</i>	<i>1648.5±508.9</i>	<i>1658.3±377.8</i>	<i>1687.2±393.5</i>
CHO(g)	Breakfast	57.4±34.4	55.7±34.7	56.0±34.9
	Lunch	68.2±57.5	64.2±54.3	65.2±52.2
	Dinner	107.9±45.2	110.8±42.8	112.5±46.2
	Total	233.6±68.4	230.7±60.7	233.6±56.1
<i>Mean CHO</i>	<i>Males</i>	<i>226.5±84.3</i>	<i>241.7±72.3</i>	<i>233.2±68.6</i>
	<i>Females</i>	<i>240.7±52.3</i>	<i>219.7±40.9</i>	<i>234.1±44.4</i>
Fat(g)	Breakfast	8.9 ±10.6	9.2±9.4	7.7± 7.5
	Lunch	16.9±21.3	16.5±17.9	18.4±22.8
	Dinner	29.1±35.3	31.4 ±31.1	29.8±25.3
	Total	54.9±47.7	56.9±30.8	55.9±30.5
<i>Mean Fat</i>	<i>Males</i>	<i>61.3±25.4</i>	<i>59.2±41.6</i>	<i>48.9±30.0</i>
	<i>Females</i>	<i>48.6±25.4</i>	<i>54.6±24.5</i>	<i>62.8±31.1</i>
Protein(g)	Breakfast	13.1±7.7	12.9±8.3	13.1±8.5
	Lunch	26.0±33.4	24.0±30.8	25.1±30.5
	Dinner	44.8 ±28.0	37.9±18.0	36.2±17.0
	Total	83.9±41.7	74.8±29.1	74.4±26.8
<i>Mean Protein</i>	<i>Males</i>	<i>99.1±18.7</i>	<i>83.7±36.3</i>	<i>84.4±27.4</i>
	<i>Females</i>	<i>68.7±18.7</i>	<i>64.6±31.0</i>	<i>64.3±23.5</i>

Table 4.2. Mean time trial performance (seconds)

	Trial			p	E.S.
	FAM	CHO+P	CHO+C		
male	1671±242	1632±245 ^β	1592±268 ^β	p<0.05	0.310
female	1927±181	1816±142	1795±169	p<0.05	0.144
Average	1799±246	1724±216	1694±241 *	p<0.05	0.231

Time, in seconds to complete 20 km on a cycle ergometer. Note that both the CHO+P and CHO+C were completed in significantly less time than the FAM trial, however, because that trial was only used for familiarization, statistical analysis compared mean times of the CHO+P and CHO+C trials only. Data are presented as means ± SD. ^β = males took significantly less time than females to complete the 20 km in both trials (p<0.05). * = significantly faster than the CHO+P trial (p<0.05).

Figure 4.1. Performance time.

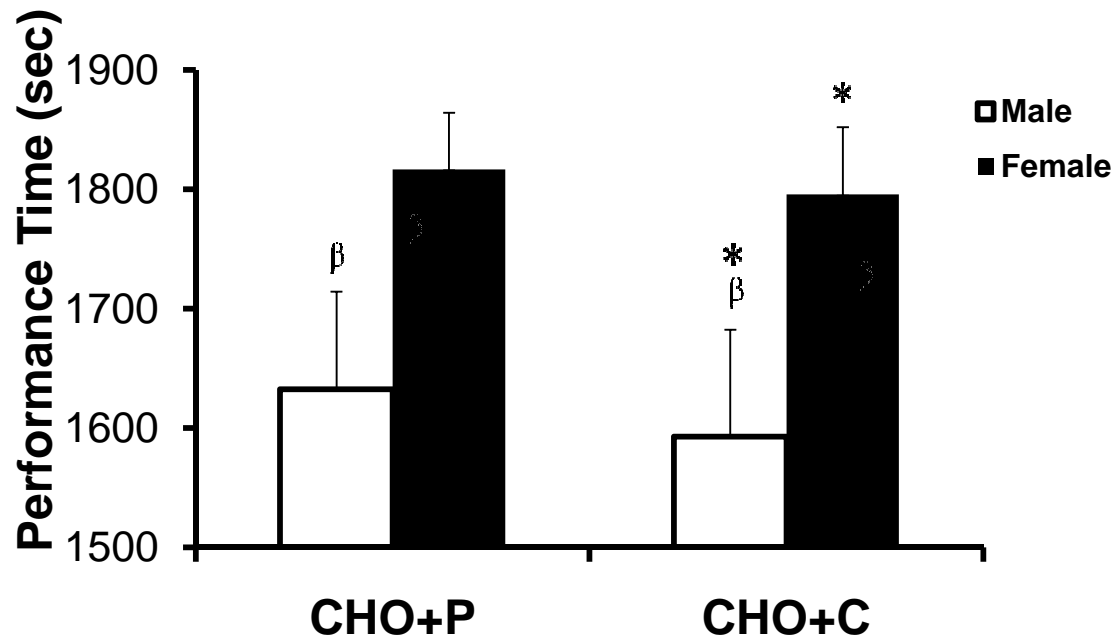


Figure 4.1. Performance time was significantly faster for males compared to females in both exercise trials ($p < 0.05$). β = significantly faster for males. CHO+C given 1 hour before exercise produced significant performance improvements compared to CHO+P for both males and females. * = significantly faster than the CHO+P trial.

This performance improvement between trials was not different between males and females ($F(1,16)=0.455$, $p>0.05$). Whether the participant was a caffeine high-user or not had no impact on performance between trials ($F(1,16)=3.553$, $p>0.05$). The variables of $VO_{2\text{peak}}$, weight, caffeine use and drink color produced no interactions with any of the test variables.

4.3 Ratings of Perceived Exertion

There were no significant main effects for RPE between trials ($F(1,16)=0.461$, $p>0.05$), nor were there any interactions between trial and sex ($F(1,16)=0.115$, $p>0.05$) (Figure 2.4A). Within each trial, there were significant main effects for RPE, where perceived exertion levels increased throughout the exercise bout ($F(1,16)=96.045$, $p<0.05$, $ES=0.857$) (Figure 4.2 B).

4.4 Physiological Measures

Blood Glucose

There was a significant interaction between trial and time (pre-and post exercise) for blood glucose measurements ($F(1,16)=6.257$, $p<0.05$, $ES=0.281$), where blood glucose levels were increased post exercise compared to rest in the CHO+C trial ($p<0.05$) but not in the CHO+P trial (Figure 4.3). There were no significant differences in blood glucose levels between males and females ($F(1,16)=0.702$, $p>0.05$), and no interactions between trial and sex ($F(1,16)=0.026$, $p>0.05$).

Figure 4.2. RPE values in each exercise trial.

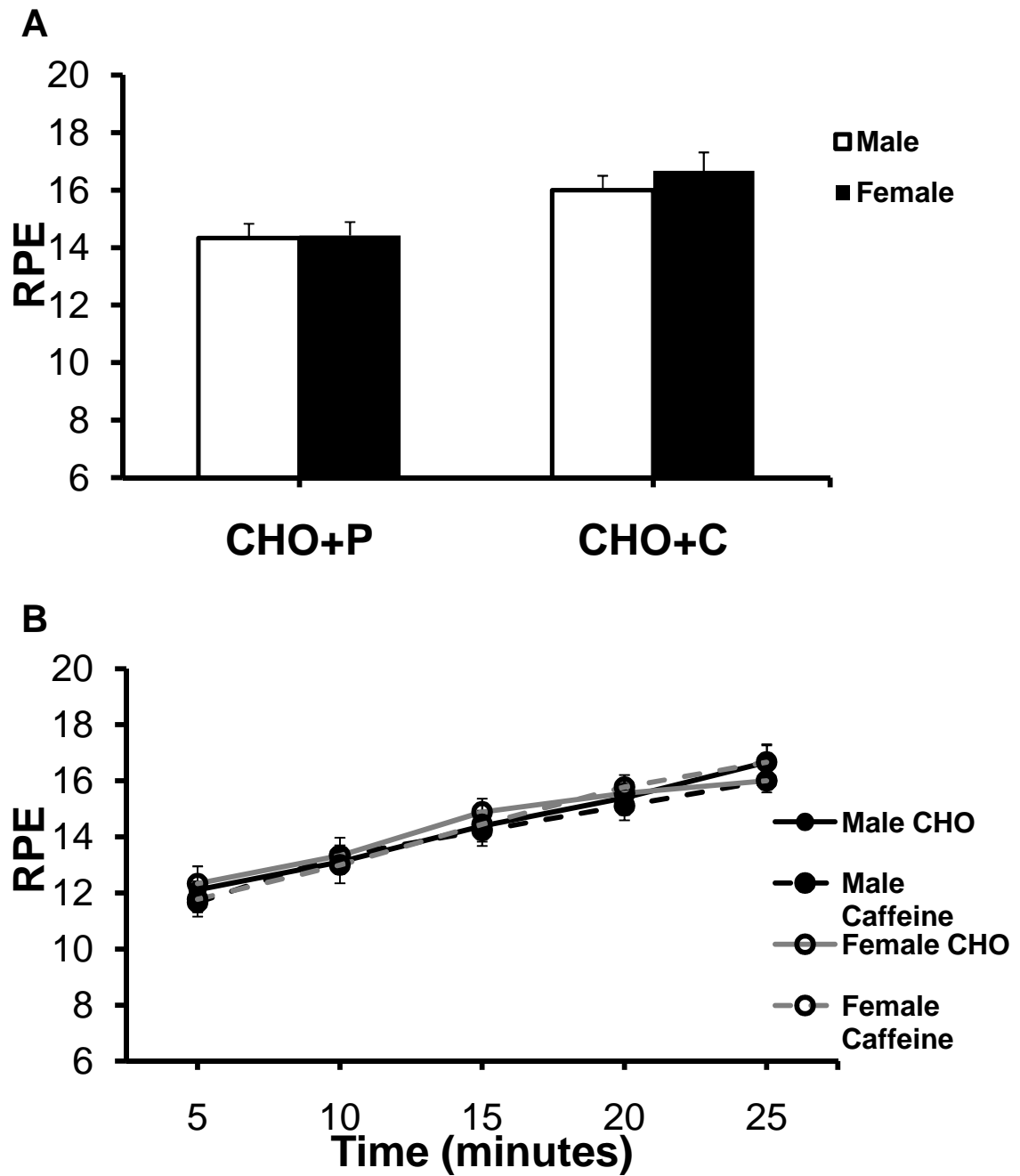


Figure 4.2. Ratings of Perceived Exertion (RPE). A, Mean RPE values recorded during the time trials. Subjects pointed to a RPE value on a scale at 5 minute intervals throughout exercise. There were no significant differences when either CHO+P or CHO+C was ingested 1 hour before exercise, indicating that fatigue and sense of effort experienced during exercise was unaffected by caffeine. B, RPE values recorded at 5 min intervals during the 2 time trials for the first 25 min. All participants took at least 25min to complete the 20 km. For this reason, data points are only shown to 25 min for comparison of non-continuously monitored variables. At each recording interval, RPE values increased throughout the exercise bout in a similar fashion for both trials. The dark circles represent the males and the clear circles the females, while the solid line is the carbohydrate only trial and the dotted line is the caffeine trial. There was a significant main effect for time within each trial such that RPE values increased. Each data point represents means and standard deviation.

Figure 4.3. Blood glucose values in each exercise trial.

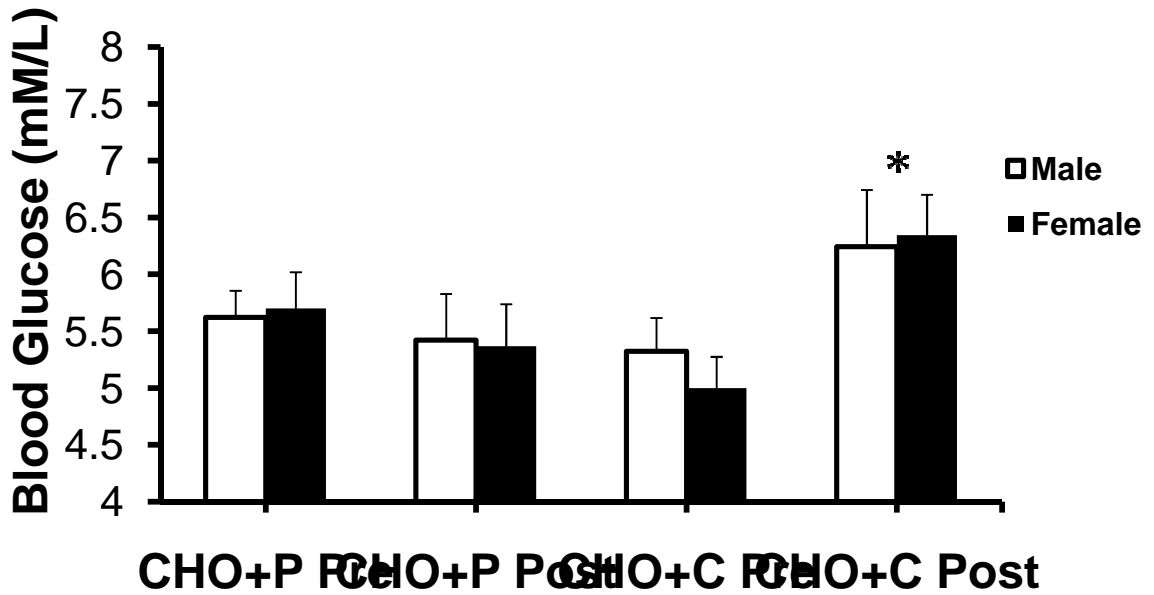


Figure 4.3. Blood glucose levels were measured pre and post exercise in each exercise trial. Blood glucose levels were significantly increased post exercise when CHO+C was ingested 1 hour before exercise but not when CHO+P was ingested 1 hour before exercise ($p < 0.05$). * = significantly greater than pre exercise in the CHO+C trial.

Respiratory Exchange Ratio (RER)

There were no significant main effects between the CHO+P trial and the CHO+C trial for RER when values were taken at 5 minute intervals ($F(1,16)=1.033$, $p>0.05$) nor when the RER was averaged throughout the trial ($F(1,16)=0.568$, $p>0.05$) (Figure 2.7A). There were significant differences in RER between sexes ($F(1,16)=5.265$, $p<0.05$, $ES=0.248$), where females had lower RER values during exercise, which was evident in the final two recording periods at 20 min ($F(1,34)=10.239$, $p<0.05$) and 25 min ($F(1,34)=20.881$, $p<0.05$). There were no significant differences in RER when taking percentage time point values at four points (25, 50, 75 and 100%) throughout the trial ($F(1,16)=0.267$, $p>0.05$) (Figure 4.4B). There were changes in RER which were observed within each trial. This change in RER was evident when comparing the RER values at 25% to those at 50 and 75% ($p<0.05$). Further, there were significant differences between values at 50% to those at 75% ($p<0.05$) and between those at 75% and 100% ($p<0.05$) (Figure 4.4B).

Figure 4.4. RER values in each exercise trial.

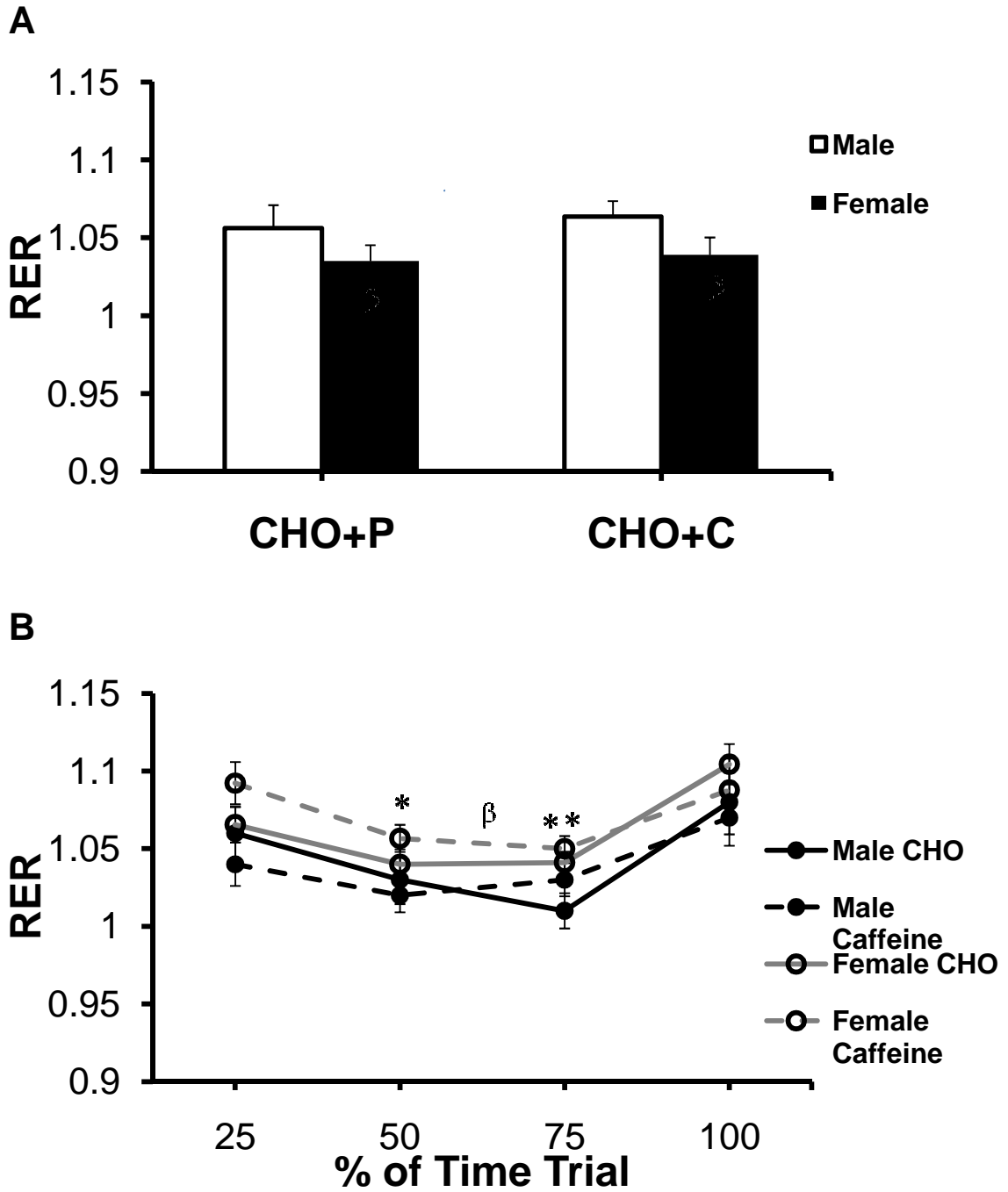


Figure 4.4. Respiratory Exchange Ratio (RER). A, Mean RER values between trials. Mean RER values were determined from values taken at 5 min intervals throughout each exercise bout. There were no significant differences between trials when either CHO+P or CHO+C was ingested 1 hour before exercise. Means for males were significantly higher than females during both trials ($p < 0.05$). Means during the time trials were 1.056 and 1.035 for males and females in the CHO+P trial and 1.063 and 1.039 in the CHO+C trial. β = significantly different than males. B, The RER taken at different percentage points throughout the trials revealed significant differences at several different time points ($p < 0.05$). *=significantly different than 50 and 75% of total time. **=significantly different than 75% of total time. The dark circles represent the males and the clear circles the females, while the solid line is the carbohydrate only trial and the dotted line is the caffeine trial.

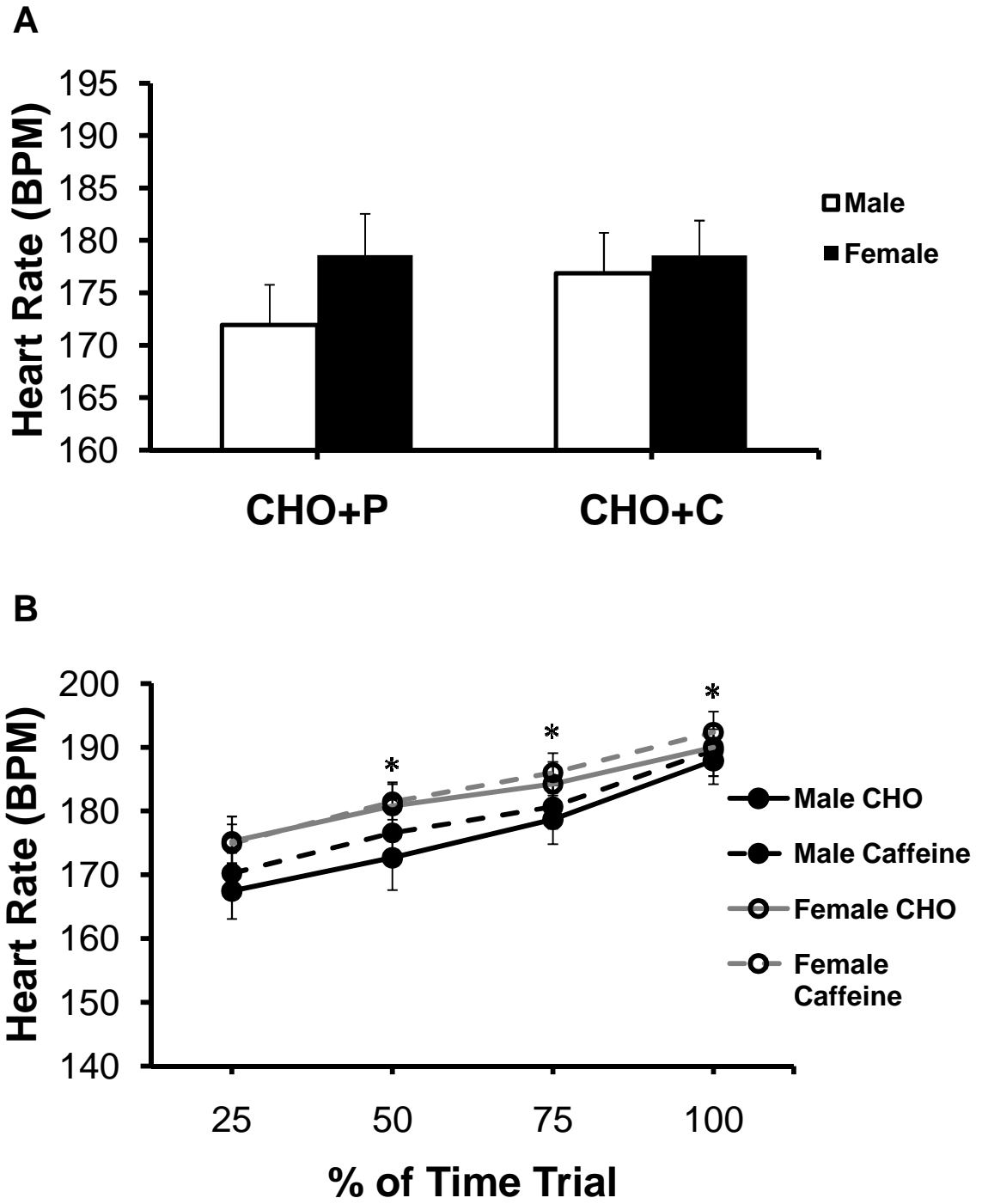
Heart Rate

There were no significant main effects between trials for heart rate when values were taken at 5 minute intervals ($F(1,16)=1.939, p>0.05$), nor when heart rate was averaged throughout the trial ($F(1,16)=1.939, p>0.05$) (Figure 4.5A). There were no significant differences when taking percentage time point values at four points (25,50,75 and 100%) throughout the trial ($F(1,16)=0.593, p>0.05$) (Figure 4.5B). Further, there were no interactions between trial and sex ($F(1,16)=1.974, p>0.05$). There was a significant main effect for heart rate within each trial ($F(4,13)=93.830, p<0.05, ES=0.854$), as heart rate increased throughout exercise. Heart rate taken as a percentage of maximum heart rate showed significant differences between males and females regardless of treatment. Females exercised at a significantly higher percentage of their maximum heart rate than males ($F(1,16)=5.352, p<0.05, ES=0.251$) (Figure 4.5C)

Tympanic Temperature

There were no significant main effects between trials for temperature when values were taken at 5 minute intervals ($F(1,16)=0.314, p>0.05$) or when temperature was averaged over the duration of the trial ($F(1,16)=1.03, p>0.05$) (Figure 4.6A). There were no significant interactions between trial and sex ($F(1,16)=1.083, p>0.05$). There were significant main effects for time within each trial ($F(1,16)=55.441, p<0.05, ES=0.810$), as temperature increased progressively throughout the exercise bout (Figure 4.6B).

Figure 4.5. Heart rate values in each exercise trial.



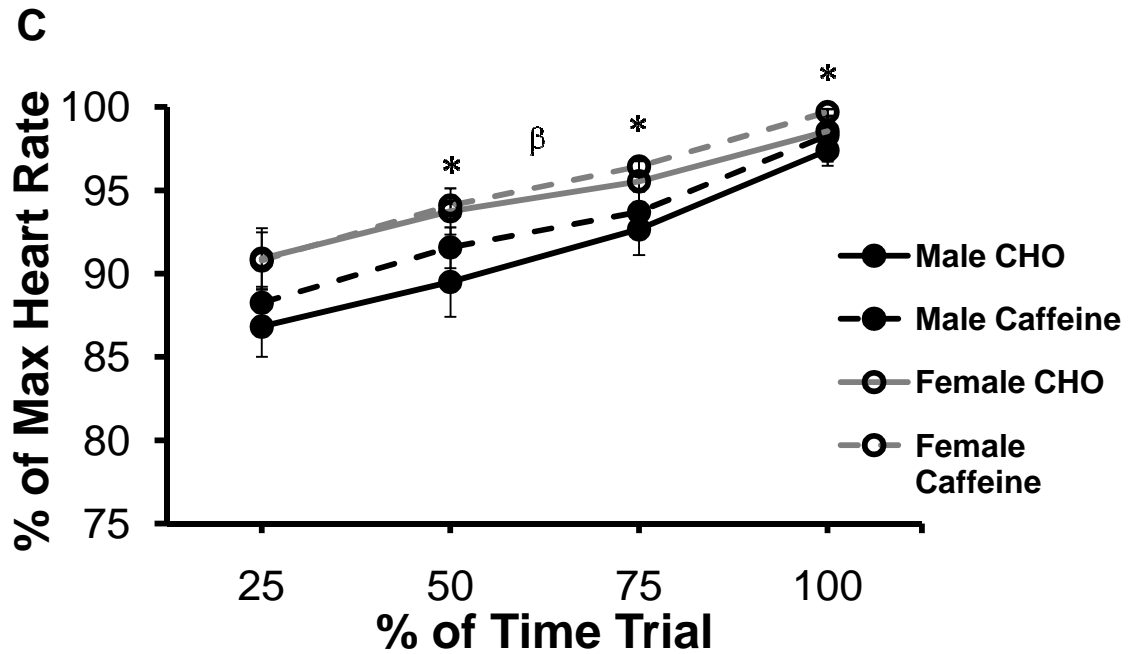


Figure 4.5. A, The mean of heart throughout each exercise trial. Heart rate was not significantly different between trials when either CHO+P or CHO+C was ingested 1 hour before exercise. B, Heart rate taken at different percentage time points throughout the trial. Heart rate increased throughout the exercise bout in the same manner for each trial and was not significantly different between sexes. *= Heart rate was significantly increased at each percentage time point throughout all trials. C, Heart rate taken as a percentage of maximum heart rate showed significant differences between males and females regardless of treatment. β = significantly higher percentage of maximal heart rate for females. The dark circles represent the males and the clear circles the females, while the solid line is the carbohydrate only trial and the dotted line is the caffeine trial.

Figure 4.6. Tympanic temperature values in each exercise trial.

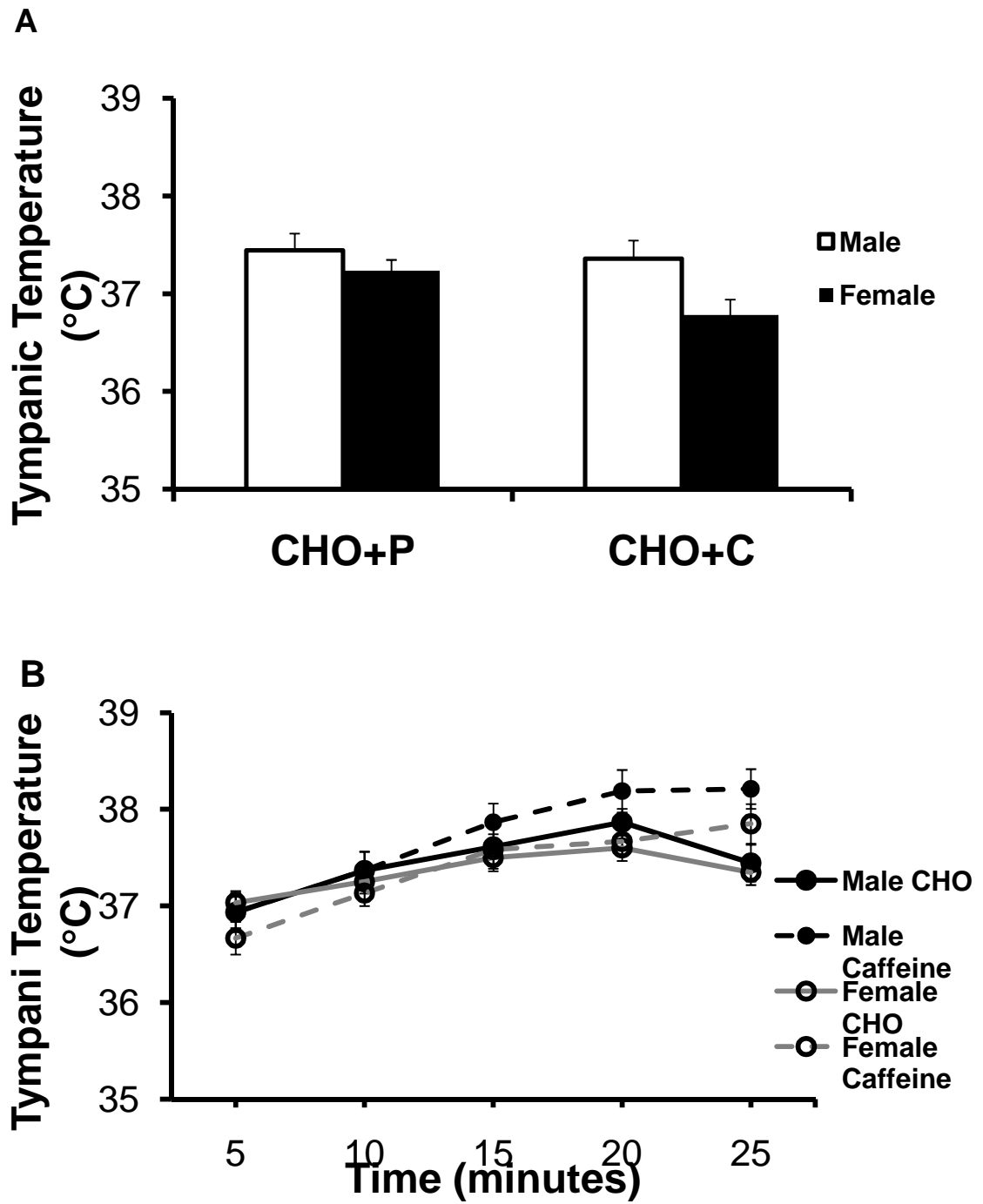


Figure 4.6. A, Mean tympanic temperature between trials. Tympanic temperature was not significantly different between trials when CHO+P or CHO+C was ingested 1 hour before exercise. B, Tympanic temperature taken at 5 min intervals throughout exercise of the 2 time trials. Tympanic temperature increased in a similar fashion throughout each exercise trial, and was not different between sexes. The dark circles represent the males and the clear circles the females, while the solid line is the carbohydrate only trial and the dotted line is the caffeine trial.

Long term excitement

The Emotiv EPOC malfunctioned for 6 participants. Consequently, data are presented for only 12 individuals. There were no significant main effects between trials for LTE ($F(1,10)=0.002$, $p>0.05$) (Figure 4.7), and no interactions between trial and sex ($F(1,10)=0.313$, $p>0.05$). There were significant main effects for LTE within each trial ($F(1,10)=10.770$, $p<0.05$, $ES=0.519$), as LTE decreased throughout the exercise bout. This decrease in LTE was most pronounced in the last two recording periods between 20 and 25 min (Figure 4.7). There were also significant increases in LTE between rest and the initial 5 min of exercise and significant decreases between the initial 5 min and the value taken at the very end of exercise ($F(1,2)=29.707$, $p<0.05$, $ES=0.748$).

4.5 Pearson Correlations

There were no correlations between VO_{2peak} and improvements made between the CHO+P and CHO+C trials ($r = -0.084$, $p>0.05$). Heart rate showed a positive correlation with RPE in the CHO trial ($r = 0.625$, $p<0.05$) and caffeine trial ($r = 0.692$, $p<0.05$). Heart rate showed a positive correlation with temperature in the CHO trial ($r = 0.502$, $p<0.05$), and in the caffeine trial ($r = 0.536$, $p<0.05$). Heart rate showed a negative correlation with LTE during the CHO trial ($r = -0.464$, $p<0.05$) and the caffeine trial ($r = -0.279$, $p<0.05$). RPE showed a positive correlation with temperature in the CHO trial ($r = 0.397$, $p<0.05$) and the caffeine trial ($r = 0.488$, $p<0.05$), and showed a negative correlation with RER ($r = -0.536$, $p<0.05$), only in the caffeine trial. RPE showed a negative correlation with LTE in the CHO trial only ($r = -0.416$, $p<0.05$). Temperature during the exercise trial showed no correlation with RER, yet did showed a negative

correlation with LTE only during the caffeine trial ($r = -0.480$, $p < 0.05$).

Figure 4.7. LTE values in each exercise trial.

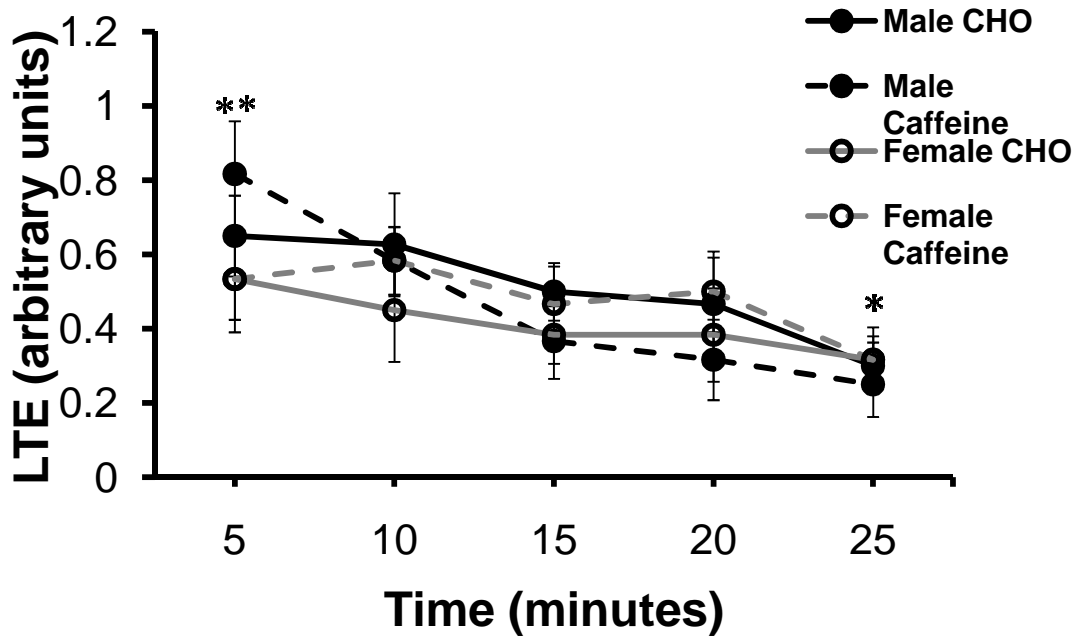


Figure 4.7. Long Term Excitement (LTE). LTE was taken at 5 min intervals throughout the exercise bout. LTE decreased in a similar fashion in all exercise trials and was not significantly different when either CHO+P or CHO+C was ingested 1 hour before exercise. LTE was not different between sexes. *= Significantly different than 5 min, $p < 0.05$. **=significantly different than rest (not shown), $p < 0.05$. The dark circles represent the males and the clear circles the females, while the solid line is the carbohydrate only trial and the dotted line is the caffeine trial.

5. DISCUSSION

5.1 Performance Measures

The major finding in this study was that caffeine combined with a carbohydrate drink significantly improved performance during a 20 km biking time trial for both males and females, compared to carbohydrate alone. This performance improvement equaled a 2% improvement over the CHO trial alone. This 29.5 second improvement compared to the CHO only trial, would have significant performance implications for competitive athletes. World class races in aerobic sports such as running, cycling and swimming are often won by the narrowest of margins, making this finding from a practical perspective even more important. For example, the difference between first and fifth was approximately 30 seconds at the 2011 Canadian Interuniversity Sport (CIS) Cross Country Championships. Our results are in agreement with a recent meta-analysis done by Conger et al. (2011) who found that the average performance difference reported in various studies suggests that a CHO drink combined with caffeine might result in a 6% performance improvement compared to CHO alone (Conger et al., 2011). It is important to note that most of the investigations used in the analysis were done on lower intensity aerobic exercise lasting longer than 35 min or a high intensity time trial which followed prolonged low intensity exercise (Conger et al., 2011). For example, Kovacs et al. (1998) found that caffeine users (trained athletes) completed a 1 hour time trial significantly faster and with higher mean work output after ingesting a CHO electrolyte solution plus 225 mg of caffeine compared with placebo water, placebo CHO electrolyte solution or CHO electrolyte solution plus 150 mg of caffeine (Kovacs et al., 1998). However, a limitation of that study was that caffeine dosage was not dependent on body weight,

thereby resulting in lighter individuals getting a higher relative amount of caffeine. Other studies found similar results in time trials of 15 and 45 min, however were done following prolonged low intensity exercise (135 and 105 min) making them hard to compare with the current investigation (Cureton et al., 2007; Hulston & Jeukendrup, 2008). The mechanism by which caffeine improved performance in these studies may be through sparing of muscle glycogen rather than an effect on the central nervous system, as participants would likely be in a state of glycogen depletion at the beginning of the time trial. Thus, it may be more practical to compare the current results to those studies which employed time trials of similar exercise intensity, yet did not use a combination of CHO and caffeine. Our results are in agreement with Bridge & Jones (2006) who found that caffeine (3mg/kg) significantly improved 8 km running performance by 1.2% in male distance runners and McNaughton et al. (2008) who found that cyclists rode significantly further in a 1 h time trial after ingesting caffeine (6 mg/kg) compared to a placebo.

While non-caffeine users would have been preferred for this study because they have been shown to produce greater performance improvements when ingesting caffeine prior to exercise (Bell & McLellan, 2002), a mixture of caffeine high-users and low-users participated in the present study. The improvement in performance with caffeine ingestion was greater in caffeine high-users (54.5 seconds) compared to low-users (6.5 seconds), however this difference was not statistically significant. These findings are in contrast to Bell & McLellan (2002) who found performance was significantly improved in non-users compared to users of the drug. Further, none of the participants in the current study showed any indication of negative effects toward the caffeine, making this finding difficult to explain. Consequently, the 24 h caffeine washout period may have

been sufficient to normalize all participants before each trial or caffeine is able to improve performance regardless of prior caffeine use.

To our knowledge, this is the first investigation to measure the effect of caffeine on exercise performance using sex as an independent variable. It was hypothesized that males would improve more than females since low dose caffeine (100 mg) induces greater arousing effects in males compared to females at rest (Adan et al., 2008). The present results indicate that there are no significant differences in time trial performance between males and females when caffeine was ingested prior to the trial. It is clear that caffeine can improve high intensity aerobic performance in females (Anderson et al., 2000; Macleod et al., 2004), however, according to our data this is not different between sexes. Males did show a greater improvement with caffeine ingestion (40 seconds for males and 21 seconds for females) however, it would take a larger sample size for this to reach statistical significance. Thus, further research is required to determine if caffeine results in differences in time trial performance between males and females.

As expected, RPE values progressively increased throughout the exercise bout in both trials. In disagreement with our hypothesis, RPE levels were not different between trials. This is in agreement with Anderson et al. (2000) who found that caffeine had no effect on RPE taken after exercise in elite female rowers. In contrast, a meta-analysis revealed that there is a 6% reduction in RPE when caffeine is ingested compared to a placebo, and that the mean change in RPE is significantly related to mean change in performance (Doherty & Smith, 2005). However, the authors indicated that there is no difference in RPE at the conclusion of exhaustive exercise done at a constant load, suggesting that fatigue and sense of effort experienced at the end of exhaustive exercise

(e.g. a time trial) is the same regardless of caffeine ingestion (Doherty & Smith, 2005). It is possible that RPE may only be influenced by caffeine during low intensity exercise or submaximal exercise. For example, RPE was lower during the caffeine condition (5 mg/kg), during exercise 10% below the anaerobic threshold (AT), and not exercise 10% above the AT (Denadai & Denadai, 1998). Further, the type of exercise test may have an influence on the RPE. Nineteen of the twenty one studies used in the analysis by Doherty & Smith (2005) were trials done to exhaustion at a certain percentage of an individuals' VO_{2max} . Consequently, caffeine may affect ones perception between trials, since the exercise intensity is not changing. In contrast, during a time trial such as the one administered during our investigation, the participant was exercising to their maximum capacity throughout the entire trial, resulting in perceived exertion values that were similar between trials, and not influenced by caffeine ingestion. It is concluded that caffeine ingestion does not influence RPE during a high intensity time trial performance and that RPE values are not different between sexes.

The finding that caffeine increased blood glucose levels post exercise is consistent with other research (Bell & McLellan, 2002; Graham & Spriet, 1995; Trice & Haymes, 1995) of similar intensity and confirms our hypothesis. In contrast, other investigations found no change in blood glucose levels post exercise (Graham et al., 1998; Greer et al., 2000). It is interesting to note that in a review by Graham (2001), the authors indicate that there are a few reports of increased blood glucose levels due to caffeine, but most show no change. The authors, however, provide six studies which agree that caffeine can influence blood glucose during exercise, and none to back up the statement that most studies show no change. Thus, it is clear from our study, and others

of similar exercise intensity, that caffeine has the ability to increase blood glucose levels post exercise compared to a placebo trial. As reviewed earlier, this may be the result of caffeine's effect on intestinal glucose absorption through direct action on the GLUT 1 transporter (Van Nieuwenhoven et al., 2000), induced catecholamine release or adenosine receptor antagonism which would inhibit glucose uptake into the muscle (Pizziol et al., 1998).

The finding that caffeine had no effect on the RER during exercise is consistent with other research and is in agreement with our hypothesis (Bell & McLellan, 2002; Hulson & Jeukendrup, 2008). This was confirmed by Graham et al. (2008) who showed that caffeine had no effect on key metabolic intermediates which are proposed to be increased by fat oxidation during exercise. Further, muscle biopsy analysis revealed no sign of glycogen sparing during exercise (Graham et al., 2000). Further, it is clear that glucose rate of appearance and disappearance is unaffected by caffeine, suggesting that caffeine's proposed glucose sparing effect may not be evident during high intensity exercise (Roy et al., 2001). In fact, it was suggested earlier that performance improvements during short duration exercise (< 1 h) are likely due to changes in neural activation (Jackman et al., 1996), and not the sparing of muscle glycogen when glycogen depletion is not a limiting factor. When the FAM trial was included in the analysis, RER values were significantly higher in the CHO and the CHO combined with caffeine trial. This may have been due to the ingestion of CHO prior to exercise, possibly increasing the amount of CHO used as a fuel. It could also be the result of the participants being unfamiliar with the exercise trial and not producing a true maximal effort, thus resulting in a lower RER. This is confirmed when looking at the means of RPE values between

trials. The mean RPE values were lower in the FAM trial (13.85) compared to the CHO+P trial (14.37) and the CHO+C trial (14.20). This provides further justification to why this trial was removed from the final analysis. It is important to note however, that individuals in the fed-state have not shown performance improvements when ingesting CHO before and during exercise (Jeukendrup et. al. 2008) whereas the participants in the current did. As noted earlier, it is possible that the fed state masks the performance benefitting effects a CHO supplement, however, the participants in the current study were not fasted and showed improvements in exercise times.

The RER was lower in females compared to males and was not influenced by caffeine ingestion. The wealth of the published literature indicates that females burn a greater proportion of fats and less CHO than males during sub-maximal and low intensity exercise (Isacco et al., 2012). However, no studies were found investigating the effect of a high intensity time trial on fuel metabolism in men and women. Therefore, it is interesting to note in our study that females burned a higher percentage of fat during high intensity aerobic exercise which is represented by decreased RER values throughout the exercise bout. Further research should be conducted in order to confirm that this finding is consistent with sub-maximal and low intensity exercise.

Caffeine had no effect on heart rate during exercise, nor were there any differences between sexes. This is in contrast to findings from other investigations (Bridge & Jones, 2006; Kovacs et al., 1998), which suggest that caffeine increases heart rate during high intensity aerobic exercise, possibly due to a higher work output achieved during the exercise bout (Kovacs et al., 1998). It is difficult to explain why these findings are in contrast to the current investigation, especially since these studies employed time

trial tests and not trials to exhaustion, and all participants were caffeine users. However, the participants in both studies were highly trained athletes, and those in the current study were a mix of trained and untrained individuals, which may have accounted for variations in heart rate. Further, the type of exercise may have influenced the findings, as Bridge & Jones (2006) used an 8 km running time trial, while Kovacs et al. (1998) used a 60 min cycling time trial, both of which are different from the current investigation. Interestingly, it has been found that the administration of 5 mg/kg of caffeine resulted in lower heart rates during exercise at 30-70% VO_{2max} , but no difference in heart rate when participants exercised above 75% VO_{2max} (Gaesser & Rich, 1985). In the present study, participants spent the majority of the time above this threshold. Consequently, because of the intensity of the trials, HR differences may have been washed out. Nonetheless, it is clear that more studies are required to investigate the effect of caffeine on heart rate during high intensity aerobic exercise, as well as whether heart rate changes are different between sexes.

There were no changes in LTE between trials, and no differences between sexes as a result of caffeine ingestion. However, LTE decreased within each trial which is in agreement with pilot studies out of our lab. It is difficult to compare these results to other investigations which monitored EEG during fatiguing exercise, as the current study used a novel portable neuroheadset which monitored arousal using proprietary software and algorithms. Nonetheless, the LTE appears to closely monitor arousal as purported. Most studies analyzed the α/β index to monitor fatigue during exercise, and found that the ratio is increased at the end of exhaustive exercise due to decreases in beta activity while alpha

activity remains unchanged (Ftaiti et al., 2010; Nybo & Nielson, 2001). Further, it is clear that these changes in the α/β index represent decreased arousal (Nielsen & Nybo, 2003). It is not clear how the decrease in LTE in our study is related to changes in the α/β index with fatiguing exercise, however, it is likely that LTE changes during exercise observed in our study are associated with decreases in arousal levels with fatiguing exercise. It is possible that the decrease in LTE observed during exercise in our study is better represented by the “inverted U” hypothesis proposed by Hebb (1955), stating that arousal levels increase at the onset of exercise and decrease as an individual approaches fatigue (Kamijo et al., 2004). This is consistent with our finding that LTE was significantly increased between rest and the initial 5 min of exercise, and decreased between the initial 5 min and the value taken at the end of the time trial. Our results also show a significant negative correlation between LTE and tympanic temperature, possibly indicating that an increase in body temperature with exercise may be related to decreases in LTE which accompany fatigue. Given these results, it is necessary for future research to be done using the Emotiv Epoc to evaluate EEG changes during exercise. Also, while differences in LTE were not observed between trials, it may be beneficial for future studies to examine blood borne neurotransmitter precursors such as tryptophan to establish links to fatigue, as increases in tryptophan may be linked to arousal levels (Davis & Bailey, 1997).

In conclusion, it is clear that CHO combined with caffeine has the ability to improve performance over CHO alone, with this improvement being the same between males and females. To our knowledge, this was the first study to evaluate the effect of

caffeine on differences in exercise performance between sexes. Blood glucose levels, which were significantly, increased post-exercise in the caffeine trial and not the CHO only trial was the only other measure which was affected by caffeine ingestion. Thus, it is still unclear how caffeine can improve performance over carbohydrates alone in a high intensity aerobic time trial. It may be that performance improvements are associated with an effect of caffeine on skeletal muscle and subtle changes in neuromuscular recruitment (Anderson et al., 2000), however, more research is needed to confirm these hypotheses. It is clear that this ergogenic aid is able to enhance exercise performance, but the manner in which caffeine provides this advantage still remains elusive.

6. LIMITATIONS

A limitation of this study is that we did not include a caffeine alone condition. We only included a placebo, a CHO trial and a CHO combined with caffeine trial. We did not deem it necessary to include a caffeine only condition as it may have created problems with participant recruitment and retention, as participants would be forced to come into the lab on 5 different occasions and to control menstrual cycle phase would have necessitated an additional month between trials. Further, the differences between a caffeine condition and caffeine combined with CHO would likely be minimal during high intensity aerobic exercise as CHO depletion is not a limiting factor during a 10 km race.

The purpose of this study was to evaluate the effectiveness of caffeine and a CHO beverage to provide practical information to athletes given that this would be the most likely scenario during competition (e.g. an energy drink with caffeine, rather than a coffee). Untrained participants were used in this study as a result of availability, and the

difficulty in scheduling around an athlete's season. Nonetheless, the potential for detecting small but meaningful changes in performance may have been higher with elite athletes. Elite athletes are usually highly reliable at performing during an exercise test and produce a small coefficient of variation between exercise trials, increasing the precision between the true intervention effects from everyday differences in performance (Burke, 2008). Interestingly, we found a significant correlation between $VO_{2\text{peak}}$ and mean performance time (Appendix C) indicating the importance of prior fitness to this test, but this did not turn out to be factor in our analysis.

Another limitation is that the pre-exercise meal was not controlled for each individual; rather a simple dietary recall was used to ensure that caffeine was not ingested 24 h before exercise. In a practical sense, every person has their own meal preference prior to exercise, and interfering with that may have negatively impacted their exercise test. Nonetheless, nutritional analysis of a 24 h dietary recalls prior to testing were not statistically different.

Finally, while the Emotive EPOC has undergone internal validation against standard EEG machines, this was not done in the current study. Further, some of the LTE data was lost due to connection issues with the headset and data receiver. Inaccurate/incomplete data was received from 6 of the 18 participants, and was therefore removed from the data analysis. Further, it was difficult to compare our analysis of the LTE to other methods (i.e. α/β ratio) used to analyze EEG patterns during exercise. Thus, it is clear that further research using the Emotiv EPOC needs to be done in order to determine its effectiveness during exercise.

7. FUTURE DIRECTIONS

Future studies should evaluate the effect of caffeine combined with CHO on high intensity aerobic exercise as it has generally been ignored throughout the literature. These studies should evaluate differences between men and women as it is unclear if sexes respond differently to caffeine ingestion during exercise, and if exercise duration/intensity has any implications. Further, the optimal dosage of caffeine and CHO is still debated, and may be different between sexes. These findings could have great implications for elite middle distance athletes (i.e 5000 m or cross country runners), as they want to know the exact combination of caffeine and CHO to consume prior to competition in order to get the greatest performance improvement.

Interestingly, the major sports supplement companies such as Gatorade™ and Powerade™ do not have popular drinks which contain caffeine combined with CHO. Moreover, most energy drink companies which sell beverages that contain both caffeine and CHO are more focused on promoting their drinks to the general population who require caffeine to promote wakefulness. If these drinks are targeted more towards athletes, they may become more widespread.

The WADA will be interested in these results, as they are continuously trying to determine the legal amount of caffeine allowed by professional athletes. Currently, caffeine is off the banned substance list, partially due to inconclusive research, and the fact that ingesting high amounts of caffeine may not improve performance beyond ingesting normal amounts. If WADA is aware of the optimal dosage of caffeine needed to

produce performance improvements, maybe they will implement legal limits.

8. CONCLUSION

In conclusion, it is clear that caffeine can improve performance during high intensity aerobic exercise. This could have great implications for athletes competing in aerobic sports who want to gain a legal advantage over competitors who may not be taking caffeine prior to exercise. There are still some question marks in regards to caffeine ingestion and sport performance. There is no agreement throughout the literature to what dosage is optimal for performance improvement and if this dosage is different between males and females. It is clear that further research needs to be done in order to determine performance differences between sexes. If there is consensus throughout the literature, maybe WADA and the IOC will implement legal limits for caffeine ingestion during competition. It will be interesting to follow the progression of caffeine ingestion in sport and how opinions and regulations change as more and more research is done on the effect of caffeine on sports performance. Clearly, the world's most consumed drug has powerful effects for the general public and athletes alike.

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Appendices

Appendix A. Caffeine Consumption Questionnaire.

Subject ID _____

Adapted from the CCQ by R.E. Landrum 1988

CAFFEINE CONSUMPTION QUESTIONNAIRE (CCQ)

Please answer the following questions as completely and honestly as you can. This information is **STRICTLY CONFIDENTIAL** – do not write your name anywhere on this page. Thank you for your cooperation.

Please answer the following questions about your caffeine usage. Respond to items that you consume at least once a week.

COFFEE (5 oz servings/week)	MORNING 6am-12pm	AFTERNOON 12pm-6pm	EVENING 6pm-2am	NIGHT 2am-6am
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Regular brewed

Percolated

Drip-brewed

Regular instant

Decaffeinated

Instant

TEA

(5 oz serv/week)

COCOA

(5 oz serv/week)

CHOCOLATE

(8 oz serv/week)

SOFT DRINKS

(12 Oz. Serv/Week)

Coca-Cola

Diet Coca-Cola

Dr. Pepper

Diet Dr. Pepper

Mountain Dew

Diet Mountain Dew

Mr. Pibb

Tab

Pepsi Cola

Diet Pepsi Cola

RC Cola

Mello Yello

Diet Mello Yello

Root Beer

Red Bull

Rockstar

Other Energy
Drinks

Appendix B. Par Q form

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of any other reason why you should not do physical activity?

**If
you
answered**

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT _____
or GUARDIAN (for participants under the age of majority)

WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.



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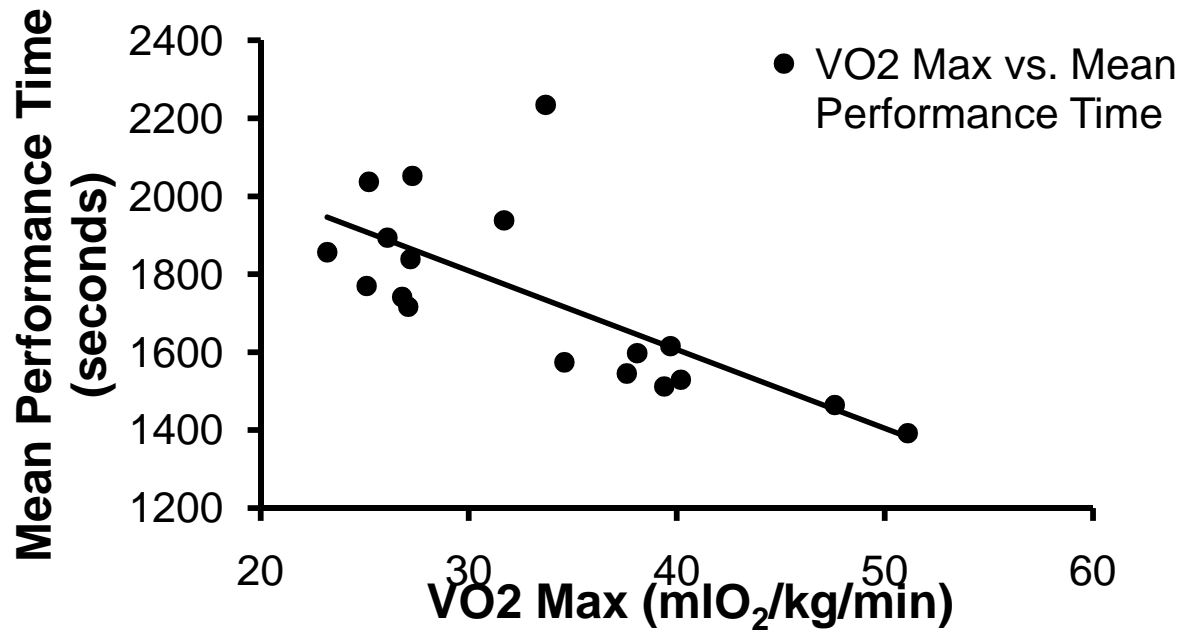


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continued on other side...

Appendix C. VO2 max vs. performance time.



VO2 max vs. mean performance time. VO2 max showed a significant negative correlation with mean 20 km cycling performance time ($p < 0.05$, $r = -0.712$). This indicates that those individuals with the highest VO2 max values had the fastest mean performance times over the 3 trials.

Vita Auctoris

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