Carbohydrate ingestion and exercise performance in the heat: Neuromuscular aspects of fatigue

By

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To honour my subjects...
If you worried about falling off the bike, you’d never get on. **Lance Armstrong**

Só pedala quem aguenta. Quem não aguenta joga futebol.  
**Cycling is just for those that can endure. Those that can’t, play soccer.**  
**Carlos Leônidas da Silva**

Life is a Hell of a Ride... you never know what will come next... **Camila Nassif**

One minute you’re pedalling along a highway, and the next minute, boom, you’re facedown in the dirt. **Lance Armstrong**

This is not Disneyland, or Hollywood. I will give you an example: I’ve read that I flew up the hills and mountains of France. But you don’t fly up a hill. You struggle slowly and painfully up a hill, and maybe, if you work very hard, you get to the top ahead of everyone else. **Lance Armstrong**

Everything hurts. Your back hurts, your feet hurt, your hands hurt, your neck hurts, your legs hurt, and of course, your butt hurts. **Lance Armstrong**

But one of the redeeming things about being an athlete – one of the real services we can perform – is to redefine what’s humanly possible. **Lance Armstrong**

We cause people to reconsider their limits, to see that what looks like a wall may really just be an obstacle in the mind. **Lance Armstrong**

Chemo has made the worst climb in the Alps seem flat. **Lance Armstrong**

When you have lived for an entire year terrified of dying, you feel like you deserve to spend the rest of your days on a permanent vacation. **Lance Armstrong**

The best discoveries... are made out of your comfort zone. **Anonymous**

Keep smiling; it makes people wonder what you are up to. **Anonymous**

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1.1 FLUID INGESTION, CARBOHYDRATES AND FATIGUE

Fluid ingestion has been one of the most studied strategies for maintaining and improving endurance performance in a warm environment, in addition to other strategies such as precooling (Schmidt & Brück, 1981; Lee & Haymes, 1995) and acclimation (Nielsen et al., 1993; Pandolf et al., 1977). Many investigations have been conducted which have examined the effects of various sports drinks on performance (Coyle et al., 1986; Powers et al., 1990; Febbraio et al., 1996; Kang et al., 1996; Maughan et al., 1996; Nassis et al., 1998; Burke et al., 2000; McConell et al., 2000 and Silami-Garcia et al., 2004), however, the type and amount of fluid to be ingested as an ergogenic aid is still very much under debate (Noakes, 2007b).

Dehydration as a consequence of prolonged physical activity is still regarded as one of the main limiting factors of physical performance in addition to a reduced heat tolerance (Kay & Marino, 2000; Sawka, 1992 and Sawka et al., 1992). However, despite the importance of the ingestion of carbohydrate (CHO) before and after physical activities having already been established, controversy still exists regarding CHO ingestion during exercise (ACSM, 2007; DeMarco et al., 1999). Many studies dealing with fluid replacement with added CHO during prolonged exercise have attempted to verify the possibility of attenuating glycogen usage as this has been thought to delay fatigue (Coyle et al., 1986; Coggan & Coyle, 1988; Hermansen et al., 1967; Hultman, 1967; Langenfeld et al., 1994 and Maughan et al., 1996). There has also been the suggestion that glycogen usage is increased during exercise in the heat, and thus a reliance on glycogen under these conditions might also promote premature fatigue (Febbraio et al., 1994a; Febbraio et al., 1994b).
Many studies using 2 – 7% CHO solutions have suggested enhanced exercise performance when CHO were ingested during exercise (Coggan & Coyle, 1987; Coyle & Coggan, 1984, Coyle et al., 1986; Febbraio et al., 1996; Kang et al., 1996; Maughan et al., 1996; McConell et al., 1999; Tsintzas et al., 1996). In contrast, other studies have shown this not to be the case (Silami-Garcia et al., 2004; Burke et al., 2000; Gomes, 1999; McConell et al., 2000; Nassis et al., 1998; Powers, et al., 1990; Robinson et al., 2002; Timmons et al., 2000).

At present, there are very few studies which link CHO availability to central nervous system (CNS) fatigue. These data are sparse and it is far from conclusive that CHO play a significant role in CNS fatigue during prolonged exercise. Studies by Fritzsc 

he et al. (2000), Nybo (2003) and Winnick et al. (2005) are the most recent studies linking the possible effects of CHO to CNS fatigue. Fritzsc 

he et al. (2000) found an attenuated decline in neuromuscular power when water was ingested during prolonged moderate-intensity exercise in a warm environment. They also concluded that ingestion of water with CHO attenuates the decline in maximal power more so than water alone, and ingestion of CHO alone does not attenuate the decline in neuromuscular power compared with a placebo (PLA).

Nybo (2003) studied prolonged exercise with or without glucose supplementation and its effects on CNS activation. It was concluded that exercise-induced hypoglycaemia attenuates CNS activation during a sustained maximal muscle contraction, whereas central activation appears to be unaffected by three hours of moderately intense exercise in endurance trained athletes when blood glucose is maintained with the ingestion of CHO.

1.2 JUSTIFICATION AND BACKGROUND

Although some studies suggest that the ingestion of CHO restores glycogen and, therefore, attenuates fatigue during prolonged exercise (Burke et al., 2000; Costill et al., 1973;
McConell et al., 1999), there is still controversy in relation to its ingestion during exercise (ACSM, 2007; DeMarco et al., 1999). As some studies show an enhanced performance when CHO are ingested (Coggan & Coyle, 1987; Coyle et al., 1986; Febbraio et al., 1996; Maughan et al., 1996; Tsintzas et al., 1996), others have not observed the same results (Carter et al., 2004b; Burke et al., 2000; Nassif et al., 2008; McConell et al., 2000; Nassis et al., 1998; Powers et al., 1990; Robinson et al., 2002; Timmons et al., 2000). These differences, which can also be observed in more recent work, suggest that researchers may need to design studies so evaluation of the overall effect of CHO on exercise performance can be made. That is, when attempting to test the real effects of a substance, the PLA effect should be taken into account as the expectations of subjects in relation to a certain substance can determine the intrinsic feedback (Wilmore & Costill, 2001). Clark et al. (2000) evaluated the PLA effect of CHO beverages and showed a better performance when subjects believed they were ingesting CHO but were actually drinking PLA. These authors also suggest using a Latin square design for a more robust control of blind treatments. Notably, the studies by Gomes (1999) and Nassif et al. (2008) used a Latin square design, which is not a common design used in CHO studies. Interestingly, studies that have used this design did not report a physiological effect of CHO on performance.

Another aspect that should be taken into account is the form of ingestion which most of the studies use. Most studies have used beverages with 2 - 7% CHO, with what is termed a “matching placebo”. Recent studies by Carter et al. (2004 a, b) have examined the CHO effect on performance using different methods of fluid administration. These authors evaluated the effect of CHO infusion on exercise performance and did not observe any effects. Also, Carter et al. (2004a) observed an improvement in performance using a CHO mouth rinse. This study suggests that a mouth rinse alone can increase the central drive or motivation of the individual. Therefore, it is critical to consider what method of ingestion is to be used when the PLA effect might be present.
The studies by Silami-Garcia et al. (2004), Gomes (1999), Nassif et al. (2008), and Timmons et al. (2000) used gelatine capsules as a method of ingesting CHO. Gomes (1999) compared the effects of ingesting water or a solution of 6% CHO during sub maximal exercise to fatigue, in a thermoneutral, and in a warm and humid environment during exercise at 50% of the peak power output (PPO). Differences in time to fatigue in both environments were not observed, although Burke (2001) confirmed that there is a higher demand of replacing CHO during exercise in the heat.

Silami-Garcia et al. (2004) researched the effect of the similar ingestion as that by Gomes (1999) using capsules (CHO at 6%) on the maximal anaerobic power during a Wingate test followed by prolonged exercise at 60% of PPO of 90 min duration. The PPO during the Wingate test after the prolonged exercise was similar in both PLA and CHO treatments. The study by Timmons et al. (2000) also did not show an enhancement in time to fatigue when CHO were ingested in a capsule form, which is similar to that of Gomes (1999) and Silami-Garcia et al. (2004). However, the duration of the exercise test in this study was not prolonged exercise.

Nassif et al. (2008) studied the ingestion of CHO in a warm environment during prolonged exercise at 60% PPO until fatigue in a double blind fashion using the ingestion of capsules. This study showed no physiological effect, as there was no difference in time to fatigue between the PLA and the CHO ingested as capsules. This finding confirms the results of Gomes (1999) and Silami-Garcia et al. (2004) which also found no difference in time to fatigue and power output in a Wingate test after 90 min of cycling, respectively. However, Nassif et al. (2008) had subjects ingest CHO and in addition were informed that they were drinking CHO, which showed an increase of 24% in time cycled compared to the PLA treatment.

As shown previously, capsules have been successfully used to study the effect of CHO ingestion on performance accounting for the role that taste might play in the results. According to the study by Nassif et al. (2008) capsules take around 2 minutes to dissolve in the stomach and start digestion and absorption. This is important information to assure that although CHO
are being ingested in different forms, its effect on the organism and timing is compatible with the ingestion of CHO in a beverage form. The use of the two methods is critical in order to elucidate if taste plays a role in determining the individual’s performance and also to evaluate if capsules are a useful method to be used. Capsules are also important to ensure subjects are not able to distinguish the difference between the beverage that contains CHO and the one that does not.

As for the concentration of the beverage to be used, a number of studies have examined the effect of different concentrations of CHO ingestion on performance. Most studies have found that lower concentrations have a more positive effect than ingestions of higher concentrations (Galloway & Maughan, 2000; Jentjens et al., 2005). Most studies use a CHO concentration of 6%. Another important aspect that should be taken into account is the composition of the CHO being used. Previous studies have shown that the ingestion of a combination of CHO is more efficiently absorbed than when ingesting one type of CHO alone (Murray et al., 1989; Jentjens & Jeukendrup, 2005; Jentjens et al., 2006; Jentjens et al., 2005). For this reason, a combination of glucose and sucrose was used in the present study to guarantee optimized absorption of the CHO ingested.

The studies presented in this thesis aim to investigate the PLA effect and the effect of CHO on fatigue parameters, particularly CNS fatigue, using a Latin square design in a double-blind fashion, on well trained fed subjects during exercise in a hot environment (32°C and 50% RH). The methods to deliver the CHO and PLA will be in a beverage form, which most other studies have used and in a capsule form as recently used (Silami-Garcia et al., 2004; Nassif et al., 2008). Both capsules and beverages will deliver 6% CHO concentration, containing glucose and sucrose.
Chapter 2
REVIEW OF LITERATURE

2.1 IMPORTANCE OF HYDRATION DURING EXERCISE

Water is a structural element of macromolecules; it plays a role in digestion, absorption, circulation and excretion. It is important as a means of transport of nutrients and all body substances (McArdle et al., 1998). According to standard physiology texts such as Guyton and Hall (1997), water is the main component of the human body and can represent approximately 60% of body mass.

Some researchers have shown that physical performance can be improved with fluid ingestion compared to no ingestion during exercise (Guimarães & Silami-Garcia, 1993). This improvement in performance is associated with the maintenance of blood volume that allows an improvement in the cardiovascular and thermoregulatory function compensating partially or totally for the fluid loss caused by sweating. Although in the past researchers believed the ingestion of fluids attenuated the increase in heart rate, core temperature, preventing the reduction of cardiac output and increased the exercise time tolerated, mainly because of the maintenance of blood volume (Armstrong et al., 1997; Guimarães & Silami-Garcia, 1993) not much evidence can be found to support these ideas. Recently this has been shown by Watt et al. (2000) who found that an expansion in plasma volume in the heat in moderately trained men did not enhance thermoregulatory function and exercise performance during moderate intensity exercise. They also advised that the rate which core temperature increases is an important factor in determining fatigue in the heat. In the anticipatory control model, changes in exercise intensity, mainly unconscious reduction in intensity of self-paced exercise is important to reduce heat production preventing the body from overheating. Although reaching a critical core
temperature would affect performance, it has been suggested that performance is not determined by the critical temperature but by its rate of increase (Marino et al., 2004). Furthermore, exercise fatigue cannot be determined by a critical limiting temperature as Tucker et al. (2004) show that rectal temperature of individuals was not different in the first 15 km of a 20 km self-paced cycling trial in hot and cool conditions, but differences were only observed at the end of the trial, although power was always lower during the hot condition. These authors also showed a reduction in muscle recruitment much earlier in the trial than when they reached 40°C. For this, Marino et al. (2004) suggest that some studies show the participation of “a central component in the fatigue process” during exercise in the heat but that the mechanisms by which this occurs are not understood and need further investigation. However, Watt et al. (2000) challenged the wisdom that hydration prevents a drop in blood volume and therefore attenuates the rise in core temperature ultimately reducing exercise performance.

The premise that sufficient hydration will reduce hyperthermia and prolong exercise is generally agreed upon by the sporting community. The effects of water deficits during exercise have been the basis for much of the research in this area and therefore strategies which can minimise these effects have been employed. However, the majority of the studies in this area have utilised exercise protocols which require the subjects to exercise at a fixed workload or intensity. With these types of protocols it has been shown that exercise and environmental heat stress is thought to result in competition for blood between the working skeletal muscle and the skin; the cardiovascular model of exercise physiology and thermoregulation. Therefore, during exercise with heat stress, blood flow to the active skeletal muscle would be reduced due to the elevated skin blood flow. Maintaining fluid ingestion in warm ambient conditions (33 °C, 50% relative humidity; rh) has been shown to attenuate the reductions in stroke volume and reduce thermal strain over a period of 2 h when cycling at 62-67% VO_{max} (Montain & Coyle, 1992a). In addition, it has been shown that the infusion of a blood volume expander to maintain blood volume at similar levels to those when ingesting fluid alone, resulted in comparable core
temperatures, forearm blood flow, elevations in serum osmolality and sodium concentration during 2 h of exercise, whereas, complete fluid replacement resulted in lower core temperatures only in the second hour of exercise (Montain & Coyle, 1992b). These studies show that physiological strain was augmented when fluids were restricted, even though the 2 h of exercise was completed.

Other studies (González-Alonso et al., 1995, 1997, 1998, 1999) provide good evidence regarding the relationship between hydration, the development of hyperthermia and fatigue. These studies showed that when subjects exercise for 2 h at ~ 62% VO$_{2\text{max}}$ in the heat (35 °C), fluid ingestion which preserves up to 95% of body fluid loss compared with the reduction in body mass of ~ 5% when drinking was restricted (dehydration), cardiac output declined by 18% while systemic vascular resistance increased by up to 17% (González-Alonso et al., 1995). Although physiological strain was augmented significantly during the dehydration trial compared with the hydration trial, subjects were able to complete the trials within the allotted 2 h so that time to exhaustion was not different between trials. It was concluded that dehydration in hyperthermia reduces the capacity of dehydrated athletes to cope with hyperthermia. An alternative explanation would be that the subjects handled the dehydration by employing physiological responses that would enable them to continue exercising rather than stop.

A more recent study directly compared the physiological responses of 5% hypohydration with euhydration during exercise in both temperate (23 °C) and hot (33 °C) environments at 60% VO$_{2\text{max}}$, finding that the physiological consequences of hypohydration during exercise are exacerbated in the heat whilst exercise duration was not altered (Buono et al., 2000). In addition, when cycle exercise was compared to running at a fixed workload for ~ 90 min and replacing 60% of the water deficit in thermoneutral conditions, rectal temperature during running was attenuated but this difference was not observed during cycling exercise either with either hydration or no hydration (Nassis et al., 2002). These findings suggest that there might be a
difference in how water replacement might be utilized according to the mode of physical exercise.

More recent studies have used self-paced exercise protocols and provide contrasting findings to the studies discussed above which used constant load exercise. For example, by using a water replacement strategy to match the predicted reductions in body mass during exercise compared with no water replacement in both moderate (~20 °C) and warm (~33 °C) ambient conditions whilst exercising in self-paced mode over 60 min, there were no differences in thermal strain or distance completed in 60 min (Kay & Marino 2003). Similarly, Bachle et al. (2001) showed that power output during 60 min of cycling under three randomized hydration conditions, water and Gatorade were similar and that fluid replacement during 60 min of moderately intense cycling exercise did not enhance performance if subjects were normally hydrated before exercise.

In the study by Bothorel et al. (1990), five male subjects exercised in the heat (36°C) with and without fluid ingestion. The highest increase in heart rate observed during exercise without fluids confirmed that dehydration promotes a cardiovascular stress, originating from the reduced plasma volume and by consistent rises in rectal temperature. For this reason, hydration is considered one of the most efficient strategies to enhance physical performance and prevent hyperthermia in hot environments (Burke, 2001; Kay & Marino, 2000).

Schroeder et al. (1997) compared three strategies to maintain hydration level during one hour of exercise at 50% VO$_{2\text{max}}$, in an environmental chamber at 27°C and 50% relative humidity in 10 subjects. In one of the strategies the subjects ingested 200 mL of water every 15 min; in the other trial subjects ingested water *ad libitum* every 15 min, and in the third they ingested *ad libitum*. The authors concluded that the best strategy to maintain the body hydration during exercise should be ingesting 200 mL every 15 min. Even though hydration has been believed by some to be one of the most efficient strategies to prevent dehydration and hyperthermia, one should be clearly aware of the capacity of the brain to regulate exercise intensity to actually
reduce heat production to avoid damage to the organism. Hydration can be seen as a way of minimizing the increase in core temperature and heat storage rather than preventing hyperthermia. Studies have shown how important the level of hydration is in relation to the tolerance of prolonged exercise, verifying that hydrated individuals better support the increases in body temperature when compared with hypo-hydrated individuals (Byrne et al., 2006; Lambert et al., 1996; Maughan & Shirreffs, 1997; Noakes et al., 1990; Sawka & Greenleaf, 1992; Sawka et al., 1992).

Gastric emptying proceeds primally because of a pressure gradient between the stomach and duodenum, thus, a higher amount of fluid will be emptied faster than a smaller amount; volumes higher then 600ml do not seem to result in an additional increase in the speed of the gastric emptying (Lambert et al., 1996). Maughan and Noakes (1991) suggest that to obtain a high rate of gastric emptying a full stomach (500 - 600 mL) should be maintained by repeated ingestion of fluid. The rate of gastric emptying can range from 0.8 to 1.2 l/h (Coyle & Hamilton, 1990).

The classic study by Hunt and Spurrel (1951) concluded that the addition of glucose to the water can reduce the rate of gastric emptying. Concentration of sports beverages will directly affect the rate of gastric emptying. Beverages that contain more than 7% CHO result in a significant slower gastric emptying (Maughan et al., 1993). This can make the rate of gastric emptying slower than the fluid loss, not minimizing dehydration and increasing the risk of gastric discomfort to the athlete (Rehrer et al., 1989). The American College of Sports Medicine (2007) also suggest that the addition of CHO and/or other nutrients in beverages decreases gastric emptying in the same proportion as the caloric content of the solution.

Costill and Saltin (1974) observed that exercise on an ergometer seems to have a reduced effect on gastric emptying at intensities of 50 to 70% of maximal oxygen consumption (VO$_{2\text{max}}$), but when exercise exceeded 70% of VO$_{2\text{max}}$, gastric emptying was inhibited. As exercise intensity during races can easily exceed 70% of VO$_{2\text{max}}$, it could result in even less
absorption of any CHO or from higher energy content drinks compared to plain water, as fluids with a higher energy content tend to be absorbed slower than those with a lower energy content (Hunt & Spurrel, 1951; Maughan et al., 1993) Therefore, as the nature of a race is more similar to a time trial, subjects ingesting CHO drinks will tend to absorb less of this beverage as the intensity is too high, inhibiting gastric emptying.

It is also important to call attention to the fact that beverages with combinations of CHO tend to be more beneficial compared to those with just one CHO component. Jentjens et al. (2004) studied the effect of the ingestion of a mixture of glucose and fructose compared to glucose alone and water. Eight trained cyclists completed three trials ingesting the mixed combination of CHO, glucose alone or water. These authors found that a combined mix of CHO showed higher rates of exogenous CHO oxidation and fluid availability compared with glucose as well as a lower endogenous CHO oxidation with the CHO mixture when compared to water.

In relation to the temperature of the fluid ingested during prolonged exercise with the intention of replacing the fluid lost through sweat, some authors recommend that temperatures should be 4 to 10 °C (Wimer et al., 1997). Between these values; fluids are absorbed faster because of an increase in stomach motility (Williams, 1999). Although with other animal studies it has been shown that the preferred temperature is approximately body temperature (30 - 37 °C); the preference of adult males is to ingest water at a temperature of approximately 15 °C (10 - 20 °C) (Boulze et al., 1983).

According to the study by Costill and Saltin (1974), the volume of fluid remaining in the stomach 15 min after the ingestion was consistently a smaller amount when ingested solutions were cooler, indicating increased rate of gastric emptying when the temperature of the fluid was 5 °C as opposed to 15 °C. In addition, this same study found that approximately 50% of a cold solution (5 °C) was emptied by the stomach in the first 15 min after ingestion, although just 27%
was emptied when the temperature of the solution was 35 °C, that is, a cold solution tends to leave the stomach faster than a warmer solution.

Guimarães and Silami-Garcia (1993), studied the effects of cold water ingestion (12°C) on the hydration state and thermoregulatory responses, in a hot, humid environment (20°C and 68.8% RH), before and after submaximal exercise in a cycle ergometer. These authors found an increase of 15% in exercise time in the group that ingested water, when compared with the group without water. They attributed this result on a cooling of the body promoted by the ingestion of cold water.

Viveiros (1996) studied the effects of the ingestion of water at 10, 24 and 38 °C on the total time of sub maximal exercise to fatigue and concluded that the ingestion of water at different temperatures did not significantly modify cardiovascular, respiratory, metabolic and neural parameters during experimental trials or at the point of fatigue.

2.2 DEHYDRATION

The effects of water loss and dehydration have been researched for many years and the knowledge base in this area extends into the early 1900’s. In a classic paper by Adolph and Dill (1938), dehydration was shown to decrease performance of men during prolonged exercise in hot environments. Only in the 1970’s did scientists and authorities within the sporting industry begin to be concerned about the detrimental effects of dehydration on physical performance and the health of people engaging in physical exercise generally. The American College of Sports Medicine (ACSM) published for the first time in 1975 guidelines relating to preventing exercise-induced dehydration (ACSM, 2007; Barr, 1999). The current recommendation by The ACSM is that hydration should be as close as possible to the amount of sweat lost during exercise (ACSM, 2007). The water ingestion should be counterbalanced with the daily loss of body fluids
without physical activities. A person can lose approximately 2.3 l of water a day. From this amount, approximately 700 mL are lost through the lungs and the skin, 1400 mL through urine, 100 mL through faeces and 100 mL through sweat.

Moderate physical exercise training typically results in a loss of sweat between 0.8 to 1.4 l/h, but the highest sweat rate reported in subjects was approximately 3.7 l/h (Armstrong & Dziados, 1986). An elevated sweat rate during exercise can result in dehydration particularly when the ingestion of fluids does not replace all fluid lost and when the sweat rate is higher than the rate of gastric emptying (0.8 to 1.2 l/h) (Coyle & Hamilton, 1990; Burke & Hawley, 1997).

Dehydration has also been shown to contribute to the amount of heat retained by the body in addition to a reduced heat tolerance (Guimarães & Silami-Garcia, 1993; Sawka, 1992) primarily because of the reduction in blood volume as a result of insufficient distribution of fluids and by redistribution of blood volume from the central circulation to the periphery, with the overall result being heat dissipation and a prevention in core temperature reaching undesirable values (Armstrong et al., 1997). Dehydration, thus, contributes to fatigue in accordance with the high sweat rates which results by the need of cooling during sub maximal exercise of long duration, mainly in hot and humid environments (Maughan & Noakes, 1991).

Gisolfi and Wenger (1984) observed that the highest thermoregulation demand that follows dehydration seems to be the result of reduced plasma volume and increased osmolality which both result from the loss of hypotonic fluid through the sweat glands. Osmolality is affected by changes in water content of the body. Loss of plasma fluid will lead to a higher osmolality which will impact on physiological and thermoregulatory functions, with increases in core temperature (McArdle et al., 1998). The recent ACSM guidelines suggest that greater than 2% dehydration should be avoided (ACSM, 2007).

Dehydration equivalent to only 2% loss in body mass is thought to compromise physiological function and negatively influence performance; whereas, values above 5% can decrease workload capacity by approximately 30% (Maughan & Noakes, 1991). Adolph and Dill
(1938) have suggested the possibility of death when dehydration levels are greater than 15%. Lower levels of dehydration do not show the same risk but can present as a challenge, affecting exercise in levels as low as 2% (ACSM, 2007). This level of dehydration is common in a variety of sports and can be reached rapidly when the athlete starts the exercise session dehydrated. Thirst, irritability, general discomfort followed by headaches, weakness, dizziness, cramps, feeling cold, nausea and decrease in performance are signs and symptoms of dehydration (NATA, 2000). Sawka et al. (1992) verified that trained and heat acclimatized subjects show a lower total time of exercise in a hypo hydrated state compared to a euhydrated state.

As it can be observed in recent studies there is not enough evidence that core temperature determines fatigue. It plays a role in activating thermoregulation changes such as sweating to try to maintain homeostasis but there is not a set consensus that fatigue ensures when a certain core temperature is reached. Sawka (1992) suggests that an increase in the level of hypohydration promotes a systemic reduction in sweat rate for a certain core temperature during exercise in the heat, and increases the temperature threshold for the onset of sweating (Sawka, 1992; Sawka et al., 1996).

In the classic study by Pitts et al. (1944) subjects marched from one to six hours in a hot environment with a 10 min rest between each hour. Subject’s heart rate and core temperature increased constantly during exercise and they reached exhaustion when ingestion of fluids was completely restricted. However, when fluid loss was completely replaced, the heart rate and the core temperature were stable during exercise, with attenuation in the signs of fatigue. This study showed that the increase in workload accompanies a strain on the cardiovascular system characterized by a rise in heart rate. Recent studies on hydration also show that one of the consequences of dehydration is an increase in cardiovascular strain, which involves a decrease in ejected blood volume from the heart, increased heart rate, increase in systemic vascular resistance and possibly a reduction in cardiac output (Armstrong et al., 1997; Montain & Coyle, 1992a; Sawka, 1992). However when individuals exercise at their own pace some studies have
observed changes in intensity and in pacing strategies, sometimes not conscious that consequently results in reductions in heart rate, core temperature and heat storage acting as a protective system to the organism (Kay et al., 2001). Unconscious decisions might have been taken by subjects by feedback to reduce intensity as a form of protection.

According to Wilmore & Costill (2001), the feeling of fatigue during prolonged exercise matches the decrease in muscle glycogen. With individuals running on a treadmill at 70% of VO$_{2\text{max}}$, these authors only noticed severe fatigue when the muscle glycogen concentration was near depletion. The ingestion of fluids during physical exercise has been associated with an enhancement of performance and consequently in a delay of exhaustion, but these mechanisms still need to be verified (Kay & Marino, 2000).

Kay and Marino (2000) suggest that the mechanism by which the ingestion of fluids enhances human performance during exercise is, in part based on an increase in the capacity to store heat. This mechanism allows thermoregulation, metabolic and cardiovascular alterations to occur to enhance performance in the heat. However, these same authors have evaluated this “heat sink” hypothesis and found no differences in the final rectal temperature recorded, and they suggest that other factors than the “heat sink” might be responsible for controlling exercise performance and fatigue development in moderate and warm environments when exercise is performed as self-paced (Kay & Marino, 2003). These findings indicate that a heat sink is more likely when exercise is performed at a fixed intensity rather than during self-paced exercise. This was recently shown by Lee et al. (2008) where subjects were able to cycle for longer when ingesting cold drinks (4 °C) compared to warm drinks (37 °C) before exercise, confirming the precooling effect of cold drink ingestion.

As hydration state of subjects is an important factor for drawing from studies which evaluate dehydration effects on performance, an easy and non invasive method has been used to assess hydration status. The urine specific gravity (USG) and the urine colour are non invasive methods used to determine the hydration state of individuals. The USG is measured
using a small sample of urine droplet from an automatic 20µ pipette on a refractometer, which has a scale that can be read when the refractometer is directed at a light source. Armstrong (2000) has suggested that hyper-hydrated individuals should show values below 1.013, euhydrated individuals should show values between 1.013 and 1.029 and hypo-hydrated individuals should show values above 1.029. The more concentrated the urine is, the higher the refractometer reading, indicating different levels of hydration.

2.3 METABOLISM DURING EXERCISE AND PACING

As traditionally taught, the human body has three avenues of energy source during physical activity, these being: anaerobic alactic, anaerobic lactic and aerobic, with aerobic being the predominant source during prolonged exercise. Proteins, lipids and CHO are available substrates for the resynthesis of adenosine triphosphate (ATP), the energy necessary for muscle contraction. The utilization of reserves of substrate for the supply of the energy depends on the intensity and duration of exercise and the nutritional state and physical conditioning of the subject, so that a more conditioned subject might better utilize fat and less glycogen as an energy source for a certain intensity of exercise when compared with less conditioned subjects (Dennis et al., 1997; Holloszy & Kohrt, 1996; Maughan et al., 2000; McConell et al., 1997).

Glycogen is synthetized by CHO from meals through a process called glycogenesis following the digestion of a meal. Meals ingested from 1 – 4 h before prolonged exercise can increase the availability of CHO to increase hepatic and muscle glycogen storage (Coyle et al., 1985). The largest store of glycogen is in skeletal muscle, but during exercise mainly the glycogen in the active muscle fibres is mostly and firstly utilised as an energy source. Another source of storage of glycogen is in the liver, known as hepatic glycogen (Romijn et al., 1993). According to Maughan et al. (2000) the depletion of hepatic glycogen can limit performance in
two ways, one by causing a faster depletion in muscle glycogen, or two, by causing the
development of hypoglycaemia which might inhibit neurologic function.

During exercise a high increase in the utilization of blood glucose occurs by those
muscles in action. Therefore, some metabolic processes such as glycogenolysis and lipolysis in
the fat tissue have a very important role in regulation of blood glucose concentration (Vranic,
1992) adding to the fact that endurance training can spare plasma glucose and liver glycogen
(Baldwin et al., 1975; Coggan et al., 1990). At the beginning of exercise and as intensity
increases the muscle glycogen plays an important role in the contribution of energy expenditure,
and of plasma glucose increases, but as exercise duration increases, there is a significant
increase in free fatty acids contribution to the production of energy (Romijn et al., 1993).

Endurance training is responsible for a variety of changes and adaptations in the aerobic
energy system so that endurance trained individuals have a more efficient aerobic metabolism
and a better fat metabolising system which allows these individuals to use more free fatty acids
as a source of energy compared to untrained individuals. This is possible because of an
increase in blood flow and activated enzymes used to metabolise fat (McArdle et al., 1998).
Also, according to Hurley et al. (1986), aerobic training enhances the individual's ability to burn
fat and uses it as a source of energy and therefore a corresponding decrease in CHO
breakdown. This ability to spare CHO is believed to be due to a facilitated release of free fatty
acids from adipose tissue deposits and also because of an increase in intramuscular fat in
endurance trained individuals (Hurley et al., 1986). Highly trained endurance athletes have the
ability to spare carbohydrate oxidation that is inhibited by their high efficient fat oxidation
capacity and also when exogenous CHO is ingested (Jansson & Kaijser, 1987; Spriet & Watt,
2003; Jeukendrup et al., 1998). During exercise at a fixed intensity, aerobic metabolism is most
responsible for the energy necessities of the active muscles. Under these conditions there is
very little or no accumulation of lactate in the blood (McArdle et al., 1998; Nassif et al., 2008).
During exercise, the muscle glycogen reserves are rapidly depleted mainly because of the duration and intensity of exercise (Maughan et al., 2000). According to Powers and Howley (2000) and McArdle et al. (1998), the predominant substrate during exercise of low to moderate intensity is fat. Although fat utilization is predominant during prolonged exercise at this intensity, as exercise intensity increases the utilization of glucose and muscle glycogen also increases. Prolonged exercise with a duration that can vary from 1 to 2 hours reaching fatigue can completely deplete muscle glycogen reserves, thus there is an unquestionable contribution of CHO toward the end of exercise (Maughan et al., 2000).

2.4 FIXED INTENSITY AND SELF-PACED EXERCISE

For many years scientific studies have concentrated in evaluating a number of variables in relation to exercise undertaken as fixed intensity. As this type of exercise has contributed to sports science in understanding body responses and the effects of different environmental conditions, a closer model of exercise to the athlete’s reality is necessary for the application of the knowledge being produced by athletes and coaches to improve performance and minimise fatigue.

Traditionally, sub-maximal exercise performance has been assessed by treadmill running or cycling at a fixed power output to exhaustion although these protocols have reported poor reproducibility with a test-retest co-efficient of variation (CV) ranging from 17.4% to 39.5% (McLellan et al. 1995; Jeukendrup et al. 1996). These CV values most likely reflect the untrained status of the subjects or lack of experience with the particular protocol (McLellan et al., 1995). In contrast to constant load trials, protocols that allow subjects to voluntarily select and alter intensities typically demonstrate greater test-retest reliability. For example, Palmer et al. (1996) had 10 subjects perform 3 x 20 km and 40 km time trials using their own bicycle mounted to a
cycle ergometer so that subjects were able to monitor elapsed distance resulting in a CV of ~1.0%. In addition, Marino et al. (2002) have shown the CV to range from ~1.3 – 3.5% in a time trial lasting 60 min where subjects could alter their cadence and gear ratio. Therefore, given the available evidence it would be prudent to evaluate the effects of either CHO ingestion or placebo using a self-paced exercise protocol.

A variety of exercise protocols have been used. In fixed intensity protocols, subjects are usually required to maintain the intensity of exercise using one of the measurements used to monitor exercise intensity such as power and speed. These values are usually determined based on parameters measured during the athlete’s VO$_{2\text{max}}$ test. Also a percentage of VO$_{2\text{max}}$ using gas exchange can be used to control exercise intensity. This control of exercise intensity allows researchers to observe body responses to different hydration strategies and the effects of environmental conditions so that investigators have all the control.

On the other hand, self-paced exercise gives the athlete control over intensity. This is much closer to the athlete’s reality during competition and what makes the use of this type of exercise protocol in research studies easier to transfer and apply knowledge and results found in scientific studies to improve performance in real life situations. During a self-paced laboratory trial, subjects are free to change gears, power, cadence and speed as needed and thus able to adjust to the conditions of exercise as necessary to produce the best performance possible in those conditions.

Even though there are different modes of exercise, duration and intensities, it is more likely that the ingestion of CHO would be of benefit in exercise performance of longer duration. Maughan et al. (2000) suggests exercise intensities that can be sustained for periods of 30 to 180 minutes can produce responses which agree with the central fatigue hypothesis and have enough duration to observe possible changes in the body.

Recently, studies evaluating central fatigue have provided evidence that athletes tend to reduce muscle recruitment during exercise to prevent damage to the organism and in an attempt
to maintain homeostasis. There is also evidence that athletes can reach intensities at the end of exercise similar to those produced at the beginning of exercise (Kay et al., 2001). According to Noakes (2007a), athletes usually anticipate and choose their pace according to the expected duration of the exercise. He argues that the periphery can not solely explain fatigue during these conditions but rather the CNS deals with the requirements of the exercise by integrating many signals which ultimately allows the athlete to pace themselves according to the energy requirements of the exercise bout. Tucker et al. (2006a) have shown that athletes tend to speed up at the end of races longer than a mile even though the lactate levels might be high at the end of exercise; athletes are still capable of increasing intensity at the end of exercise. This gives pacing a very important role in determining the best performance possible and protecting the organism, which is ultimately controlled by the CNS. Noakes (2007a) calls attention to the fact that if at the end of the exercise the body is fatigued and might have high levels of lactate as proposed by the peripheral fatigue model, how are muscles still able to increase intensity at the end of exercise as shown by Kay et al. (2001)? Noakes (2007a) suggests that this increase in intensity can only be accomplished by information sent by the body informing the brain that the distance to be covered is almost over and the brain is able to then give the command to the muscle fibres to actually increase exercise intensity.

According to Weir et al. (2006) the central governor model as proposed by Noakes (2007a) presents the brain as a subconscious regulator of the pacing strategy which subjects choose to change power output with the objective of preserving the organism and any physiological failure (eg. rigor). Weir et al. (2006) considers fatigue as any reduction in force and power during exercise. This view shows how subjects are subconsciously changing pacing strategies from possible messages sent by the brain after evaluating changes in body homeostasis to allow the body to perform the task as well as possible without causing any damage. This model proposes how a self-paced exercise protocol is more meaningful even in a laboratory setting since it is a lot closer to what athletes will face in real life situations.
Even though most studies examining CHO ingestion have used fixed intensity exercise protocols whilst recently a few studies have used self-paced exercise protocols, controversy still exists regarding the effects of CHO in improving performance and delaying fatigue. A number of studies with different exercise protocols have been used to evaluate the effect of CHO ingestion on performance. This has not helped investigators elucidate if CHO ingestion really plays an ergogenic role as the use of different methods, exercise protocols feeding state and controls make it difficult to compare studies. For this, when CHO ingestion is observed during exercise in a thermoneutral environment (Burke et al., 2000; Coyle et al., 1986; Powers et al., 1990, St Clair Gibson et al., 2001b; Wright et al., 1991) or in the heat (Silami-Garcia et al., 2004; Bilzon et al., 2000; Carter et al., 2003) controversies still exist about the possible CHO ergogenic effect. The same can be observed when fixed intensity exercise protocols (Galloway & Maughan, 2000; McConnell et al., 2000) are compared with self-paced protocols (Abbiss et al., 2008; Burke et al., 2000; St Clair Gibson et al., 2001b). The fed state might play a psychological part in the results of these studies, but further investigation is needed to clarify this possibility.

Recently, Theurel and Lepers (2008) evaluated the effect of endurance cycling in a variable exercise protocol compared to fixed intensity exercise in 10 well trained cyclists. Subjects executed 2 x 33 min trials with the same average power output, but one in a constant power output of 70% $\text{VO}_{2\text{max}}$ and the other in a variable power output. Maximal voluntary contraction (MVC) torque and voluntary activation were significantly reduced after exercise in the variable power output trial when compared with the exercise at a constant output. The authors suggested that the results are possibly explained by a higher contribution of anaerobic sources and higher muscle recruitment during the variable power output exercise. This is another indication that self-paced exercise could be a more robust protocol, mimic real life situations for athletes and assist researchers in elucidating all possible fatigue mechanisms.

It is also important to note that heat accumulation is different between fixed intensity and self-paced exercise. During fixed intensity exercise the subjects have no control over intensity as
higher heat accumulation is expected to be reached during prolonged exercise. As for heat accumulation during self-paced exercise, changes in intensity by subjects due to their control over intensity, will alter heat production in a way that a maximal level is never reached so not to compromise performance and with the purpose of maintaining homeostasis and protecting the organism (Tucker et al., 2006b; Marino et al., 2004; Tucker, 2008).

2.5 FATIGUE

The term fatigue is normally used to describe general sensations of tiredness and also a reduction in muscle performance. Generally, the causes of fatigue are believed to be associated with energetic systems (ATP-CP, glycolysis and oxidation), accumulation of metabolic products, nervous system fatigue and failure of the contractile mechanism within the muscle fibres; although these factors are not universally accepted as discrete explanations of fatigue (Wilmore & Costill, 2001).

There are some difficulties in identifying the mechanisms that cause fatigue and this reflects the difficulty to define the word fatigue and exhaustion (Kay & Marino, 2000). According to Noakes (1998) fatigue is the defence mechanism that prevents impairment of the human organism and exhaustion is really what is used to describe the termination of exercise (Kay & Marino, 2000). According to Guyton and Hall (1997) fatigue is reached by the hard and prolonged contraction of the muscle that increases in an almost direct proportion to the speed of depletion of the muscular glycogen. This depletion results simply in an inability of the contractile and metabolic systems of the muscle fibres to maintain the same workload. More recently, Noakes et al. (2005) suggested that fatigue should be seen as a sensation or emotion separated from a physical symptom such as a decrease in exercised muscle force production and if the exercise muscles have any energy remaining when exhaustion is reached, then causes of
fatigue cannot be peripherally controlled but rather related to CNS regulation of the motor unit in the muscles. Gandevia (2001) considers Bigland-Ritchie and Woods’ (1984) definition of fatigue as any activity that causes a decline in muscle force or power production regardless of whether it can be sustained. According to Weir et al. (2006) fatigue is task dependent and that the proposed model by Noakes et al. (2005) is more usefully applied in endurance exercise. For this, Weir et al. (2006) discuss task dependency and suggest factors that will influence fatigue response such as exercise intensity, type of contraction, muscle fibre distribution type, environment among others.

Central fatigue can also be evaluated using submaximal voluntary contractions even though a great number of studies have used maximal voluntary contractions. Taylor and Gandevia (2008) have called attention to this possibility. According to Taylor and Gandevia (2008) the disproportionate increase in perception of effort by individuals when executing a low target force is the best indication of how important central fatigue is during submaximal activities. According to these authors, when one attempts to evaluate fatigue using submaximal exercise, fatigue occurs but no decrease in performance can be observed as other motor units end up being recruited to compensate for fatigued motor units. According to Maluf and Enoka (2005) when subjects perform sustained submaximal voluntary contractions of ~ 15-20% of their maximal force they reach what is termed “task failure” and are not capable of maintaining the target force.

Exhaustion can be related to an increase in body temperature (González-Alonso et al., 1999), an increase in accumulation and depletion of metabolites (MacLaren et al., 1989) and an alteration in the recruitment pattern of muscle fibres (Astrand & Rodahl, 1987). Another critical factor in performance during prolonged exercise in the heat seems to be an increase in body temperature (combination of the average body temperature and core temperature) (Kay & Marino, 2000; Rodrigues & Silami-Garcia, 1998). Gonzáles-Alonso (1999) suggests that the time
to exhaustion is inversely related to the initial body temperature and directly related to the rate of heat accumulation.

Heat exposure can affect many areas of the body and even more so when one is trying to reach maximal prolonged exercise performance. Central and peripheral fatigue can play a significant role in determining how well one will perform and how the nervous system might limit performance. Central fatigue can be seen as a failure in the brain or/and in the message being carried by the spinal cord to the exercised muscles and peripheral fatigue can be seen as a failure in the muscle apparatus, this being in its electrical or chemical responses. At present, it is clear that the CNS does play a role in fatigue and so does the response within the periphery, but it is not clear if CHO prevents the fall in performance, although there is consensus that it does avoid hypoglycaemia and maintain blood glucose levels as close as possible to the fed state levels when ingested during prolonged exercise. Some suggest that maintaining the energy available to the brain and the muscle prevents a failure in the muscle apparatus and also in the CNS command of the task being executed. CHO ingestion would help maintain the body’s capacity to produce the same 100% drive to the muscle contraction and in the muscle might provide enough energy to allow its full contraction capacity, producing as much force as possible. On the other hand, it’s clearly shown in the literature how CHO ingestion does maintain blood glucose levels but it is still not clear how some results show improvement in prolonged exercise performance in the heat and what mechanisms are responsible in these cases (Coyle et al., 1986; Coggan & Coyle, 1987; Febbraio et al., 1996a; Nassis et al., 1998), although other studies have not observed this improvement (Burke et al., 2000; McConell et al., 2000; Nassif et al., 2008; Robinson et al., 2002).

Fatigue is a multivariable complex phenomenon that can be caused by many different factors occurring in the periphery and CNS. A large amount of research has been done in an attempt to explain the causes of fatigue, but a definitive cause or mechanism is still unclear. Recently, most of the studies evaluating the possible mechanisms of fatigue in the heat have not
found enough evidence to support their findings without turning to the possible role of the CNS. Although for many years limitations in endurance exercise have been focused on peripheral factors particularly on reductions in substrate availability when studying the ingestion of CHO as an ergogenic tool. Recently the central fatigue model has been the one closer to explaining reductions in performance and fatigue during prolonged exercise. St Clair Gibson et al. (2001b) suggest that there are neural strategies in place to maintain muscle reserve and reduce exercise in order not to have any permanent damage to muscle and organs. As fatigue can not be completely explained only by peripheral factors, Noakes and St Clair Gibson (2004) suggest that the peripheral model to explain fatigue needs to be replaced since they have shown that fatigue can develop even when not all the muscle fibers of the exercised muscles have been recruited. Vøllestad (1997, p.220) presents a table (Table 1) with different terms and definitions used to define fatigue. Maximal Voluntary Contraction and Maximal Power Output: this thesis agrees with these definitions by the author. The subjects were asked and motivated to produce a maximal amount of force for each maximal voluntary contraction they were required to execute as quickly as possible.
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>Maximal voluntary contraction</td>
<td>The force generated with feedback and force encouragement, when the subject believes it is a maximal effort.</td>
</tr>
<tr>
<td>Maximal evocable force</td>
<td>The force generated by a muscle or group of muscles when additional electrical stimulation does not augment force.</td>
</tr>
<tr>
<td>Maximal Power Output</td>
<td>The power generated with feedback and encouragement when the subject believes it is a maximal effort.</td>
</tr>
<tr>
<td>Muscle Fatigue</td>
<td>Any exercise-induced reduction in the capacity to generate force or power output.</td>
</tr>
<tr>
<td>Central Fatigue</td>
<td>Any exercise-induced reduction in maximal voluntary contraction force which is not accompanied by the same reduction in maximal evocable force.</td>
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Noakes (2000) has outlined models which try to explain exercise fatigue. The models discussed by Noakes (2000) are the cardiovascular/anaerobic model; the energy supply model; the energy depletion model; the muscle recruitment (central fatigue)/muscle power model; the biomechanical model and the psychological/motivation model. Noakes (2000) provides arguments which suggest that most of the classical understandings based on metabolic capacity to explain fatigue do not give sufficient support for the mechanisms of fatigue in many varied situations (Noakes, 2000). In this paper (Noakes, 2000) a possible “central governor” has been proposed which could be used to explain the reduced intensity or termination of exercise before any damage is caused to the organism. According to Noakes (2000), this concept was first suggested by A.V. Hill, A. Bock and D.B. Dill, but has been completely ignored by modern exercise scientists (Noakes, 2000). Kayser (2003) also agrees with this concept that exercise capacity of the muscle is not only determined at the muscle level and that a cerebral command also plays an important role. According to Kayser (2003) the “central governor” not necessarily has an anatomical location, but may be of just functional nature. He suggests that it would receive information from many different systems which are linked with the exercise being executed, gathering and relating this information to each other to result in effects on the cortex to force the individual to stop exercising when all the information received by the governor goes far beyond a determined threshold. Kayser (2003) also suggests that when exercising at a high intensity where there is also high regional neural activity, a higher demand of energy compared with what is actually being supplied may cause an imbalance in some regions of the brain which are activated during exhaustive exercise. For this, he believes is what might differ between a winner and a loser and not just differences in VO$_{2\text{max}}$ but also how big a safety margin the CNS has to keep the organism out of danger. Kayser (2003) concludes that exercise starts and ends in the brain suggesting that there has been an increase in the amount of experimental proof that the CNS is perhaps the real limiting aspect in exercise performance. He also suggests that the
level of performance that can be produced for a given time is determined by the cardio-
respiratory and metabolic capacity of an individual.

Heat exposure can affect many areas of the body and even more so when one is trying
to reach maximal prolonged exercise performance. Central and peripheral fatigue can play a
significant role in determining how well one will perform and how the nervous system might limit
performance. Central fatigue can be seen as a failure in the brain or/and in the message being
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CHO are ingested during prolonged exercise in the heat (Burke et al., 2000; McConell et al.,
2000; Nassif et al., 2008; Robinson et al., 2002)

Weir et al. (2006) state that central and peripheral mechanisms are in action
simultaneously to limit force and power and that fatigue changes depending on the task being
executed. These authors suggest that more research needs to be conducted to identify what
factors in different tasks are responsible for different levels of central and peripheral factors contributing to fatigue.

Booth et al. (1997) evaluated Ca$^{2+}$ uptake and its association with a possible prolongation of half relaxation time after prolonged exercise on the contractile function and homogenate sarcoplasmic reticulum Ca$^{2+}$ uptake and Ca$^{2+}$ - adenosinetriphosphatase activity. After 10 untrained men exercised to fatigue at ~75% peak oxygen consumption, these authors observed a reduction in Ca$^{2+}$ uptake but did not observe changes in the contractile function of the quadriceps muscle.

Lepers et al. (2000) tested eight well trained subjects that exercised at 65% of their maximal aerobic power and studied its effect on vastus lateralis and vastus medialis electromyography (EMG) activity, maximal concentric, eccentric and isometric contractions, muscle compound action potential (M-waves) and isometric muscle twitch of the quadriceps muscle pre and post-exercise. Parallel decreases in peak torque in all types of contraction were observed. Increase in M-wave duration in both vastus lateralis and vastus medialis after exercise was observed but M-wave amplitude decreased. Decreases in maximal twitch tension, total area of mechanical response and maximal rate of twitch tension development post-exercise were observed. These authors suggest that a reduction in muscle activity after prolonged exercise is due to a decrease in neural input and failure of contractile peripheral mechanisms of the exercised muscles.

Also, Lepers et al. (2002) studied the effect of prolonged exercise in neuromuscular variables in nine trained subjects during 5 h exercise at 55% VO$_{2\text{max}}$. Reduction of 18% in MVC torque of the quadriceps muscle post-exercise was observed. Significant reductions in peak twitch torque, contraction time and total area of mechanical response were observed in the first hour, although M-wave changes were only observed after 4 h of exercise. A decrease in muscle activation of 8% was also observed at the end of exercise. These authors suggest that changes
in the muscle contractile properties are apparent after the first hour of exercise but that the central drive is more affected in the latter stages of exercise lasting 5 h.

For ethical reasons measurements of central mechanisms in humans are very difficult to undertake but that does not stop researchers studying the role of the central nervous system in performance capacity. Methods such as the interpolated twitch technique, nutritional strategies and pharmacological interventions are used.

The interpolated twitch technique is a technique developed to compare the voluntary muscle force of an individual with the force produced by this same individual when submitted to a supramaximal electrical stimulation (Saboisky et al., 2003; Martin et al., 2004; Morrison et al., 2004; Nybo & Nielsen, 2001a). According to Weir et al. (2006) the interpolated technique shows increases in force higher than the voluntary level and indicates that not all motor units used for that particular maximal voluntary contraction were recruited.

Within the nutritional strategies that affect the availability of tryptophan to the brain, the most used strategy is the ingestion of branched chain amino acids, or CHO, or a combination of these substances prior or during exercise (Davis et al., 1992; Mittleman et al., 1998; Watson et al., 2004)

Drugs and medications that change the availability of monoamines in the neurons synaptic cleft of the central nervous system are used as pharmacological interventions (Pannier et al., 1995; Strachan et al., 2004 and Watson et al., 2005).

For the purpose of this thesis, this review will present only the twitch interpolated technique and the nutritional strategy of CHO ingestion, as these are the typical and popular interventions used to study fatigue.
2.6 METHODS OF MEASURING NEUROMUSCULAR MUSCLE FATIGUE

Vøllestad (1997) discusses a variety of measures to evaluate human muscle fatigue. Some of these methods are maximal voluntary force, power output, tetanic force, twitch interpolation, endurance time and EMG. After reviewing each different method, the maximal voluntary contraction force or power output was defined as a “gold standard”, since it is a direct measure that can be used with very low variability if used to evaluate knee-extensors. The other methods should be used as additional information and EMG should be used carefully as its validity is questionable under some conditions.

2.6.1 Muscle voluntary contraction

Voluntary muscle contraction has been used to access the capacity of the individual to activate muscle fibers and produce force. This measurement is achieved by using a dynamometer or a force transducer attached to a customized chair. Muscle contractions can be either eccentric, concentric or isometric, considering that subjects are capable of producing more force and activate more muscle fibers when maximal isometric contractions are used (Babault et al., 2001). When using maximal voluntary contraction to evaluate force generation one should consider that this measurement can be influenced by motivation (Secher, 1987; Rube & Secher, 1981). Muscle force production is useful in evaluating the effects of prolonged exercise on the capacity of recruiting muscle fibers and how the CNS performs with the exposure of exercise in the heat.
2.6.2 Electrical stimulation technique and voluntary activation

The electrical stimulation technique, commonly known as twitch interpolation, uses the delivery of one or more external supramaximal stimulus (electrical) on the nerve or belly of the muscle being studied during a maximal voluntary contraction (Babault et al., 2001, Folland & Williams, 2007, Davis & Bailey, 1997). The twitch interpolation technique has been used to verify an individual's capacity to activate skeletal muscle and is used to differentiate if the nature of fatigue after exhausting exercise is central or peripheral in origin (Paillard et al., 2005). According to Folland and Williams (2007), as the maximal voluntary force can be influenced by psychological factors (e.g. attention, motivation), the interpolated twitch technique should be used to measure the true capacity of the muscle to activate all its muscle fibres, and would be more reliable and reduce the effect of possible psychological factors. But it has been shown that encouraged healthy subjects can produce a muscle activation of less than 100% during a maximal isometric contraction, indicating that individual motor unit firing frequency was below maximal capacity and that the central nervous system was not capable of activating all motor units (Gandevia, 2001). Folland and Williams (2007) suggest that when applying two superimposed twitches during each MVC, the first twitch applied produces more reliable values for the true force produced. Nørregaard et al. (1997) confirmed the reliability of this technique considering it a reliable method to be used in research. Muscle contractions can be eccentric, concentric or isometric, considering that subjects are capable of producing higher levels of activation when maximal isometric contractions are used (Babault et al., 2001). Also, Hales and Gandevia (1988) point out that when using the interpolated twitch technique some important factors should be considered. These are the selection of the muscle and site to be stimulated, immobilization of the muscle and how stable the stimulus conditions are.

The determination of voluntary activation is done by the relation between the torque generated by the muscle when stimulated during rest and during a maximal voluntary
contraction with a superimposed electrical stimulus (Merton, 1954). Recently, interpolated twitches with more sensitive devices have shown that even healthier individuals might not possibly activate all muscle fibers even during a maximal voluntary contraction attempt (Shield & Zhou, 2004).

However, according to Shield and Zhou (2004), the use of the interpolated twitch technique in a voluntary contraction was proposed initially by Denny-Brown (1928). Merton (1954) also showed this technique that in healthy individuals was capable of activating the majority of the muscle, but more recent and sensitive techniques have shown that total activation does not occur very often and in a lot less muscle as was previously thought (Shield & Zhou, 2004).

According to Paillard et al. (2005), even though the study of Rutherford et al. (1986) has shown that the twitch interpolation technique and the percutaneous superimposed electrical stimulation technique are accurate ways of measuring muscle activation, it has been observed that this technique might fail in fully activating certain muscles in healthy subjects when the torque recorded is not higher than the torque generated during the voluntary contraction. Even so, one should consider that even though the superimposed technique is a valid method to be used, the accuracy of the measurements might be influenced by many parameters such as the nature of the current, the muscle being tested, the muscle action and the subject characteristics. Kooistra et al. (2007) reported that when an individual succeeds in improving their MVC in a later attempt during a same session one can observe a good increase in torque than in the level of voluntary activation reached. As a result, Kooistra et al. (2007) recently examined the relationship between the calculated voluntary activation and the voluntary torque produced by knee extensors during high intensity contractions. They reported that subjects presented consistently higher levels of maximal voluntary contraction than the maximal voluntary level of 90% that is usually observed in healthy subjects when the interpolated twitch technique is used. Therefore, these authors concluded that if voluntary activation in healthy subjects is ~90%, this
measurement is probably an overestimation of their ability to contract their quadriceps muscles maximally. This technique evaluates what occurs within the central command after exercise, showing if there is failure in the process between the message sent by the CNS and the action being executed as the muscle contracts. Calculations of voluntary activation using the difference between the maximal voluntary force and the maximal voluntary force of the same muscle with a superimposed electrical stimulus as one of the methods to evaluate the possible reduction in central activation (Nybo & Nielsen, 2001a).

Recently, an increase in the number of studies about the role of the central nervous system in the development of fatigue has increased and the interpolated twitch technique has been extensively reported. Nybo and Nielsen (2001a) showed a reduction in performance and in the percentage of central activation when subjects exercised under hot conditions. Other studies such as those of Tucker et al. (2004), Saboisky et al. (2003) and St Clair Gibson et al., (2001b) describe a reduction in muscle recruitment, reduction in muscle activity and production of force and central activation reduction.

### 2.6.3 Resting Twitch Contraction

Resting twitch contraction (Rt) is the measurement of the isometric force profile generated by the muscle when a single electrical stimulus is applied (Fitts, 2003). One of the characteristics observed in a Rt is the peak twitch force produced, which is the highest isometric force produced after a supramaximal stimulus. The Rt is a measurement that allows observation of how the musculotendinous unit responds. This evaluates how much and how fast Ca⁺ is released and sequestered in the muscle being evaluated. This provides the investigator with information with respect to the capacity of the muscle to produce force and it is very dependent
on the number of cross-bridges that can be formed during the muscle coupling process (Fitts, 2003).

2.6.4 Compound muscle action potentials

Compound muscle action potentials are commonly known as M-waves and used to assess the quality and amount of neuromuscular propagation (Saboisky et al., 2003). According to Weir et al. (2006) M waves provide information about the stability of the neuromuscular propagation following electrical shocks to the peripheral motor nerve. The measurement of M-wave amplitude gives investigators information about the amount of motor units that are actually activated at the neuromuscular junction during exercise whilst the M-wave duration gives information in relation to the time taken for the action potential to be generated and to propagate to the motor unit in the synaptic cleft in the presence of the neurotransmitter Acetylcholine (Ach). In relation to the technique, Weir et al. (2006) suggest that surface EMG amplitude is currently the best available technique. These authors suggest that values of surface EMG amplitude are normalized using either the peak to peak amplitude or the M-wave area.

2.6.5 Electromyography

Since the studies of Piper (1912) and Cobb and Forbes (1923) and more recently by Dimitrova and Dimitrov (2003), a number of studies have used the surface EMG technique to evaluate signs and causes of fatigue. Weir et al. (2006) define surface EMG as a sum of all propagated action potentials in the area where the recording is being done. Surface EMG has been used to assess muscle function by evaluating the electrical signal of the muscle during contraction using electrodes attached to the skin (Cram & kasman, 1998; Farina et al., 2004). This electrical signal comes from the action potential generated in the muscle by the motor unit
and as a high number of motor units are activated during a voluntary contraction, the signal which is captured as the EMG signal is a sum of all action potentials generated by that contraction (Kamen & Caldwell, 1996). EMG can provide information in relation to the activation of the motor units during contraction and especially, the amount of motor unit activity (Gandevia, 2001) in addition to demonstrating both central and peripheral components of the neuromuscular system (Farina et al., 2004).

In the study by St Clair Gibson et al. (2001b) a reduction in EMG was observed during exercise. Although causes of fatigue during exercise in the heat are not clear, recent studies have shown a reduction in EMG activity of exercised muscles in the heat (Tucker et al 2004). Others have also shown a reduction in the EMG activity during cycling exercise in the heat from the beginning to the final stages after which the EMG activity was reported reaching values close to pre-exercise (Kay et al., 2001). However, these authors used self-paced exercise rather than fixed intensity exercise. In addition, these same authors have reported a reduction in central activation of exercised muscles in the heat using the interpolated twitch technique (Saboisky et al, 2003).

Weir et al. (2006) point out that even though a number of studies such as St Clair Gibson et al. (2001b) and Kay et al. (2001) have measured EMG during a cycling protocol, one should interpret the results with caution. For this, they suggest the following actions should be taken into account when interpreting these results: “(a) surface EMG amplitude cancellation; (b) M-wave normalization; (c) motor unit firing rate; (d) fatigue related reflex inhibition; (e) spectral compression; (f) EMG amplitude dependence on muscle action velocity.” Weir et al. (2006) do not suggest that EMG signals are not useful information to be used but attention to the limitations in the methods should be carefully considered.

Farina et al. (2004) point out how important the location of the electrode is since some locations are better than others. For this reason, these authors suggest that all studies which evaluate EMG should always describe the placement location of the electrodes.
2.6.6 Quantification of EMG

The amplitude of the EMG signal is dependent on the number of motor unit action potentials recorded and is qualitatively related to muscle torque. EMG is typically quantified as either the root mean square (RMS) or integrated EMG. For RMS EMG, the signal is quantified by squaring all data points, summing the squares and dividing by the number of data points, and then calculating the square root (Cram & Kasman, 1998). For integrated EMG, the signal is quantified by calculating the area under the EMG-time curve by rectifying the signal and summing all data points over time (Cram & Kasman, 1998). Changes in the amount of muscle recruitment could clarify and help understand fatigue mechanisms and possible changes in pacing strategies during prolonged cycling in the heat (Kay et al., 2001).

2.6.7 Spectral Compression

Spectral compression (SC) is used to measure changes in the muscle fiber conduction velocity and can be a method used to evaluate muscle fatigue (Lowery et al., 2000). According to Farina et al. (2004) studying muscle fatigue to identify the type of motor units being used and describe the pattern of the activity of the motor unit, the relationship between the average muscle fibre conduction velocity and power spectral frequencies is be used. Farina et al. (2004) also state that analysis of the EMG spectrum gives information about what type of fibers are being recruited by the motor unit during a determined activity. For this, SC can be a non invasive way to measure shifts of recruitment of slow or fast twitch muscle fibers. Considering the type of fibers and changes in fiber recruitment during exercise could be important and indicative of how the CNS is controlling the intensity of exercise and commanding the individuals to perhaps reduce intensity when the organism is close to fatigue aiming to maintain homeostasis.
2.7 EXERCISE IN THE HEAT, PERFORMANCE AND CENTRAL FATIGUE

2.7.1 Exercise in the Heat

For the maintenance of thermal homeostasis, the organism has different avenues of heat gain and loss such as, radiation, convection and conduction, which essentially depend on a gradient of temperature between the skin and the environment. Evaporation of sweat is dependent upon water vapour pressure gradients which are related to the relative humidity, workload and metabolism (Powers & Howley, 2000; Silami-Garcia et al., 1999; Stitt, 1993).

The major avenue for producing endogenous heat is via increased metabolism which can be over 20 times resting values during intense physical activity. Physical exercise increases metabolic rate in the process of supplying energy for muscle contraction. Depending on the mode and kind of exercise, between 70 and 90% of the metabolic heat is released so that body temperature does not rise above dangerous levels (Sawka, 1992; Sawka et al., 1996). The mechanical efficiency during activities such as running and cycling is approximately 20%; around 80% of the total energy spent is released as heat. If a person was unable to lose heat to the environment, the body temperature could increase by 8°C with fatal consequences within 35 minutes; therefore, heat dissipation is critical during exercise in humans (McArdle et al., 1998; Nielsen, 1996).

With the increase in environmental temperature the contribution of conduction and convection in heat dissipation processes decrease drastically and radiation is reduced to negligible levels, although evaporation of sweat prevails as the principal mechanism of dissipation of body heat (NATA, 2000; Silami-Garcia, 1997; Silami-Garcia et al., 1999). The evaporation of sweat is an efficient way of cooling the body in hot conditions, although as humidity rises above 60%, it becomes harder for sweat to evaporate so that it drips from the
body without evaporating and adding to overall water losses (Basset Jr. et al., 1987; Nielsen, 1996; Silami-Garcia, 1997; Silami-Garcia et al., 1999; Maughan, 1992).

Minimizing body water loss and consequently cardiac overload is critical in minimizing the possibility of hyperthermia and fatigue caused by dehydration (Armstrong et al., 1997; Guimarães & Silami-Garcia, 1993; NATA, 2000; Sawka, 1992). Soares (1993) verified a decrease in exercise tolerance in subjects with core temperature elevated by immersion in hot water when compared to the control group, which resulted in an average decrease of 27.9% in exercise time which was also associated with higher ratings of perceived exertion. Secretion of sweat also represents the loss of vital fluids, which if not replaced properly with the ingestion of fluids can lead to dehydration (Shi & Gisolfi, 1998). Factors such as intensity of exercise, level of physical fitness, acclimation level, environmental conditions (dry temperature, humidity and air velocity), type of clothing used and hydration state can influence the sweat rate (Burke & Hawley, 1997; NATA, 2000).

According to Armstrong et al., (1997) and Sawka et al. (1992), the maximal rectal temperature that can be tolerated is about 39.5ºC. However, temperatures as high as 40-41ºC have been recorded in some instances (Pugh et al., 2002; Gonzáles-Alonso et al., 1999b; Nybo & Nielsen, 2001a). Moseley and Gisolfi (1993) in a review have concluded that an excessive rise in core temperature by heat exposure can lead to circulatory shock and multiple organ failure.

Gonzáles-Alonso et al. (1999) have shown that hyperthermia is more responsible for inducing fatigue than dehydration during prolonged exercise in the heat. They also showed that higher internal body temperatures are associated with fatigue in trained cyclists during prolonged exercise in the heat, suggesting that time to exhaustion is inversely proportional to the initial temperature of the subject which is directly related to the rate of heat storage. Galloway and Maughan (1997) have shown that ambient temperature has a direct effect on prolonged exercise in man. They found an inverse-U relationship between performance and ambient temperature. In
this study, eight subjects cycled in four different environments which resulted in reduced duration of prolonged exercise as environmental temperature increased up to 30.5 ± 0.2 °C.

### 2.7.2 Exercise in the Heat and Central Fatigue

Recently, a number of studies about the role of the CNS in the development of fatigue have been reported. Nybo and Nielsen (2001a) after evaluating 14 males that exercised at 60% VO\(_{2\text{max}}\) at two different environments (40 vs 18 °C), observed a reduction in performance and in the central activation percentage, amount of muscle activated by the CNS following exercise in the heat. Other studies by Tucker et al. (2004), Saboisky et al. (2003) and St Clair Gibson et al. (2001b) report reductions in muscle recruitment, muscle activity, force generation and reductions in central activation.

Tucker et al. (2004) evaluated skeletal muscle recruitment during dynamic exercise in hot compared with a cool environment. Ten male subjects performed 2 x 20 km time trials, one in the hot and the other in the cool environment. A reduced integrated electromyography (iEMG) was observed early in the hot environment, when core temperature, heart rate and RPE were still similar between trials. They also observed a reduced iEMG from 10 km to 20km, where a reduction in power was also observed in the heat compared with the cool conditions. They attribute this result to an anticipatory response of the brain, causing a reduction in muscle recruitment and thus a reduction in heat production in order to maintain homeostasis.

Martin et al. (2004) studied the effect of exercise in the heat in 13 subjects that cycled at 60% maximal oxygen consumption until voluntary exhaustion. Voluntary activation of the leg extensors and forearm flexors was assessed during shortening and lengthening contractions before and after exercise. A significant reduction in voluntary activation for both leg extensors and forearm flexors was observed at the end of the endurance contractions after the prolonged
exercise. According to Martin et al. (2004) there is a reduction in CNS voluntary drive to the active skeletal muscle that is performing shortening and lengthening contractions after exercise in the heat. This reduction was observed only after a series of dynamic movements which suggests that the CNS permits brief ‘re-activation’ of the skeletal muscle in the heat.

In the study by St Clair Gibson et al. (2001b) a reduction in muscle recruitment (iEMG) was observed during exercise. Seven endurance trained cyclists completed 2 x 100 km time trials with CHO dietary manipulation; one rich in CHO whilst the other not rich in CHO combined with the ingestion of a 7% CHO beverage or a corresponding PLA. No differences in force output, power output or iEMG between CHO and PLA treatments were observed. They observed a decrease in power output followed by a reduction in neuromuscular activity during the bouts of high-intensity exercise. Also, others have shown a reduction in the EMG activity during exercise in the heat at the start of cycling exercise up to the final stages, where the EMG activity was restored to values close to those measured at the beginning of exercise (Kay et al., 2001). That is, Kay et al. (2001) evaluated neuromuscular changes during self-paced exercise in the heat. Eleven subjects cycled for 60 min punctuated by six 1-min sprints at 10 min intervals. These authors observed a reduction in the iEMG activity during cycling exercise in the heat from the beginning until the final stages; where the iEMG increased to values close to initial exercise values.

Saboisky et al. (2003) have also reported reduced central activation in muscles exercised in the heat. Thirteen subjects were tested for central activation of the leg extensors and the elbow flexor muscles before and after exhaustive exercise in the heat to determine if exercise-induced hyperthermia affects the CNS drive to exercised (leg extensors) and non-exercised (elbow flexors) muscle groups. Subjects exercised in an environmental condition of 39.3 ± 0.8°C and 60.0 ± 0.8% rh for ten minute periods at 50%, 40%, 60%, 50% of PPO and then at 75% of PPO until exhaustion. Before and after each exercise trial, subjects performed an MVC with the selected group of exercised and non-exercised muscles and superimposed electrical stimulation
was also used to assess voluntary activation. After finding a reduced central activation ratio in the exercised muscles compared to the non-exercised muscles, these authors suggested that the CNS selectively reduced central activation to specific muscles as a result of exercising while exposed to the heat.

Morrison et al. (2004) after evaluating the effects of passive hyperthermia on MVC and voluntary activation (VA) demonstrated that a high core temperature is the primary factor contributing to central fatigue during isometric contractions. These authors also suggest that skin temperatures, cardiovascular and psychophysical strain do not seem to be a major modulator of hyperthermia-induced fatigue. Cheung (2007) has noted that in the past decade, an emerging hypothesis seems to suggest that there is an effect of heat exposure on neuromuscular activation. Although for many years it has been thought that one of the explanations of fatigue was the reaching of a critical core temperature, along with other metabolic explanations, some have shown an important effect of the CNS in fatigue (St Clair Gibson et al., 2001b; Saboisky et al., 2003) and also note an anticipatory mechanism in which the subject selects a certain intensity to adapt to the changes in body heat storage and avoid a possible cellular catastrophe (Marino, 2004).

As more literature has shown there is a relation between CNS and the capacity to exercise, this study elucidates the possible effect of CHO on performance and on peripheral and central fatigue. We examined how tasting or not tasting the beverage affects the subjects’ capacity to perform and if CHO ingested in different forms would actually change the response to CHO ingestion. It is also important to point out that this study was undertaken when subjects were fed and using a self-paced protocol, which is a protocol closer to competition reality and thus add important information about exercise physiology under these situations.
2.8 CHO INGESTION AND CENTRAL FATIGUE

Since the appearance of sports drinks in the early 1970’s there has been an increasing interest in studying their effect on exercise performance in humans and animal models (Fernstrom & Fernstrom, 1993; Pitsiladis et al., 2002; Huffman et al., 2004). Much research has been conducted on the effect of CHO ingestion and possible nutritional interventions to prevent the early onset of fatigue and enhance human performance during exercise. It is clear how the ingestion of CHO during exercise will increase blood glucose concentration but not necessarily improve performance. About 20 years ago, Newsholme et al. (1987) first suggested that the increase in brain serotonergic activity caused by prolonged exercise may possibly increase lethargy, alter the sensation of effort, change the tolerance to discomfort and also reduce drive and motivation, which could impair physical and mental performance. According to this theory, one of the mechanisms to support the central fatigue hypothesis is the importance of the plasma concentration ratio of free tryptophan to branched-chain amino acids on serotonin synthesis (Newsholme et al., 1987). This suggestion opened doors for possible nutritional interventions with the manipulation of neurotransmitter precursors with the objective of delaying onset of fatigue and possibly improving performance (Meeusen et al., 2006). Although this aspect is beyond the scope of this thesis it is something that researchers should have in mind when studying the possible CHO ergogenic effect, as it could also be more evidence of the effect of CHO on the CNS.

The foundation of the “Central fatigue hypothesis” came from previous studies that linked the diet being consumed and the availability of substrates to the brain (Fernstrom & Wurtmain, 1971). Serotonin has been one of the main neurotransmitters studied during prolonged exercise. An increase in the synthesis of serotonin, most studied neurotransmitter, during prolonged exercise has been associated with reduction in central command of the exercised muscles, fatigue, lethargy and sleepiness (Young, 1991). Newsholme et al. (1992) suggest that higher
tryptophan levels would be necessary to increase the serotonergic activity of the central nervous system. Therefore, this increase in tryptophan levels (precursor of serotonin) would increase the serotonergic activity in the central nervous system, and cause less lethargy and fatigue.

Blomstrand et al. (2005) also evaluated the effects of CHO ingestion on brain amino acids during prolonged cycling, which was calculated by the difference between the arterial-jugular venous concentrations multiplied by plasma flow. Subjects who fasted for 12 h, exercised at 60% VO₂max in two experimental conditions for 3 h with ingestion of a 6% CHO solution or placebo every 15 min. Blood analysis showed higher glucose levels and lower free fatty acids with CHO ingestion when compared with the corresponding PLA. The authors also observed an increase in free tryptophan concentration of 66% when PLA was ingested, which was higher than the values observed at the end of exercise when CHO were ingested. These authors suggest that CHO ingestion attenuated the increase in free tryptophan.

The effect of branched chained amino acids (BCAA) and tryptophan ingestion was also studied by Van Hall et al. (1995) during prolonged exercise to exhaustion. Subjects exercised at 70-75 % of peak power in four trials ingesting a 6% CHO solution, a combination of a 6% CHO solution and tryptophan (3g/l), a solution with low BCAA concentration (6g/l) and one with high BCAA concentration (18g/l). Even though there was a higher availability of free tryptophan with the ingestion of tryptophan and BCAA was higher when BCAA was ingested the time to exercise to exhaustion was not different between treatments (122 ± 3 min). It is important to note the fact that a PLA treatment was not performed and that the CHO treatment was used as a control.

Davis et al. (1992) suggest that the ingestion of CHO reduces lipolysis and lowers the concentration of free fatty acids in the circulation. Davis et al. (1992) believes that its ingestion will be a way of reducing the cerebral tryptophan uptake resulting in better performance with CHO, by attenuating the increase in plasma free fatty acids and free tryptophan. It is important to call attention to the fact that Davis et al. (1992) did not use a PLA when testing CHO, but rather
water was used. This can affect the performance of subjects as the PLA effect was not completely taken into consideration.

Meeusen et al. (2006) also suggest that it is clear how the ingestion of CHO affects the CNS as a number of studies have shown how important an increase or maintenance in substrate delivery to the brain might be, since hypoglycaemia can affect brain function and cognitive performance. Accordingly, Meeusen et al. (2006) suggest that many brain factors that influence signal transduction such as chemicals for brain cell communication could limit performance such that the complex nature of brain neurochemistry that has not been investigated in detail makes it difficult to define central fatigue. At present, there are some studies which link CHO availability to central nervous system function. However, these data are sparse and it is far from conclusive that CHO plays a role in CNS fatigue during prolonged exercise.

Recently, Green et al. (2007) showed that glucose ingestion increases Na\(^+\)-K\(^+\)-ATPase activity when subjects exercised at ~57% VO\(_{2}\)peak until fatigue. These authors suggest that exercise with repetitive contractions as in cycling can strain the excitability capacity of the muscle membrane and also affect the capacity of the muscle to maintain Na\(^+\) and K\(^+\) homeostasis during prolonged exercise. It is thought that this increase might be due to hormonal changes related to the ingestion of CHO and that it could be favourable to protect the membrane excitability depending on the demand of the activity. Also, Stewart et al. (2007) studied the effect of CHO ingestion on membrane excitability and isometric properties of the quadriceps muscle pre-exercise, after 90 min and post-exercise during cycling at 60% VO\(_{2}\)peak. The duration of exercise was longer with CHO ingestion and the reduction in MVC was less pronounced, although changes in muscle activation were not observed between conditions. An improved muscle function and possible protection of the muscle’s membrane excitability as a result of CHO ingestion is suggested by these authors (Stewart et al., 2007).

Fritzsche et al. (2000) studied exercise in the heat and concluded that during prolonged moderate-intensity exercise in a warm environment, ingestion of water attenuates the decline in
neuromuscular power. Fritzsche et al. (2000) also concluded that ingestion of water with CHO attenuates the decline in maximal power more so than water alone, and ingestion of CHO alone does not attenuate the decline in neuromuscular power compared with a PLA.

Nybo (2003) tested if prolonged exercise with or without glucose supplementation affected CNS activation. Eight endurance-trained males executed 2-min of sustained maximal knee extension in a baseline condition and also after two trials of 3 h cycling. Nybo (2003) concluded that exercise-induced hypoglycaemia attenuates CNS activation during a sustained maximal muscle contraction, whereas central activation appears to be unaffected by 3 h of moderately intense exercise in endurance trained athletes when blood glucose is maintained with the ingestion of CHO. Winnick et al. (2005) studied the effect of ingestion of CHO on CNS function during intermittent high intensity exercise similar to team sports. Twenty subjects (ten men and ten women) with experience in team sports completed two trials with the ingestion of a 6% CHO beverage and another with a corresponding PLA after familiarization. CNS function was tested using the Stroop Color and Word Test. Force sensation was also assessed by measuring force output over 3 min which subjects were instructed to maintain the sensation with an initial force output equal to 40% MVC. A motor skills (MS-test) was also assessed. No differences were observed in MVC and in the MS-test but all other tests showed a positive effect of CHO ingestion at the final stages of exercise. Winnick et al. (2005) concluded that CHO feedings during intermittent high-intensity exercise similar to that of team sports benefited both peripheral and CNS function late in exercise compared with a flavoured PLA.

Also Abbiss et al. (2008) evaluated the effect of CHO ingestion and ambient temperature on muscle fatigue in endurance cyclists. In this study, 10 male cyclists performed 4 x 90 min constant pace cycling trials at 80% of secondary ventilatory threshold, followed by a 16.1 km trial with CHO or PLA gels ingestion. Muscle activation using superimposed electrical stimulation was measured before and after exercise and iEMG of vastus lateralis was measured during exercise. No differences were observed in percent muscle activation although improvement in
the 16.1 km trial was observed in the hot condition but not in the temperate condition. These authors concluded that the increase in heat stress and CHO ingestion may interfere in the athletes’ pacing strategy and that further research is needed to evaluate if the athletes’ capacity of judging proper pacing strategies is affected by the environment or CHO ingestion.

Recently published, Jeukendrup et al. (2008) evaluated the effect of CHO ingestion on a 16 km time trial protocol. Subjects were asked to cycle for 25 min at 85% of their maximal power output as fast as possible. For this study the authors clarify that the exercise was called a 16 km time trial as the amount of work covered matched the subjects’ personal best in a 16 km time trial. These authors did not observe differences in performance when CHO was ingested. It should be noted that the duration of the time trial was less than 30 min, which may not have been enough time for the body to actually need to utilize the CHO ingested as the ACSM (2007) recommend CHO ingestion for physical activity longer than 1 h duration, although these guidelines have been questioned by Noakes (2007b). The duration of the study by Jeukendrup et al. (2008) might not have been enough to observe changes in physiological measurements that could influence exercise performance. Blood glucose level were also not measured in this study and subjects exercised after a 3 h fasting.

Generally, these studies show that the relationship between CNS fatigue and CHO ingestion is a relatively recent area of study. Clearly, understanding mechanisms which might alter CNS fatigue with CHO ingestion would be advantageous in athletic performance and can possibly elucidate if there is an ergogenic effect when CHO are ingested.
2.9 CHO INGESTION, PLACEBO EFFECT AND PERFORMANCE IN THE HEAT

A summary of the findings of key studies which have examined CHO supplementation during exercise is displayed in Table 2. A more detailed discussion of these studies follows.
Table 2. Summary of major studies examining the effect of CHO ingestion during exercise.

<table>
<thead>
<tr>
<th>Study</th>
<th>Environment Conditions</th>
<th>Nutritional State</th>
<th>Exercise Protocol</th>
<th>Ingestion Method / CHO content</th>
<th>Glucose Response</th>
<th>Outcome / conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbis et al. 2008</td>
<td>Thermoneutral / Heat</td>
<td>Fed</td>
<td>16.1 km time trial</td>
<td>25% CHO solution and gel</td>
<td>▲ CHO</td>
<td>Improved performance in time trial in the heat, but no differences in muscle activation between PLA and CHO.</td>
</tr>
<tr>
<td>Burke et al. 2000</td>
<td>Thermoneutral</td>
<td>Fed</td>
<td>100 km time trial</td>
<td>7% CHO solution</td>
<td>Not measured</td>
<td>No differences in time to complete trial and power output.</td>
</tr>
<tr>
<td>Carter et al. 2004a</td>
<td>Thermoneutral</td>
<td>4 hours fasting</td>
<td>Set amount of work as quickly as possible</td>
<td>6.4% CHO solution mouth rinse</td>
<td>Not measured</td>
<td>Performance time improved with CHO, Power output higher in the first 3 quarters on the CHO compared with PLA. 5 subjects could not distinguish solutions but 4 guessed correctly which had CHO, of these 4, 3 performed faster with CHO and 1 slower.</td>
</tr>
<tr>
<td>Carter et al. 2004b</td>
<td>Thermoneutral</td>
<td>12 hrs fasting</td>
<td>Set amount of work as quickly as possible</td>
<td>Infusion of 20% CHO in saline and 0.9% saline (PLA)</td>
<td>▲ CHO</td>
<td>No differences in time to complete work or power output.</td>
</tr>
<tr>
<td>Clark et al. 2000</td>
<td>Not mentioned</td>
<td>Fed</td>
<td>40 km time trial</td>
<td>7.6% CHO solution</td>
<td>Not measured</td>
<td>Placebo effect observed, improved performance when told to be ingesting CHO, but CHO were not being ingested.</td>
</tr>
<tr>
<td>Davis et al. 1988</td>
<td>Heat</td>
<td>Fasting</td>
<td>2hrs cycling at 75% VO₂max – 20 min interval -30 min at 75% VO₂max (task 1:10800 and task 2: 2700 pedal revolutions respectively as quick as possible)</td>
<td>6% and 2.5% CHO solution</td>
<td>▲ CHO</td>
<td>Better performance with 2.5% CHO on task II compared with PLA</td>
</tr>
<tr>
<td>Study</td>
<td>Environment Conditions</td>
<td>Nutritional State</td>
<td>Exercise Protocol</td>
<td>Ingestion Method / CHO content</td>
<td>Glucose Response</td>
<td>Outcome / conclusion</td>
</tr>
<tr>
<td>-----------------------</td>
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</tr>
<tr>
<td>Davis <em>et al.</em> 1992</td>
<td>Not mentioned</td>
<td>Fasting</td>
<td>Cycling to fatigue or for up to 255 mins at a constant workload of VO\textsubscript{2} on lactate threshold</td>
<td>6% and 12% CHO solution</td>
<td>▲ CHO</td>
<td>Longer time cycled on CHO trials</td>
</tr>
<tr>
<td>El-Sayed <em>et al.</em> 1996</td>
<td>Thermoneutral</td>
<td>Fasting</td>
<td>1 hour time trial</td>
<td>8% CHO solution</td>
<td>No differences in glucose</td>
<td>Greater distance cycled on CHO trial</td>
</tr>
<tr>
<td>Frütsche <em>et al.</em> 2000</td>
<td>Heat</td>
<td>Fasting</td>
<td>122 min exercise bout</td>
<td>6% and 42% CHO solution</td>
<td>▲ CHO</td>
<td>PLA and CHO attenuates the decline in maximal power output more than H\textsubscript{2}O</td>
</tr>
<tr>
<td>Jeukendrup <em>et al.</em> 2008</td>
<td>Thermoneutral</td>
<td>Fasting</td>
<td>Fasting and 4 ml/kg of the drink of the day prior to trials</td>
<td>25 min at 85% maximal power output as fast as possible</td>
<td>6% CHO solution</td>
<td>Not measured</td>
</tr>
<tr>
<td>Millard-Stafford <em>et al.</em> 1992</td>
<td>Heat</td>
<td>no solid food on the day and 400mL of the drink of the day 30 min before exercise</td>
<td>35km run in an outdoor course in a self-selected pace and last 5km in a race pace.</td>
<td>7% CHO solution</td>
<td>▲ CHO</td>
<td>Better performance on the last 5km run of CHO compared with PLA</td>
</tr>
</tbody>
</table>
Table 2 continued.

<table>
<thead>
<tr>
<th>Study</th>
<th>Environment Conditions</th>
<th>Nutritional State</th>
<th>Exercise Protocol</th>
<th>Ingestion Method / CHO content</th>
<th>Glucose Response</th>
<th>Outcome / conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nybo 2003</strong></td>
<td>Not mentioned</td>
<td>Fasting</td>
<td>3 hrs cycling at a constant output of 200 ± 8 W</td>
<td>6% CHO solution</td>
<td>↑ CHO</td>
<td>Lower MVC and level of voluntary activation in PLA</td>
</tr>
<tr>
<td><strong>St.Clair Gibson et al. 2001</strong></td>
<td>Thermoneutral</td>
<td>Fed</td>
<td>100 km time trial</td>
<td>7% CHO solution</td>
<td>Not measured</td>
<td>No differences in MVC between PLA and CHO. No differences in time trial performance</td>
</tr>
<tr>
<td><strong>Winnick et al. 2005</strong></td>
<td>Not mentioned</td>
<td>Fasting</td>
<td>4 x 15 min shuttle running (walking, running, jumping) lasting ~ 60 mins.</td>
<td>6% CHO solution</td>
<td>Not measured</td>
<td>CHO resulted in faster 20-m sprint times and higher average jump height compared with PLA. CHO also reduced force sensation, enhanced motor skills, and improved mood late in exercise compared with PLA.</td>
</tr>
</tbody>
</table>
The addition of CHO to the water with the purpose of enhancing performance has been widely investigated. Most of the initial studies were undertaken in a thermoneutral environment (Coyle et al., 1983; Coyle et al., 1986; Powers et al., 1990, Wright et al., 1991). Only in the last decade researchers have been more concerned about evaluating the effect of ingestion of these beverages on physical performance during prolonged exercise in a hot environment (Silami-Garcia et al., 2004; Bilzon et al., 2000; Carter et al., 2003; Gomes, 1999; Febbraio et al., 1996; Galloway & Maughan, 2000). It was theorized that an increase in the availability of CHO during exercise would result in a reduction in fatigue. Some research is still undertaken in the thermoneutral environment (Burke et al., 2000; Carter et al., 2004b; Chryssanthopoulos et al., 2002; DeMarco et al., 1999; Maughan et al., 1996; McConell et al., 2000; Nassis et al., 1998) whilst there are a number of studies that do not document which environment was studied (Kang et al., 1996; McConell et al., 1999; Timmons et al., 2000; Robinson et al., 2002).

The studies that used a thermoneutral environment present inconsistencies when looking at enhancement of performance when beverages with CHO are ingested during exercise. Some studies have verified enhancement of performance (Coggan & Coyle, 1987; Coyle et al., 1983; Coyle et al., 1986; Chryssanthopoulos et al., 2002; DeMarco et al., 1999; Maughan et al., 1996; Tsintzas et al., 1996) while others have not (Burke et al., 2000; Gomes, 1999; McConnel et al., 2000; Nassis et al., 1998 and Powers et al., 1990,). That is, time to fatigue was maintained when subjects exercised ingesting a PLA. All studies cited above, except for Gomes (1999) used the method of liquid solutions for the ingestion of CHO and minerals. The study by Gomes (1999) which used the method of gelatine capsules with fluid had subjects exercise at 50% PPO until exhaustion in the heat and in a thermoneutral environment which showed no differences in time to fatigue when capsules were used rather of a beverage.

Coggan and Coyle (1988) also studied the effect of CHO ingestion during high intensity exercise and observed prevention in the decline of blood glucose and also a delay in fatigue of ~30 min. Subjects exercised until fatigue in 15 min bouts that varied from 60% to ~85% of their
They also observed that subjects are capable of exercising at up to 75% of their VO$_{2\text{max}}$ and to oxidize CHO in a rate of up to 2 g/min in the later stages of the prolonged high intensity exercise. These authors suggest that fatigue can be delayed by the ability that very well trained endurance athletes have to oxidize CHO at elevated rates from other sources than just muscle glycogen at the end of high intensity prolonged exercise (Coyle et al., 1986). Coyle et al. (1983) observed a delay in fatigue in 7 of 10 subjects when fed CHO with an exercise duration of 134 ± 6 min for the PLA treatment and 157 ± 5 min for the CHO treatment.

According to Burke (2001), the need for CHO during exercise increases with a hot environment because of an increase in oxidation of this substrate. Although physical activities in a hot environment do not always lead to the best performance, nutritional strategies have an important role in assisting subjects to perform as well as possible during prolonged exercise. The studies in a hot environment have shown inconsistencies in relation to enhanced performance when solutions with CHO were ingested. The studies by Bilzon et al., (2000); Carter et al., (2003) and Galloway and Maughan, (2000) did not use capsules for the ingestion of CHO. But the studies by Silami-Garcia et al. (2004), Gomes (1999) and Nassif et al. (2008) which used gelatine capsules did not observe enhanced performance in a warm (28°C) and humid environment (79%). Silami-Garcia et al., (2004) evaluated anaerobic performance after prolonged exercise with the ingestion of CHO and minerals in capsules in a hot and humid environment. In this study, after exercising for 90 mins at a fixed intensity of 60% of PPO subjects undertook a Wingate test. No differences in power produced during the Wingate test were observed when comparing PLA and CHO treatments.

In the study by Carter et al. (2003) the main purpose was to clarify the effect of the supplementation of CHO in moderate to intense exercise in the heat. The individuals participated in four experimental treatments ingesting a solution of 6.4% CHO and a PLA solution at intensities of 60 and 73% VO$_{2\text{max}}$ in a hot environment (35°C) and 30% relative humidity. At both intensities there was an increase in total time exercised when CHO solution was ingested, with
an increase of 14.5% at 60% VO$_{2\text{max}}$ and of 13.5% at 73% VO$_{2\text{max}}$. Carter et al. (2004b) have also investigated the effect of glucose in a 1 h time trial exercise. Six endurance cyclists were instructed to complete the most amount of work possible during one hour of exercise whilst they were infused with either glucose or saline. No difference in performance was observed although there was an increase in plasma glucose concentration.

Desbrow et al. (2004) investigated the effect of CHO ingestion on high intensity cycling performance. Nine well-trained subjects were asked to complete a set amount of work as quickly as possible in a thermoneutral environment. During exercise, subjects ingested a 6% CHO beverage or a corresponding PLA. No differences were observed in either exercise time or power output when CHO was ingested compared to the PLA. Millard-Stafford et al. (1992) investigated the effect of a 7% CHO electrolyte beverage on 40 km running time trial in the heat. Eight highly trained runners completed the time trials in an outdoor measured course so that 35 km was completed in a self selected pace and the last 5 km was completed in a race effort. Results showed a positive effect of the CHO beverage on performance compared to the PLA, although no effects were observed in thermoregulation, hydration or other physiological responses such as heart rate, blood lactate, RPE or rectal temperature.

Davis et al. (1988) investigated the effect of CHO ingestion completing three trials in the heat with either a 6% or a 2.5% CHO beverage or a water PLA beverage. Each trial was composed of two tasks with a 30 min rest. One task was a 2 h trial (10800 pedal revolutions in the fastest time possible) whilst the other was a 30 min trial (2700 pedal revolutions in the fastest time possible) which both elicit exercising at an intensity of 75% VO2max, if subjects pedalled at 90 rpm. No differences were observed in the first task of two hours cycling but the 30 min ride of the second task was significantly faster when the 2.5% CHO beverage was ingested compared with the PLA. The authors attributed the result on the capacity of that beverage to maintain blood glucose and possibly sparing muscle glycogen.
The effect of CHO ingestion during an 80 km cycling time trial was investigated by Langenfeld et al. (1994). Fourteen trained cyclist performed two trials each, one ingesting a 7% CHO beverage and another ingestion of a non-caloric PLA. The time to complete the 80 km time trial with the ingestion of the CHO beverage was 5% faster than the time to complete the trial with the ingestion of the PLA beverage. Febbraio et al. (1996) studied the ingestion of a 7% and 14% CHO solution on metabolism and performance in cold and hot temperature conditions and also the efficacy of the ingestion of low hypotonic solutions with or without CHO on metabolism and performance in a hot environment. This experiment showed an enhancement of performance with the ingestion of CHO, but only in the cold environment with a solution of 7% being better than 14%.

Galloway and Maughan (2000) studied the effect of the ingestion of a CHO and electrolyte beverage with concentrations of 2% and 15% CHO against a PLA on exercise capacity, thermoregulation and metabolic responses in a hot environment (30°C) whilst subjects exercised at 60% of VO2max until fatigue. The authors observed that the ingestion of a drink at 2% CHO increases exercise duration more than a drink with a 15% but both were better than no fluid. The median exercise duration was 70.9 min (39.4-97.4 min) in the no-drink trial, 84 min (62.7-145min) in the 15% CHO trial and 118 min (82.6-168 min) in the 2% CHO trial.

The studies by McConell et al. (1999), Robinson et al. (2002), Timmons et al. (2000), Wright et al. (1991) and Palmer et al. (1998) did not report which environment was used in their experiments. However, two studies observed an improvement in performance (McConell et al., 1999; Wright et al., 1991) whilst the other two did not report any differences (Robinson et al., 2002; Timmons et al., 2000). The study by McConell et al. (1999) evaluated the improvement on metabolism and total time of exercise when a solution of 8% CHO was ingested during exercise at approximately 69% VO2peak until exhaustion. They observed an increase of 30% in time to fatigue during CHO ingestion when compared to PLA.
Wright et al. (1991) studied the effect of a combination of CHO ingestions on performance. Nine cyclists exercised at 70% VO$_{2\text{peak}}$ until fatigue on four different occasions; no CHO (PP), pre-exercise CHO feeding (CP), CHO feedings during exercise (PC), and the combination of CHO feedings before and during exercise (CC). Time to exhaustion was 44% (CC), 32% (PC), and 18% (CP) greater than PP. Flynn et al. (1987) also studied the effect of a combination of CHO ingestion on performance and also glycogen usage. After subjects completed a “depletion ride” at 70% VO$_{2\text{max}}$ two days prior to each trial, they were fed a CHO diet. The combination of CHO for ingestion during exercise were H$_2$O (artificially flavoured and sweetened), MF (maltodextrin and fructose), MHF (maltodextrin and high fructose corn syrup) and MG (maltodextrin and glucose). Differences in amount of work completed and the glycogen usage were not observed between trials, which makes the authors suggest that cyclists with high glycogen stores might have attenuated dependence on exogenous source of CHO during 2 h of exercise.

The effect of a CHO electrolyte solution on time to fatigue on a treadmill was studied by Robinson et al. (2002). Ten runners participated in two experimental trials until fatigue at 100% VO$_{2\text{peak}}$ after the ingestion of a PLA or a 6% solution of CHO and electrolytes in a bolus of 500 mL 1 h prior to the exercise. No differences were observed in time to fatigue between treatments. Similarly, Timmons et al. (2000) studied the effect of a beverage called SPORT™ in relation to performance while exercising at 100% VO$_{2\text{peak}}$. In their study, which also used gelatine capsules, subjects ingested 2 x 500 mg capsules 1h before exercise and then after each load increase to 60, 80 and 100 % VO$_{2\text{peak}}$, giving a total of eight capsules ingested per trial and four grams of each substance per trial depending on the treatment. These authors did not observe enhancement in time to fatigue although exercise duration was 105 ± 44 and 103 ± 50 s in the PLA and SPORT™ trial, respectively.

Palmer et al. (1998) investigated the effect of CHO ingestion during a 20 km time trial. Fourteen well trained cyclists (eleven males and three females) completed two 20 km time trials
with the ingestion of CHO and a corresponding PLA trial. Fluids were ingested in one bolus 10 min before completing a 5 min warm-up and then subjects completed the 20 km time trial. No difference in time trial was observed. The authors suggested that the fact that performance was the same with CHO and PLA ingestion supports the concept that maximal exercise that lasts approximately 30 min is not limited by endogenous substrate availability nor does CHO mitigate the development of fatigue.

El-Sayed et al. (1997) studied the effect of CHO ingestion on metabolic responses and performance on a simulated 1 h cycling time trial. Eight trained cyclist completed 2 x 1 h time trials; one with 8% CHO solution and the other a PLA solution. A higher mean power output and a longer distance covered was observed in the CHO trial compared to the PLA trial although this can not be explained by the metabolic measures used in this study. Also, these authors found the improvement in performance in this study quite surprising as for the small amount of CHO ingested and suggest that confirmation follow-up studies are needed.

Anantaraman et al. (1995) studied the effect of a combination of a 10% glucose CHO solution on performance during 1 h of high-intensity exercise. Five moderately trained subjects participated in three trials with a combination of CHO and PLA being ingested prior and during exercise, such as, glucose pre-exercise with PLA during exercise, glucose pre-exercise and during exercise, and PLA pre-exercise and during exercise. Subjects started exercising at 90% of VO\(_{2\text{max}}\) for 1 h with reductions in power output permitted over time. Higher power output was observed at the end of exercise in the trials where glucose was ingested compared to the trial in which only PLA was ingested. The authors suggest a clear benefit of the ingestion of CHO pre-exercise but no further benefit was observed when it was ingested during exercise.

Burke et al. (2000) studied CHO loading on 100 km time trial cycling performance. Seven well-trained subjects completed two 100 km trials after either CHO loading or PLA controlled moderate-CHO diet. No differences in performance were observed. These authors suggest that the CHO loading had a small effect which raises the possibility that the ergogenic effects of CHO
evaluated in past studies have been a result of a possible PLA effect or from higher pre-exercise liver glycogen stores that possibly delay the onset of hypoglycaemia during prolonged exercise. Widrick et al. (1993) studied the effect of pre-exercise muscle glycogen concentration on CHO ingestion and exercise performance. Eight cyclists completed 4 x 70 km time trials according to the following combination: high pre-exercise glycogen with CHO ingestion, high pre-exercise glycogen with a non-CHO ingestion, low pre-exercise glycogen with CHO ingestion and low pre-exercise glycogen with a non-CHO ingestion. Performance and power output was significantly better when pre-exercise glycogen concentration was high, but did not have the same effect when pre-exercise glycogen levels were low. When studying CHO ingestion during exercise, it had no effect on performance or power output independently of a high or low pre-exercise glycogen concentration; however, the ingestion of CHO when pre-exercise glycogen levels were low seem to partially offset the effect of low glycogen levels, as performance and power output was similar to the values observed in the high pre-exercise glycogen with non-CHO ingestion. These authors concluded that the ingestion of CHO can partially compensate for low pre-exercise glycogen levels allowing subjects to maintain pace during the final stages of a time trial. But the important finding of this study is that when subjects perform a time trial with high pre-exercise glycogen levels, exogenous CHO provide little ergogenic effect (Widrick et al., 1993).

2.9.1 The Placebo Effect

The placebo effect is known to have a positive effect of what is believed by the subject to be an effective active substance but is actually an inert substance or a substance which has no effect on what is in fact being tested. The placebo effect was first studied in the early 70’s when Ariel and Saville (1972) tested the placebo effect on Body Builders with a tablet which showed an effect on the athletes body better then the real substance being tested. This study was the initial examination of placebo in the area of sport and how the belief of ingesting an active
substance can actually affect ones behaviour. Clark et al., (2000) presented a randomized study on the placebo effect when subjects cycled a 40 km time trial with the ingestion of CHO beverages. This study has also shown a clear placebo effect when testing CHO as an ergogenic aid in a beverage form. Even though the literature presents these two studies focusing on the placebo effect, a variety of studies seem to ignore this possible effect which could compromise their results.

When testing the effect of CHO ingestion in a beverage form one should be concerned with the possibility of subjects being able to distinguish which beverage has the CHO content. Many studies since the explosion of CHO beverage studies and its possible positives effects on performance do not give clear information about how researchers guaranteed subjects could not distinguish the difference between beverages.

Carter and colleagues have undertaken studies evaluating the effect of CHO ingestion that clearly showed a possible placebo effect and the significant role of taste when testing the possible positive psychological effects of CHO ingestion in performance (Carter et al., 2004a; Carter et al., 2004b). In one of the studies which used the method of venous infusion to deliver CHO, no effect on performance was observed and the taste factor was removed from the study (Carter et al. 2004b). In a companion study which examined the effect of a CHO mouth rinse without the physiological effects of the ingestion of CHO beverages showed a better performance with the CHO mouth rinse and also showed a surprising effect that 4 of the 7 subjects were able to guess correctly which beverage was the CHO beverage, showed how taste can possibly play an important part in determining ones improved performance when ingesting CHO.

More recently, a study by Nassif et al. (2008), using a fixed intensity protocol with CHO ingestion in a capsule form, showed no positive physiological effect on performance when treatments were double blinded. In the same study subjects ingested CHO and were also told to be ingesting CHO, an improvement of 24% in performance was observed. This once again calls
casts doubt on the information given to subjects in these studies and then what is believed by subjects. As no studies to date have compared different forms of CHO delivery the present study has the clear objective of comparing different methods of delivery and also to examine the placebo effect. Although a placebo effect was not observed in the present study as the design was such that this was a controlled factor, we can conclude that in a self-paced time trial exercise with a duration of approximately 2.5 h, CHO ingestion has no positive or additive effect on performance, as there was no differences in performance between the CHO and PLA treatments. It is also important to note that performance in the H₂O treatment was also not different from the CHO and PLA treatments; CHO does not play an important role in improving performance. Even though an improvement in performance was not present, this study matches the results of the previous classic studies that show CHO ingestion to maintain blood glucose levels when ingesting beverage (Abbis et al., 2008; Coyle et al., 1986; Febbraio et al., 1996; Nassis et al., 1998). Although it has been classically shown that CHO ingestion prevents the development of hypoglycaemia, allowing subjects to exercise for longer in fixed intensity protocols it is not clear what mechanisms would permit CHO to have an effect in self-paced time trials. It is also important to point out that no subjects in the present study, even on the H₂O presented anywhere close to developing hypoglycaemia.

2.10 METHODS USED FOR CHO SUPPLEMENTATION

Since CHO supplementation has been part of the sporting environment and researchers have been examining its effect on human performance, fluid preparations have been used as a method of CHO supplementation. As scientific studies showed that a possible placebo effect was possible due to motivation and psychological factors, a need for methods that would account for this would be essential. There are possibly three methods of delivery of CHO
supplementation being used by researchers, capsules or tablets, beverages and venous infusion.

A classic study by Ariel and Saville (1972) showed a clear placebo effect when researchers tested the effect of anabolic steroids ingestion. In their study, Ariel and Saville (1972) used a tablet as an ingestion method, as subjects could not taste the tablet content and therefore unable to know who had anabolic steroids. Therefore, one would assume that if taste could play an important role in how individuals would perform, acting as a motivational factor that could influence results, researchers should carefully select the method used to deliver supplementation. This possible motivational factor could have come from the media, with no scientific background, that a substance has a positive effect on performance. Even though we have evidence from the early 70’s when tablets were used, few studies have looked for methods of supplementation that will account for the possible placebo effect. A recent study by Clark et al. (2000) examining the placebo effect did not use a method such as tablets, but used beverages. Beverages have been the main method used in the studies where a CHO beverage is prepared and a placebo beverage that is suppose to match all the characteristics of the CHO beverage but the energy content has been removed. In many cases, it is not clear if subjects can tell the difference between these two beverages. It would be logical to give preference to use a beverage method to undergo this type of study as this is actually the method athletes’ use during sporting events. Even so, this can only be done if one can guarantee no differences in taste, colour and texture, tests that are not clearly stated or explained in most studies.

In order to create a method of CHO supplementation that takes the taste factor into account, and is able to be compared to the beverage, it is important to look at the rate which the body will actually have the energy of the CHO ingested available to be used. Capsules and tablets do account for the taste factor, but does that mean CHO are being delivered to the body in the same amount and rate? Blood infusion has also been a method used to test the effect of CHO accounting for the possible placebo effect (Carter et al., 2004b). No study to date has evaluated
the comparison between different methods for the ingestion of CHO to the beverage method. Verifying the effect of the method of supplementation would require gastric emptying measurements or blood glucose measurements to ascertain if CHO is being absorbed at the same rate independent of the ingestion/delivery method.

The first study published using capsules to evaluate CHO supplementation was by Timmons et al. (2000) who tested CHO ingestion during exercise at 100% of VO$_{2\text{max}}$. Expectably the duration of the exercise was very short and one could believe that there was not enough time for CHO to be absorbed after ingestion to actually have an effect on performance. A subsequent study examined the effect of CHO ingestion using blood infusion. For this, CHO or a saline solution as a placebo was intravenously infused (Carter et al., 2004b). In that study, no differences were observed in performance when CHO were ingested. These same authors published a study where the effects of a CHO mouth rinse on performance was tested and which suggested that receptors in the mouth might send a signal to the CNS about the availability of CHO for the working muscle. However, in this study there was no possibility for a direct physiological effect of CHO ingestion, as the CHO was not actually ingested, but mouth rinsed (Carter et al., 2004a). This suggests a possible role of the CNS together with taste that could affect subjects’ motivation levels. Recently published, the study by Nassif et al., (2008) used gelatine capsules for CHO supplementation evaluating prolonged exercise, which also did not find positive physiological results with CHO ingestion when compared to a PLA. None of these studies tested the possible differences in absorption and glucose responses between the methods of supplementation. Therefore, comparing the beverage method with other methods that have a better chance to account for the placebo effect and the role of taste on these studies could add substantially to the scientific literature on this topic. For this reason, this thesis tested the capsules method to be compared with the beverage method commonly used.
As gastric emptying would be a much more complex and invasive method to evaluate how CHO are being absorbed, the comparison of blood glucose measurements were used. This thesis showed no differences between blood glucose measurements when looking at the ingestion of CHO in a beverage compared to a capsule form. It is important to note that CHO ingestions occurred always in the same pre determined moment, independent of the method of supplementation. For this reason, it is also important to point out that tasting may not have such an effect on performance but rather the information given to subjects about the testing procedures and content of the supplementation. In two studies by Carter and colleagues, that using infusion and the other testing a CHO mouth rinse, do not report what participants were actually told in regards to what was being infused or mouth rinsed. In the present study, subjects were told that CHO were being ingested at all times other than the water treatment. If taste does play a role in CNS regulation and therefore how well subjects will perform, differences would have been observed when the beverage and capsules were tested, but this was not the case. For this reason, we conclude that there were no significant physiological effects of CHO ingestion when comparing capsules vs beverages. It is also important to note that taste did not play a role in determining performance in the present study.

2.11 THESIS HYPOTHESIS

As it can be observed by the literature presented, changes commanded by the CNS might be responsible for changes on pacing strategies to allow individuals to complete self-paced exercise in their best ability without compromising the organism. CHO ingestion and possible changes in pacing strategies during prolonged exercise have been studied in the past but not using a capsules method to guarantee complete control of the placebo effect. For these reasons and also for a lack of studies which look at the possible effects of CHO ingestion on CNS fatigue, the questions to be addressed by this thesis are:
1. (a) 
- Will CHO independent of its form of ingestion improve exercise performance in a 60 km self-paced time trial in the heat?

(b) 
- Will CHO independent of its form of ingestion alter pacing strategies?

(c) 
- Will alterations in pacing strategies be associated with alterations in skeletal muscle recruitment?

2. (a) 
- Will CHO independent of its form of ingestion alter parameters of neuromuscular fatigue in non-fasting participants following prolonged self-paces cycling in the heat?

(b) 
- Will the M-wave amplitude, evoked twitch contractile properties, MVC and the level of voluntary activation be altered following exercise in the heat when CHO is ingested?

3. 
- Will double blind ingestions of 6% CHO in a beverage and capsule form versus a placebo alter the neuromuscular parameters associated with sustained submaximal contractions to fatigue following prolonged exercise in the heat?
STUDY ONE

Double-blind carbohydrate ingestion does not improve 60 km self-paced cycling in warm ambient conditions
Abstract  Study One

Objective: The effect of carbohydrate (CHO) supplementation on endurance performance has been extensively studied but its placebo (PLA) effect can make the ergogenic qualities of CHO more difficult to determine. This study tested the effect of double blind ingestions of PLA and 6% CHO in capsules (c) and in a beverage (b) during a 60 km self-paced cycle time trial in the heat (32 °C and 50% relative humidity).

Methods: Ten well trained male subjects (mean ± SD: 26 ± 3 years; 64.5 ± 7.7 kg and 70.7 ± 8.8 mL.kg⁻¹.min⁻¹ maximal oxygen consumption) completed 5 x 60km cycling time trials (TT) punctuated with 4 x 1 km sprints at 14, 29, 45 and 59 km. Trials consisted of 5 treatments, PLA in beverage (PLAₐ) and capsule (PLAₐ) form and CHO (CHOₐ and CHOₐ) in similar forms and a control water trial (H₂O). Mean fluid volume ingested in each treatment was 284 ± 30 mL.

Results: There were no differences in TT among treatments (H₂O 138.2 min ± 17.5 min, PLAₐ 130.2 ± 11.2 min, CHOₐ 140.5 ± 18.1 min, PLAₐ 143.1 min ± 29.2 min, CHOₐ 137.3 ± 20.1 min). An increase in blood glucose was observed in the treatments where CHO was ingested compared with PLA and H₂O treatment (p< 0.05). No differences in blood lactate were observed between treatments. There were no differences in power output, speed and cadence among treatments although these were different (p< 0.05) between the sprints and the low intensity periods of the trial for all treatments. No differences were observed in core and mean skin temperatures among treatments. Changes in electromyography were evident between sprints and low intensity efforts with muscle recruitment for PLAₐ and CHOₐ treatments being reduced in the latter stages of the trial (> 36.5 km).

Conclusions: We conclude that CHO ingestion has no significant benefit in either capsule or beverage form over 60 km in the heat compared to either PLA or H₂O. It is evident that muscle recruitment is altered in the final stages of the trial (~ 36 km) so that subjects will not compromise homeostasis.
3.1 INTRODUCTION

Maintaining hydration status is thought to be important for achieving the highest level of exercise performance, but there is still doubt about the effect of added carbohydrate (CHO) as an ergogenic aid to the fluid to be ingested. For example, some studies show enhanced performance with the addition of CHO (Coyle et al., 1986; Coggan & Coyle, 1987; Febbraio et al., 1996a; Nassis et al., 1998) whereas others do not (Burke et al., 2000; McConell et al., 2000; Robinson et al., 2002). Supplementation with CHO has mainly been studied during prolonged exercise and in the majority of cases during fixed intensity protocols; although a time trial protocol would be closer to the athlete’s performance reality. Even though some studies have used a time trial protocol in thermoneutral and hot environments, doubt still exists about the usefulness of CHO ingestion as an ergogenic aid in these different conditions. Some studies do not report a positive effect of CHO ingestion on exercise performance in a thermoneutral environment (Burke et al., 2000; Carter et al., 2004b; Desbrow et al., 2004) whilst other studies do (Langenfeld et al., 1994; Anantaraman et al., 1995; El-Sayed et al., 1997). Some studies (Davis et al., 1988; Millard-Stafford et al., 1992) have focused on warm environments but the benefits of CHO ingestion in these ambient conditions remains equivocal (Febbraio, 2000).

However, in those studies where improvements in exercise performance have been shown with the ingestion of CHO in hot conditions, a higher energy demand has been inferred in such conditions (Davis et al., 1988; Millard-Stafford et al., 1992; Burke, 2001). Millard-Stafford et al. (1992) studied the effect of CHO ingestion during a 40 km self-paced outdoor run and observed improved performance and increases in blood glucose in comparison to a placebo (PLA). According to Burke (2001) there is an increase in CHO demand during exercise in the heat because of a shift in the utilization of substrate towards CHO oxidation.

An important aspect to be considered is whether subjects in previous studies have been fed before exercise. As most studies have tested the effects of CHO with subjects not
fed, more recent studies have examined the effect of CHO in fed subjects, which is likely to be of practical significance (Burke et al., 2000; Clark et al., 2000; Desbrow et al., 2004; Nassif et al., 2008).

More recent studies have also confirmed that the placebo effect is potentially a confounding factor when testing the effect of CHO on effort sense (Clark et al., 2000; Noakes et al., 2004; Nybo & Secher, 2004). Therefore, it would be prudent to account for this possibility when studying the ingestion of CHO or any other active substance, as subjects’ expectations could determine the intrinsic feedback as classically shown (Ariel & Saville, 1972). Clark et al. (2000) showed improved performance when subjects believed they were ingesting CHO but were actually consuming a PLA. In this particular study, 43 participants completed 2 x 40 km time trials; one trial to establish baseline performance and a second trial where the subjects ingested a CHO or a PLA fluid. These two groups were further subdivided into three groups so that subjects either knew they were ingesting CHO or they knew they were ingesting a PLA, or to a group not knowing either. The striking result from this study was that the group that ingested a PLA but was told they were ingesting CHO had a better performance than the group that was told and had actually had ingested CHO.

Furthermore, infusion of glucose (CHO) versus saline (PLA) to evaluate 1h cycle time trial performance in a thermoneutral environment, resulted in no improvement in performance with either CHO or PLA treatment (Carter et al., 2004b). This group also investigated the possible role of CHO receptors in the mouth on performance and concluded that an enhancement in performance is related more to an increase in central drive or motivation than a metabolic effect (Carter et al., 2004b). Recently, a study by Chambers et al. (2009) showed changes and activation of the parts of the brain related to rewards when subjects ingested glucose in a beverage form. Improvements in performance suggest that this might be related to the activation of specific brain regions so that oral receptors in the mouth may respond differently to CHO then those that respond to sweetness. These findings show how important it is to develop methods that do not allow subjects to recognize what is being administered since four of the nine participants in this study detected they were ingesting
CHO, and of these four, three performed better when ingesting CHO. Similarly, the fact that subjects know what is ingested may act as a motivational factor that could enhance performance more than the physiological benefit attained (Nassif et al., 2008). For that reason, infusion and capsules could be more appropriate methods to test the real effects of a substance and be able to discriminate between the effect of CHO ingestion and what is actually the placebo effect on exercise performance.

The mechanism/s by which CHO might improve exercise performance is still not clear, however, it is possible that any improvement or change in performance with CHO might be observed in the recruitment of skeletal muscle as postulated by St.Clair-Gibson et al. (2001) who found changes in neuromuscular activity and power output during prolonged exercise when subjects were CHO loaded. Changes in electromyography (EMG) measurements could be indicative of skeletal muscle recruitment and reflect the pacing selection of subjects during prolonged exercise (Kay et al., 2001; St Clair Gibson et al., 2001b).

Therefore, the purpose of this study was to examine the effect of ingesting either a 6% CHO beverage or an equivalent 6% CHO capsule versus corresponding PLA and water (H2O) as a control treatment during prolonged endurance exercise (60km time trials) in the heat when subjects were fed. A further aim was to examine whether subjects would alter their pacing strategy (speed, cadence and power output) according to the type of ingestion and if this was associated with alterations in skeletal muscle recruitment.
3.2 METHODS

3.2.1 Subjects

Ten well trained male participants took part in the study after completing a health screening questionnaire and being released by a physician. This research was approved by the Ethics in Human Research Committee of the University and each subject signed a letter of informed consent after attending an information session.

Preliminary measurements included skinfolds (biceps, triceps, chest, sub auxiliary, subscapular, suprailiac, abdominal, thigh and calf), maximal oxygen consumption ($\text{VO}_2\text{max}$), and peak power output (PPO). The mean $\pm$ SD characteristics of the participants are given in Table 3.

Table 3. Physical characteristics of the participants ($n = 10$).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Body Mass (kg)</th>
<th>$\Sigma$SF (mm)</th>
<th>PPO (W)</th>
<th>$\text{VO}_2\text{max}$ (ml.kg$^{-1}$.min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean± SD</strong></td>
<td>26 ± 3</td>
<td>170 ± 9</td>
<td>64.5 ± 7.7</td>
<td>54.2 ± 13.1</td>
<td>323 ± 41</td>
</tr>
</tbody>
</table>

$\Sigma$SF; sum of nine skinfolds (bicep, triceps, sub scapular, pectoral, mid-axilla, abdominal, supraspinale, thigh, calf), PPO; peak power output, $\text{VO}_2\text{max}$; maximum oxygen uptake.
3.2.2 VO_{2max} test

A BIOPAC® Systems (Goleta, CA, U.S.A.) spirometer was used to determine expired gas concentration during a progressive VO_{2max} testing protocol as previously described (Kay et al., 2001). Briefly, the progressive test commenced at a workload of 100 W with increments of 10 W every 30 s until voluntary termination. The VO_{2max} testing was performed in a thermoneutral environment at 22 ± 1 °C and 68 ± 6% relative humidity (rh) using a Flow Trainer (TACX®, T1684, Netherlands) with the subjects’ own bicycles attached. Ten subjects were tested totalling 50 data points for analysis. The study was balanced by order and the washout factor was controlled by treatments being completed at least 7 days apart. Throughout the test, participants were required to remain in a seated position but permitted to change gears and/or cadence throughout the test, provided that the required power increments were maintained all times. A subject prepared and ready to start the VO_{2max} is presented in Figure 1.
**Figure 1.** Subject ready to start the VO$_2$ max test protocol.
3.2.3 Research design

All subjects completed five randomized double-blind experimental treatments in two 5 x 5 Latin Squares. See Table 4 below.

Table 4. Treatment sequences for each subject in the randomized 5 x 5 Latin Square design.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 6</td>
<td>H₂O</td>
<td>PLAₐ</td>
<td>CHOₐ</td>
<td>PLAₖ</td>
<td>CHOₖ</td>
</tr>
<tr>
<td>2 and 7</td>
<td>CHOₖ</td>
<td>PLAₖ</td>
<td>H₂O</td>
<td>CHOₐ</td>
<td>PLAₐ</td>
</tr>
<tr>
<td>3 and 8</td>
<td>CHOₐ</td>
<td>H₂O</td>
<td>CHOₖ</td>
<td>PLAₐ</td>
<td>PLAₖ</td>
</tr>
<tr>
<td>4 and 9</td>
<td>PLAₐ</td>
<td>CHOₖ</td>
<td>PLAₖ</td>
<td>H₂O</td>
<td>CHOₐ</td>
</tr>
<tr>
<td>5 and 10</td>
<td>PLAₖ</td>
<td>CHOₐ</td>
<td>PLAₐ</td>
<td>CHOₖ</td>
<td>H₂O</td>
</tr>
</tbody>
</table>

H₂O; water treatment, PLAₐ; placebo beverage treatment, CHOₐ; carbohydrate beverage treatment, PLAₖ; placebo capsule treatment, CHOₖ; carbohydrate capsule treatment.
Subjects ingested either a PLA, 6% CHO, or H₂O. The PLA and CHO treatments were each administered across two treatments using different modes of ingestion; as a beverage (b) or in capsules (c) with distilled water. Therefore, each participant completed 2 x PLA trials (PLA_b and PLA_c) and 2 x CHO trials (CHO_b and CHO_c) and an additional H₂O trial as a control. Subjects were told they were ingesting CHO for all treatments. All treatments were coded and prepared by an individual that was not directly involved with the study. Treatments were revealed to the investigators at the completion of the study.

Each subject performed the treatments at the same time and day of each week to minimize any possible motivational differences and diurnal variations. Subsequent treatments were separated by 12 ± 7 days. The long gaps between treatments are explained by personal issues and illness that subjects faced during the data collection period, which was beyond the researchers control and required trials to be postponed. However, to guarantee the level of fitness of subjects, they were instructed to maintain their training routine as close as possible. The minimum intervals between treatments were 7 days and the maximum was 42 days. The variation in number of days for the intervals between treatments can be explained by the need of some subjects to undergo treatment (i.e. colds) and take medications that could affect particular measurements. Therefore, trials were delayed to guarantee reliable measurements. The responsible physician advised that subjects should be off the prescribed medication for 10 days to be able to go back to treatment testing. In addition, participants were required to maintain their usual training routine but abstain from training or any strenuous activity, caffeine and alcohol consumption for at least 24 h prior to all treatments.
3.2.4 Experimental Procedures

**Pre-testing requirements:**

For two days prior to each treatment, subjects were required to follow a diet with 60 to 70% CHO, 1.2 to 1.7 g of protein/kg of body mass, and 20 to 25% fats, as prescribed individually by a dietitian (ACSM, 2007). The purpose of this was to ensure complete recovery of muscle glycogen stores following the last day of training prior to each treatment. Subjects were not required to fast overnight prior to each treatment and were required to consume a prescribed breakfast on the morning of each treatment. Subjects were instructed to consume the same morning meal on the day of each treatment.

Data collection took place between the months of April and August. The mean time taken for subjects to complete the study was 46±13 days. As it was predicted to be quite a long study, subjects were asked to train normally during data collection and not change their training routines. Before each trial was scheduled, subjects were contacted to ensure they were following all the instructions before each treatment such as training routine maintained, rest day the day prior to each treatment, diet followed and hydration status maintained as prescribed.

**Time trial protocol:**

For the time trial, subjects mounted the cycle and commenced a five minutes warm-up at 50% of their PPO as previously determined. After the warm-up, subjects had the EMG electrodes attached to the quadriceps muscle of their dominant leg (described subsequently). Each participant completed one trial in each of the different treatments (PLA_b, CHO_b, PLA_c, CHO_c and H2O), as given in Table 2 consisting of a 60 km self-paced time trial (slope + 2, TACX® program) punctuated with 4 x 1 km sprints at 14, 29, 44 and 59 km for each trial. When not sprinting, subjects were required to complete the time between sprints as quickly
as possible (low intensity efforts, LI). Subjects were instructed and encouraged to complete each trial as quickly as possible and were permitted to alter gear ratio and cadence and pedal standing up when they wanted, other than in the sprints where they were required to remain in a seated position to prevent changes in muscle fibre recruitment patterns that can occur with posture changes. Trials took place in an environmental chamber (Russells® Technical Products, model WMD-1150-5, Holland, MI, U.S.A.) in a hot environment (32 °C and 50% rh). A fan was placed in front of the subject, positioned towards the head and torso when in a normal cycling position with a wind speed of 3 m/s, with the naturally circulating air provided by the chamber during exercise. The test was terminated when one or a combination of the following criteria were achieved; 1) core temperature reached 39.5°C, 2) RPE of 20, 3) the subject terminated exercise due to volitional fatigue, and/or 4) any sign of illness or discomfort by the subject. Exercise duration was measured using a chronometer. Subjects were given feedback about cadence at all times and distance at each 3 km interval. No feedback about time, blood results or heart rate was available to subjects during any trials until the study was completed.

Fluids were ingested five times during the trials, at 5 km, after each sprint (15, 30, 45 km) and at 55 km before the last sprint. The fluid temperature was 4 ± 1 ºC. Each subject received 284 ± 30 mL of distilled water and capsules (4 mL/kg of body mass) or beverage depending on the treatment (described subsequently) with all hydration procedures adapted as previously described (Hamilton et al., 1991). The amount of CHO and PLA powder in capsules was calculated to match the volume of water so that the mixture of the water and powder or water and capsules would correspond to a 6% CHO solution. The mean number of capsules consumed during each treatment was 116 ± 21 for PLA and 122 ± 13 for CHO. Thus, the mean number of capsules per ingestion was 24 ± 3. Each CHO capsule contained 500 mg glucose and 200 mg sucrose, whereas, each capsule of PLA contained 750 mg of placebo powder (whey protein). Differences in the amount of powder chemists managed to fit in each capsule are explained by the difference in the grain size and density of each powder. Even though there was a difference of 50 mg of powder between capsules the number of
capsules to be ingested at each ingestion was calculated according to the amount of CHO in total being ingested which would be enough to make a 6% CHO solution as stated previously, according to the amount of fluid calculated individually; rather than an absolute number of capsules.

The CHO beverage contained the same proportions of glucose and sucrose, plus whey protein, colouring and flavouring. The PLA beverage contained whey protein, colouring and flavouring. The beverage was prepared by a commercial chemist (Stenlake®, Sydney, NSW, Australia) who guaranteed that beverages matched in taste, colour and flavour. The final CHO powder provided 260 mg of sucrose plus 100 mg of glucose/g of powder. Table 5 describes the composition of beverage powders and capsules.

### Table 5. Beverage and Capsules composition

<table>
<thead>
<tr>
<th>Component</th>
<th>PLA Powder (in 100g)</th>
<th>CHO Powder (in 100g)</th>
<th>PLA capsule (00 capsule)</th>
<th>CHO capsule (00 capsule)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid dilutant</td>
<td>99.1</td>
<td>63.2</td>
<td>750 mg</td>
<td>-</td>
</tr>
<tr>
<td>Saccharin</td>
<td>0.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td>10.0</td>
<td>-</td>
<td>200 mg</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>26.0</td>
<td>-</td>
<td>500 mg</td>
</tr>
<tr>
<td>Orange flavour</td>
<td>0.74</td>
<td>0.77</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Orange colour</td>
<td>0.03</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
3.2.5 Physiological measures

When subjects reported to the laboratory they voided. The hydration status was assessed by urine specific gravity (503 Nippon Optical Warks, Japan) before and at the completion of exercise.

Blood samples were taken from the middle finger at rest, at 5 km intervals throughout each time trial and immediately post-exercise for the determination of blood lactate (Lactate Pro®, ARKRAY, Kyoto, Japan) and glucose concentrations (Accu-check Advantage®, Roche Diagnostics Corporation, Indianapolis, U.S.A.). Heart rate was continuously monitored (Polar® Electro OY M61, Kempele, Finland) and recorded at 2 km intervals. Ratings of perceived exertion (RPE) (Borg, 1982) were evaluated every 5 km and also before and after each sprint and when EMG was recorded.

Core temperature ($T_c$) was measured using the CoreTemp™ Telemetry System (HQInc. Wireless Sensing System and Design, Palmetto, Florida, U.S.A.). Participants ingested the telemetry pill the night before the trial and were advised by the researcher at what time it should be ingested according to the participants bowl movement. Skin temperature was monitored with skin thermistors attached at four sites (chest, arm, thigh, leg) and connected to a telethermometer (Zencor/Zentemp 5000, Victoria, Australia) and recorded every 2 km. Mean skin temperature ($T_\text{mean}$) was calculated as previously described (Ramanathan, 1964).

3.2.6 Pacing data

Power output, cadence and speed were monitored throughout the trial using a TACX® Flow Trainer computer (TACX®, T1684, The Netherlands) and recorded every 0.5 km during the LI stages and every 0.2 km during the sprints until the end of exercise.
3.2.7 Neuromuscular Data

EMG from the rectus femoris and vastus medialis were recorded throughout each sprint at 14, 29, 44 and 59 km and for the first 30 s of each LI effort at 7.5, 21.5, 36.5 and 51.5 km. EMG data were captured using a Delsys EMG amplifier (model Bagnoli-4, Delsys, Boston, MA, U.S.A.) with a common mode rejection ratio greater than 90dB and were stored on a host computer. Prior to electrode attachment, skin was prepared by shaving, abrading of the outer layer of epidermal cells, and removing of oil and dirt using an alcohol swab. Single differential pre-amplified recording electrodes with a bandwidth of 20-450 Hz (model DE-2.1, bar configuration of 1mm x 10mm with a 10 mm interelectrode distance) were attached to the anterior surface of the dominant thigh. For the rectus femoris, the electrode was attached half way between the superior anterior iliac spine and the base of the patella. For the vastus medialis, the electrode was attached at the distal 4/5 point between the superior anterior iliac spine and the joint space in front of the anterior border of the medial ligament at the knee. A disposable gel adhesive reference electrode was positioned on the acromial process of the right shoulder. Data were acquired using EMGWorks Signal Acquisition software version 3.0 (Delsys, Boston, MA, U.S.A.) and sampled at rate of 2000 Hz.

For the LI efforts, the entire 30 s of recorded EMG data were used for analysis. For the sprints, data analysis was performed over a 30 s duration, which was obtained from the middle of each sprint. All EMG signals were high-pass filtered using a second order Butterworth filter at 15 Hz to remove movement artefact and quantified as the root-mean-square (RMS) using a sliding window of 125 ms and an overlap of 62.5 ms. EMG signals were quantified by summing all three RMS signals then calculating the area under the curve (integrated RMS). Subsequently, RMS data during the LI efforts and the sprints were normalised against the first 30 s of RMS data recorded at the start of exercise. Power spectrum compression was estimated using raw EMG data previously described (Lowery et al., 2000) using a Fast Fourier Transformation (FFT) algorithm. Spectral power of the raw
signals was determined for each frequency between the 65th to 90th percentiles. An estimate of power shift at each frequency was then determined by normalizing data against the same frequency range obtained from the first 30 seconds of EMG data recorded at the start of exercise. Spectral compression at each interval was finally calculated as the ratio of the average change in spectral power across the entire mid frequency range relative to the data recorded at the start of exercise with the average compression ratio observed between muscles used for analysis. The time taken for neuromuscular testing was 24±8 mins pre-exercise and 38±6 mins post-exercise, considering that the post-exercise protocol includes the sub maximal protocol and 2 repeats of the pre-exercise protocol procedures, explaining the longer duration. Mean core temperature measured during pre-exercise neuromuscular testing was 37.15±0.26 for H₂O, 37.15±0.16 for PLAₐ, 37.09±0.23 for CHOₐ, 37.16±0.23 for PLAₐ and 37.11±0.20 for CHOₐ. Mean core temperature measured during post-exercise neuromuscular testing was 37.34±0.28 for H₂O, 37.25±0.37 for PLAₐ, 37.53±0.22 for CHOₐ, 37.38±0.04 for PLAₐ and 37.62±0.44 for CHOₐ. These procedures were performed using the Matlab™ gait analysis software (The Mathworks Inc, Natick, USA). Figure 2 shows all measurements that took place in this study.
Figure 2. Representation of variables measured during self-paced time trial

1. Exercise duration (mins:secs); 2. Urine specific gravity (USG); 3. Blood glucose (mmol/l); 4. Blood lactate (mmol/l); 5. Rate of perceived exertion (RPE); 6. Heart rate (bpm); 7. Mean skin temperature (°C); 8. Core temperature pill (°C); 9. EMG (Root mean square/μV and Spectral compression/ratio); 10. Pacing data computer (Power/W, Cadence/rpm and Speed Km/h); 11. Cycle trainer; 12. Fan (Wind speed: 3 m/s)
3.2.8 Statistical analysis

The Latin Square design was used to control for sources of variation that could have influenced the target responses other than treatments, such as individual training effects (Portney & Watkins, 2000). Statistical analyses were performed using a 5 x 1 factorial ANOVA with repeated measures. Where a significant main effect was observed, a LSD post-hoc test was used to identify the source of significance. Data were also separated and analysed as start, low intensity efforts, sprints, and end of exercise so that the mean ± SD were calculated for each section of exercise and compared among treatments. The statistical power was calculated using G*Power v.3 software with the following parameters: Power $(1 – \beta) = 0.85$, 5 groups x 10 subjects per group with a correlation between treatments of $r = 0.85$. These parameters yield a total sample size of 50 subjects. This is the sample size that was used in the study design where each group represents 10 subjects x 5 trials. For clarity data were collapsed at each time point for comparison, so that the sprints were compared to the low intensity efforts in addition to comparisons across time points. All statistical analyses were performed using SPSS software (SPSS for Windows 14.0, Chicago, Ill, SPSS Inc.). All data are reported as means ± SD with the level of significance set at $p< 0.05$.

An LSD correction for multiple pairwise comparisons was used for analysis as applying a Bonferroni or Sidak correction would require a $p$ value of $< 0.0006$ to identify a statistically significant difference between comparisons. Although adjustments for multiple pairwise comparisons reduce the chance of a Type I error, they inherently increase the risk of a Type II error. We propose that effectively setting the level of significance at $p< 0.0006$ is much too stringent and would actually lead to more errors of data interpretation due to inflation in the number of Type II errors. Support for this comes from Rothman (1990) who argues against adjusting for multiple pairwise comparisons and suggests each result should be interpreted as its own analysis, regardless of the number of comparisons tested. Figure 3 below shows a timeline of the study.
Figure 3. Outline of the experimental testing procedures used in the present study. The present study reports the effects of CHO ingestion on parameters of neuromuscular fatigue following a sustained submaximal isometric contraction at 20% of the pre-exercise MVC following 60km of self-paced cycling in the heat. MVC, maximal voluntary contraction, H\textsubscript{2}O; water treatment, PLAb; placebo beverage treatment, CHO\textsubscript{b}; carbohydrate beverage treatment, PLAc; placebo capsule treatment, and CHO\textsubscript{c}; carbohydrate capsule treatment.
3.3 RESULTS

3.3.1 Time trial performance

Table 6 shows the mean completion times for each treatment. No differences were observed in time to complete the 60 km self-paced time trial between treatments ($p > 0.05$). The overall mean time to complete a trial was $137.9 \pm 19.2$ min.

Table 6. Mean ± SD exercise duration for each trial in min

<table>
<thead>
<tr>
<th>Treatments</th>
<th>H₂O</th>
<th>PLA₀</th>
<th>CHO₀</th>
<th>PLA₁</th>
<th>CHO₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>138.2</td>
<td>130.2</td>
<td>140.5</td>
<td>143.1</td>
<td>137.3</td>
</tr>
<tr>
<td>±SD</td>
<td>17.5</td>
<td>11.2</td>
<td>18.1</td>
<td>29.2</td>
<td>20.1</td>
</tr>
</tbody>
</table>
**3.3.2 Hydration and metabolic responses**

The blood glucose and blood lactate response during exercise for each treatment are displayed in Figures 4A and 4B, respectively. Blood glucose was higher in both CHO_b and CHO_c treatments compared to the PLA treatments and H_2O treatment during and at the end of the time trial. However, at the end of exercise glucose was not different to values in the H_2O treatment when compared to CHO_c (p= 0.138). This is most likely due to the delay in CHO absorption from the capsules that were ingested at 55 km. No differences in blood lactate were observed among treatments (p> 0.05). Mean pre-exercise blood lactate was 1.7 ± 0.6 mmol/l. This increased to 2.5 ± 1.3 mmol/l during the LI efforts and to 6.9 ± 2.2 mmol/l during the sprints. Subjects finished trials with blood lactate levels of 5.9 ± 1.9 mmol/l. Figure 4 shows blood glucose (A) and blood lactate (B) concentrations during the 60 km time trial for each treatment.
Figure 4. Blood glucose (A) and blood lactate (B) concentrations during the 60 km time trial for each treatment. H₂O; water treatment, PLAᵦ; placebo beverage treatment, CHOᵦ; carbohydrate beverage treatment, PLAᵦ; placebo capsule treatment, CHOᵦ; carbohydrate capsule treatment. Significant differences between treatments for blood glucose indicated as: *H₂O vs CHOᵦ, H₂O vs CHOᵦ, PLAᵦ vs CHOᵦ, PLAᵦ vs CHOᵦ (p< 0.05). **Indicates significant difference in blood lactate between the sprints at 14 – 15 km, 29 – 30 km 44 – 45 km and 59 – 60 km vs low intensity efforts. d Indicates significant difference in blood lactate between the sprint at 14 – 15 km vs the sprints at 29 – 30 km, 44 – 45 km, and 59 – 60 km (p< 0.05). Values presented as mean ± SD.
3.3.3 Heart Rate and RPE

The heart rate response and RPE during exercise for each treatment are displayed in Figures 5A and 5B, respectively. Heart rate was not different among treatments (p > 0.05). However, heart rate was significantly increased during the sprints compared with the LI efforts (p < 0.05). Subjects started exercise with an average heart rate of 71 beats/min which increased to ~157 beats/min during the LI efforts and to ~181 beats/min during the sprints, finishing exercise with a heart rate of ~179 beats/min. The average heart rate during the 60 km time trial was ~160 beats/min. RPE was not different between treatments, however, as expected RPE increased during the sprints compared with the LI efforts. When data were collapsed the average RPE for each time point was 13 ± 3 during the LI efforts and 16 ± 3 during the sprints, reaching 17 ± 3 at the end of the trials. The average RPE during the 60 km time trial was 14 ± 3. Figure 5 shows Heart rate (A) and RPE (B) during the 60 km time trial for each treatment.
Figure 5. Heart rate (A) and RPE (B) during the 60 km time trial for each treatment. H$_2$O; water treatment, PLA$_b$; placebo beverage treatment, CHO$_b$; carbohydrate beverage treatment, PLA$_c$; placebo capsule treatment, CHO$_c$; carbohydrate capsule treatment. *Indicates significant difference at sprints at 14 – 15 km, 29 – 30 km 44 – 45 km and 59 – 60 km compared with low intensity efforts. **Indicates significant difference between sprints at 14 – 15 km and 29 – 30 km for the H$_2$O treatment (p = 0.013). *Indicates significant difference between sprints at 14 – 15 km and 44 – 45 km for the PLA$_b$ (p = 0.017) and CHO$_c$ (p = 0.022) treatments; bIndicates significant difference between sprints at 44 – 45 km and 59 – 60 km for the PLA$_b$ (p = 0.010), CHO$_b$ (p = 0.000), PLA$_c$ (p = 0.048) treatments.
3.3.4 Thermoregulatory Responses

Core temperature and $T_{sk}$ temperature response during exercise for each treatment are displayed in Figures 6A and 6B, respectively. The core temperature ($T_c$) was not different among treatments ($p>0.05$). Subjects started each trial with similar $T_c$ (36.8 – 37.2 °C; $p>0.05$), achieved approximately 37.3 ± 0.5 °C during the LI efforts and 38.1 ± 0.4 °C during the sprints, terminating exercise with a $T_c$ of 38.1 ± 0.5 °C. Subjects maintained an overall $T_c$ throughout exercise of 38.0 ± 0.5 °C. The corresponding $T_{sk}$ was not different between treatments. Subjects started exercise with a $T_{sk}$ of ~32.0 °C which increased to 34.0 ± 0.6 °C ($p<0.05$) during the 60 km trial. Figure 6 shows core temperature (A) and mean skin temperatures (B) during the 60 km time trial for each treatment.
Figure 6. Core temperature (A) and mean skin temperatures (B) during the 60 km time trial for each treatment. H\textsubscript{2}O; water treatment, PLA\textsubscript{b}; placebo beverage treatment, CHO\textsubscript{b}; carbohydrate beverage treatment, PLA\textsubscript{c}; placebo capsule treatment, CHO\textsubscript{c}; carbohydrate capsule treatment. *Indicates significant difference compared with the start of exercise (p< 0.05).
3.3.5 Pacing measures

Power, cadence and speed during the 60 km time trial for each treatment are displayed in Figure 7. There were no differences for power output among treatments (p> 0.05), although the power output during the sprints were consistently higher (p< 0.05) compared to the LI efforts throughout the trial. On average across the five treatments, power output during the LI efforts was 207 ± 47 W compared with 334 ± 73 W during the sprints. The overall power output during all treatments was ~230 W. There were no differences in cadence between treatments (p> 0.05). The mean cadence for the five treatments during the LI efforts was 88 ± 12 rpm which was significantly lower than the 107 ± 18 rpm (p< 0.05) during the sprints for all treatments. The overall cadence during the 60 km was 91 ± 15 rpm. No differences were observed among treatments (p> 0.05). As expected the speed during the LI efforts was reduced across all treatments (27.1 ± 5.8 km/h; p< 0.05) compared to the sprints (38.8 ± 7.9 km/h). The overall speed during the 60 km for the five treatments was 28.6 ± 7.1 km/h. Figure 7 shows power (A), cadence (B) and speed (C) during each 60 km self-paced time trial.
Figure 7. Power (A), cadence (B) and speed (C) during each 60 km self-paced time trial. H₂O; water treatment, PLAₐ; placebo beverage treatment, CHOₐ; carbohydrate beverage treatment, PLAₜ; placebo capsule treatment, CHOₜ; carbohydrate capsule treatment. *Indicates significant difference between sprints at 14 – 15 km, 29 – 30 km, 44 – 45 km and 59 – 60 km compared with low intensity efforts (p < 0.05).
3.3.6 Neuromuscular Responses

The RMS response and spectral compression ratio during each 60 km self-paced time trial are displayed in Figures 8A and 8B, respectively. During the sprints, RMS was significantly higher compared to the LI efforts throughout the trials for all treatments with the exception of PLA$_c$ treatment at 45 km which was similar for preceding and subsequent LI effort. That is, the RMS for the PLA$_c$ did not change significantly from 36.5 – 51.5 km regardless of the trial stage ($p= 0.121$). Notably, the RMS was highest at the first sprint at 15 km compared with the last sprint at 60 km for all treatments except for the PLA$_c$ ($p= 0.302$) and H$_2$O ($p= 0.095$) treatments. Interestingly the RMS was systematically reduced from one sprint to the other for the CHO$_b$ treatment beginning at 15 km. The most striking finding was that regardless of the ingestion mode the RMS recovered for all treatments for the final sprint. Although Figure 7B depicts a trend to higher ratios with increasing distance, the variability between treatments for this measure reduced the possibility of any significant differences between treatments or at any time during the trials compared to the start of the trial. Figure 8 shows root mean square (RMS) (A) and spectral compression ratio (B) during each 60 km self-paced time trial.
Figure 8. Root mean square (RMS) (A) and spectral compression ratio (B) during each 60 km self-paced time trial. H₂O; water treatment, PLA₀; placebo beverage treatment, CHO₀; carbohydrate beverage treatment, PLAₐ; placebo capsule treatment, CHOₐ; carbohydrate capsule treatment. *Indicates significant difference for RMS during the sprints compared with low intensity efforts for all treatments except for PLAₐ at 45 km (p< 0.05); aIndicates significant difference for RMS compared with previous sprint in CHO₀ (p< 0.05); bIndicates significant difference for RMS in 15 km sprint compared with 60 km except for PLAₐ and H₂O (p< 0.05) treatments.
3.4 DISCUSSION

The novel finding of the present study is that performance in a 60 km self-paced cycling time trial in the heat was not improved with CHO ingestion independent of the ingestion method. There are also two previous recent studies that have not reported differences in performance using a different form of CHO ingestion; either infusion or capsules compared with the customary fluids (Carter et al., 2004b; Nassif et al., 2008). These findings are in contrast with several other findings (Eldridge, 1974; Davis et al., 1988; Millard-Stafford et al., 1992; Langenfeld et al., 1994; Anantaraman et al., 1995; El-Sayed et al., 1997) which report enhanced performance with the ingestion of a CHO beverage. It is important to note that the present study was a randomized double blind Latin Square design to account for the placebo effect. For instance, Clark et al. (2000) have suggested that a psychological effect on performance is possible when CHO was thought to be ingested. In the present study, CHO was ingested in capsules in addition to a separate beverage form which provided a robust evaluation of the effects of this substance on exercise performance. Although some studies have used fluids with artificial sweetener as a placebo (Febbraio & Stewart, 1996), the use of this traditional method may not adequately mask the placebo and subjects could potentially discriminate between the PLA and CHO beverage by taste (Clark et al., 2000). It has also been shown that a simple mouth rinse, without actually ingesting the fluid thereby negating its potential physiological effect, can influence performance (Carter et al., 2004a). Conversely, these same authors by using venous infusion of CHO did not report enhanced exercise performance (Carter et al., 2004b), concluding that oral and pharyngeal receptors could be activated when glucose is mouth rinsed leading to a psychological effect that can influence fatigue mechanisms. A study by Chambers et al. (2009) has also suggested that oral receptors in the mouth might be capable of responding to CHO independent of sweetness. These authors showed improvement in performance and activation of reward related areas in the brain when carbohydrates were ingested in a fluid
form. The present study shows that independent of subjects being able to taste the beverages, performance remained unchanged.

In the present study, blood glucose levels were higher during the CHO treatments compared to the PLA and H₂O treatments, which is in agreement with results of other studies (Davis et al., 1988; Millard-Stafford et al., 1992). In contrast, others did not observe differences in blood glucose concentration when evaluating the effect of CHO ingestion during time trial exercise (El-Sayed et al., 1997; Desbrow et al., 2004). Therefore, given our data together with those previously reported we can only conclude that changes in blood glucose of the present magnitude do not significantly alter the exercise performance of subjects, indicating that this parameter may not be critical during long duration self-paced exercise in the heat. As expected, the blood lactate response corresponded to the intensity of exercise so that the highest values were observed during the sprints whilst these values decreased during the LI stages. The blood lactate response was clearly matched by the heart rate response so that the peak heart rate always occurred during the sprints. However, because the blood lactate and heart rate responses were not different between treatments and the values were always equivalent at each time point, this suggests that subjects at least gave an equivalent effort at each of these stages of the trial. Moreover, these data also indicate that CHO, either as a beverage or capsule form does not alter the ability to perform maximally in the heat at least over 60 km under self-paced conditions. Although, the ambient conditions were different to the present one and the distance was 16 km versus our 60 km protocol, similar findings indicating that CHO loading does not improve self-paced exercise have been recently reported (Jeukendrup et al., 2008).

Subjects rated the overall exercise as “somewhat hard” (Borg, 1982). Noakes et al. (2004) have noted the importance of RPE in understanding the mechanisms of fatigue, as some have found the same RPE at the end of exercise in subjects with both high and low intramuscular glycogen content (Baldwin et al., 2003). In the present study a similar RPE at the end of exercise was observed independent of what subjects ingested; CHO, PLA or H₂O. The present subjects had a diet prescribed to minimise variability in intramuscular glycogen
content at the start of exercise. Although this was not confirmed by muscle biopsy, it is reasonable to assume that muscle glycogen stores would be similar with dietary (high CHO content) and exercise control (Flynn et al., 1987; Jeukendrup, 2003). As it is thought that the highest RPE will be reached before complete depletion of the energy substrate (Borg, 1982), there could be a central anticipatory mechanism influencing the maximal RPE that the body can tolerate, ending exercise before this maximal value is attained. It is also important to note that RPE did not reach maximal values during the sprints thereby showing a possible anticipatory control in subject's decision making to reduce intensity in preparation for a final endspurt. However, if glycogen stores were different in any of the treatments we should have observed a difference in exercise performance, but this was not the case.

The possible effects of CHO ingestion on endurance performance should be considered with respect to the subject’s feeding status when treatments were undertaken. Many studies evaluating the effect of CHO ingestion on performance have used fasted subjects (Coyle et al., 1986; Coggan & Coyle, 1987; McConell et al., 2000; Robinson et al., 2002). As this is likely not to be what athletes actually face in real life, studies in which subjects are fed are likely to be a better representation. Interestingly, studies that have used fed subjects (Burke et al., 2000; Desbrow et al., 2004) did not observe enhanced performance, but others have (Millard-Stafford et al., 1992; Langenfeld et al., 1994; Anantaraman et al., 1995). The present findings agree with the results of the CHO loading study by Burke et al. (2000) which showed no enhancement in time trial performance. This finding suggests that exogenous CHO availability during a 60 km time trial in the heat might not influence performance. In addition, previous work has shown that endurance in a hot environment is unlikely to be influenced by CHO availability (Parkin et al., 1999; Febbraio, 2000), although others have suggested a higher demand of CHO utilization when exercise is executed in the heat (Burke, 2001).

It is also important to draw attention to the individual’s hydration status. Even though one of the objectives of ingesting CHO drinks is to provide the athlete with a source of energy, maintaining hydration is crucial, as dehydration has been found to impair
performance (Sawka & Pandolf, 1990; Sawka & Noakes, 2007). However, in the present study subjects started and ended exercise in a hydrated state (as assessed by USG), therefore, hydration could be one reason why differences in performance were not observed. Nevertheless, we have previously observed that enough fluid ingestion preventing any change in body mass does not improve performance compared to restricted fluids at least over 60 min of self-paced exercise in the heat (Kay & Marino, 2003).

Subjects started and ended exercise with similar core temperatures in all treatments, indicating that there was no CHO effect on this variable during the time trial or that the beverage alone produced any appreciable effect on thermoregulation under these conditions. Regardless of the core temperature at the end of exercise which was below 39 °C across all treatments, it is clear that CHO ingestion alone did not attenuate the increase in core temperature when exercise was undertaken in the heat. Although one would expect a more pronounced rise in core temperature over the time span of the present trial (~ 2 h) in the heat, this might have been the case if the exercise was set at a fixed intensity. That is, self-paced exercise does not produce similar physiological responses as those typically observed in fixed intensity exercise as subjects are able to alter their intensity and hence metabolic heat production so that a maximal level of heat accumulation is never reached (Marino et al., 2004; Tucker et al., 2006b; Tucker, 2008).

As no differences in power, speed and cadence were observed among treatments, there did not appear to be any differences in pacing strategies with respect to the substance ingested. Some researchers have found increases in power output with ingestion of CHO pre-exercise, independent of what was ingested during exercise (Widrick et al., 1993; El-Sayed et al., 1997; Fritzsche et al., 2000) whereas others have not (Palmer et al., 1998; Burke et al., 2000; Carter et al., 2004b; Desbrow et al., 2004) which agree with the present results when subjects are fed. Figure 5 clearly shows that during all treatments, power, cadence and speed were a function of the particular stage of the trial so that the highest value was always observed during the sprints. We can only conclude from these data that
Ingestion of CHO independent of the form of ingestion did not influence the conscious pacing strategy adopted by the subjects.

As impaired exercise performance in the heat is thought to be related to a reduction in muscle recruitment (Kay et al., 2001; Tucker et al., 2004), EMG was measured at the different stages of the trials. Figure 5A shows the mean RMS reflecting the amount of muscle recruitment. It is clear that muscle recruitment was generally higher during the sprints compared with the LI stages for all treatments except for the in the latter stages between 36.5 – 51.5 km, when muscle recruitment was similar during the LI and sprint stages. However, for the last sprint in the PLA treatment, muscle recruitment was restored to values observed at the 30 km sprint. This finding can only suggest that if subjects attempted to maintain muscle recruitment to a similar level in the subsequent sprints and LI efforts, a recovery of muscle recruitment for the last sprint might not have been possible. This is evidence that an anticipatory regulatory mechanism was in operation. Notably, muscle recruitment was systematically reduced from one sprint to the next only for the CHO treatment but recovery was evident in the last sprint. An interesting finding is that the muscle recruitment was down-regulated from the initial sprint at 15 km compared to the final sprint for both the PLA and H2O treatments but not for the other treatments (i.e. CHO, CHOc, and PLA). These data suggest that subjects were influenced by what they thought was being ingested so that a placebo effect might have been in operation. That is, with the H2O treatment there was no masking the fluid ingested, and in the CHO treatments the substances were actually CHO, whilst in the PLA no differentiation in taste between this and CHO was possible. However, the most salient result is that muscle recruitment was restored for all trials for the last sprint as previously shown (Kay et al., 2001). Also, even though a considerable reduction in EMG was observed at kilometres 45 and 60, power was very well maintained. A possible explanation for this could be a change in muscle contractibility what could be an evidence of superior muscle contraction and not an effect of fatigue responses. The fact that the pacing strategy as measured by speed, cadence and power output were identical at these distances along with heart rate can only suggests that either these
measures are less sensitive than the measure of muscle recruitment or that muscle recruitment is a tightly controlled response which occurs well ahead of the required exercise end-point. The present findings are similar to those where no differences in iEMG were observed with CHO ingestion in hot and temperate conditions (Abbiss et al., 2008). Spectral compression, which is an indirect measure of the shift from fast to slow twitch muscle fibers did not confirm that this shift occurred to any significant extent with any of the ingestion methods employed.

3.5 CONCLUSION

In conclusion, the ingestion of CHO as either capsules or beverage in a randomized double blind fashion using a Latin Square design did not significantly affect overall performance of a 60 km cycling time trial in the heat when athletes were fed. Although increases in blood glucose concentration were observed, this did not affect any pacing strategies per se although the muscle recruitment (RMS) was seemingly altered as a consequence of the placebo effect.
Chapter 4

STUDY TWO

Carbohydrate ingestion does not influence neuromuscular performance following prolonged cycling in the heat
Abstract Study Two

**Objective:** The influence of carbohydrate (CHO) ingestion on parameters of central and peripheral fatigue following prolonged exercise is yet to be fully determined. Therefore, this present study compares the effects of either a 6% CHO beverage or an equivalent 6% CHO mixture taken as capsules with distilled water versus corresponding placebo treatments and a H₂O treatment on peak isometric torque (MVC), the level of voluntary activation (VA), M-wave amplitude, and evoked twitch contractile properties (TCP) following 60 km self-paced cycling in the heat.

**Methods:** Ten trained male subjects (mean ± SD: 26 ± 3 years; 64.5 ± 7.7 kg and 70.7 ± 8.8 mL.kg⁻¹.min⁻¹ maximal oxygen consumption) completed 5 x 60 km cycling time trials punctuated with 4 x 1 km sprints (14, 29, 44, 59 km) in the heat at 32°C and 50 % relative humidity. In a randomized double blind fashion, subjects ingested either placebo (PLA) in capsules (c) or in a beverage (b) form (PLAc and PLAb) in two trials and 6%CHO in corresponding trials (CHOc and CHOb) in addition to a H₂O trial where they were neuromuscular assessments were performed pre and post-exercise.

**Results:** No differences were observed between treatments in time to complete the 60 km time trial. A comparable reduction in MVC was observed post-exercise across all treatments (p< 0.05). VA was reduced post-exercise in the H₂O treatment only (p= 0.014). M-wave amplitude was unchanged post-exercise in all treatments (p< 0.05). TCP post-exercise were altered across all treatments displaying both potentiation and fatigue-related effects (p< 0.05); however, most changed observed were compared among treatments.

**Conclusion:** The present data suggest that the development of neuromuscular fatigue following prolonged self-paced cycling in the heat was primarily related to peripheral factors affecting intrinsic force production. Furthermore, it appears that CHO ingestion may not provide an ergogenic effect on the parameters of neuromuscular fatigue following such exercise.
4.1 INTRODUCTION

A number of studies have documented the development of neuromuscular fatigue following prolonged exercise in thermoneutral and warm conditions. Such investigations report reductions in maximal voluntary isometric torque (MVC) of the knee extensor ranging from 13-50% following 30-min to 5h of constant load exercise (55-80% VO$_{2\text{max}}$) (Sahlin & Seger, 1995; Booth et al., 1997; Lepers et al., 2000; Lepers et al., 2002; Saboisky et al., 2003; Presland et al., 2005; Stewart et al., 2007). Under such conditions, the decrease in MVC post-exercise appears to be related to both central and peripheral factors. This is demonstrated by reductions in the level of voluntary activation (Nybo & Nielsen, 2001a; Saboisky et al., 2003), decreased sarcolemma excitability (Lepers et al., 2000; Lepers et al., 2002; Stewart et al., 2007), and depressed twitch contractile characteristics (Lepers et al., 2002). However, studies investigating the loss of MVC and the central and/or peripheral contributions to fatigue following long duration, self-paced exercise in the heat are limited (Abbiss et al., 2008). This is surprising given that neuromuscular fatigue is a complex phenomenon and the expression of central and/or peripheral contributions to the loss of MVC observed following prolonged exercise is likely to be task dependent (Abbiss & Laursen, 2005; Barry & Enoka, 2007).

Additionally, it is well established that maintaining adequate hydration is essential for achieving peak performance during prolonged exercise in the heat (c.f. Murray, 2007). However, conflict still exists regarding the ergogenic benefit of adding carbohydrates (CHO) to the ingested fluid (Coyle et al., 1986; Davis et al., 1988; Nassif et al., 2008). Traditionally, CHO ingestion during prolonged activity is thought to enhance performance by preventing hypoglycemia, which may result in muscle glycogen sparing and delay the onset of peripheral muscle fatigue (Coyle et al., 1986; Coggan & Coyle, 1988; Davis et al., 1988). This is thought to be especially important during long-lasting exercise in high ambient temperatures (i.e. > 27°C) due to the shift in substrate utilization (Febbraio et al., 1996b; Burke, 2001). More recent studies also indicate that CHO ingestion may reduce peripheral
fatigue during prolonged exercise by attenuating the decline in neuromuscular transmission and/or muscle membrane excitability leading to more effective neural signalling to the contractile apparatus (Stewart et al., 2007). Furthermore, it has been suggested that CHO ingestion may potentially reduce central fatigue through preventing an increase in serum nonsterified fatty acid levels (Chen et al., 2008) and the accompanying increase in concentration of brain tryptophan and serum serotonin (Fernstrom & Fernstrom, 2006). As such, it appears that the ergogenic effects of CHO supplementation during prolonged exercise in the heat may be exerted at a variety of sites within the neuromuscular system. However, the effects of CHO ingestion on the central and/or peripheral contribution to fatigue following prolonged self-paced exercise are limited (Abbiss et al., 2008).

Furthermore, it is essential that studies investigating the influence of CHO supplementation on performance adequately control for a placebo (PLA) effect as increased motivation associated with believing in a treatment may enhance performance more than the physiological benefit attained (Clark et al., 2000). Although many studies attempt to control for a PLA effect by blinding subjects to the treatments, it remains possible that subjects could correctly identify the treatment during each trial. Evidence for this is provided by Carter et al. (2004a) who had blinded subjects swill, but not swallow, with either a 6.4% maltodextrin solution or water during a 1h cycle time trial in thermoneutral conditions. These authors reported that four of nine subjects identified a difference between maltodextrin solution and water, with three of the four subjects completing time trial quicker when swilling the CHO solution. Together, these data demonstrate the importance of using ingestion methods that eliminate the ability of subjects to identify treatments. Carter et al. (2004b) controlled for the PLA effect by comparing the performance of 1h cycle time trial in thermoneutral conditions when participants were infused with CHO versus a PLA reporting that cycling performance was not significantly different between treatments despite the increased availability of plasma glucose for oxidation with CHO treatment. However, due to the invasive nature of directly infusing CHO or PLA into the bloodstream, the ingestion of CHO or PLA capsules may provide a more practical method to evaluate the effect of CHO supplementation on
neuromuscular performance whilst controlling the PLA effect and also considering a different route of carbohydrate delivery.

Therefore, the purpose of the present study was to compare the effects of either a 6% CHO beverage or an equivalent 6% CHO mixture taken as capsules with distilled water versus corresponding PLA treatments and a H\textsubscript{2}O control treatment on parameters of neuromuscular fatigue in non-fasting subjects following prolonged self-paced cycling in the heat. Specifically, the fatigue parameters examined included M-wave amplitude, evoked twitch contractile properties, MVC, and the level of voluntary activation. Evidence suggests that CHO would possibly improve exercise performance in a 60 km self-paced time trial in the heat, alter pacing strategies according to the type of ingestion, associate these strategies with alterations in skeletal muscle recruitment and alter parameters of neuromuscular fatigue in non-fasting participants following this type of exercise. M-wave amplitude, evoked twitch contractile properties, MVC and the level of voluntary activation would also possibly be altered and double blind ingestions of 6% CHO in a beverage and capsule form versus a placebo would alter the neuromuscular parameters associated with sustained submaximal contractions to fatigue following prolonged exercise in the heat.

4.2 METHODS

4.2.1 Subject Characteristics

Ten male athletes (nine mountain bikers and one triathlete) volunteered to participate in the investigation. All subjects had an extensive history of regular cycle training for at least three years prior to the study and completed a training distance of 350-400 km per week for the three months prior to data collection. All subjects were released by a physician after completing a health screening questionnaire and a physical examination. The physical characteristics of the subjects were as follows; age 26 ± 3 years, mass 64.5 ± 7.7 kg, height
170 ± 9 cm, sum of nine skinfolds 54.2 ± 13.1 mm, peak power output 323 ± 41 W, and maximal oxygen consumption (VO\textsubscript{2max}) 70.7 ± 8.8 mL/kg/min. Skinfold sites were biceps, triceps, chest, sub axila, subscapular, suprailliac, abdomen, thigh and calf. Subjects performed the progressive VO\textsubscript{2max} test using a Flow Trainer (TACX\textsuperscript{®}, T1684, Netherlands) with their own bicycle attached in a thermoneutral environment (22 ± 1 °C) using a previously described protocol (Kay et al., 2001). Prior to testing, subjects attended an information session outlining the nature of the research and possible risks involved and signed a letter of consent. The research was approved by the Ethics in Human Research Committee of the University.

4.2.2 Experimental design and time trial protocol

The details of the time trial protocol are described chapter three (study one). Briefly, however, the protocol consisted of a 60 km self-paced time trial punctuated with 4 x 1 km sprints (at 14, 29, 44 and 59 km) on five separate occasions assigned in a Latin square design. During each time trial, subjects were instructed and encouraged to complete the 60 km distance as fast as possibly by completing the stage between sprints as quickly as possible and performing a maximal effort for the duration of each sprint. Subjects were permitted to alter gear ratio and cadence and pedal standing up when they wanted, other than in the sprints where they were required to remain in a seated position. Trials took place in an environmental chamber (Russels\textsuperscript{®} Technical Products, model WMD-1150-5, Holland, MI, U.S.A.) in the heat (32 °C and 50% relative humidity). During each trial air movement through the chamber was provided by a fan positioned in front of the subject with a wind speed of 3 m/s in addition to the air circulation provided by the chamber. Subjects were given feedback about cadence at all times and distance at each 3 km interval. No feedback about time, blood results or heart rate was available to subjects during any treatments until the study was completed.
For each of the five time trials, subjects ingested either a placebo (PLA), 6% carbohydrate (CHO), or water (H₂O) in a double-blind fashion. The PLA and CHO treatments were each administered across two trials each using a different method of ingestion; as a beverage (b) or in capsules (c) with distilled water. Therefore, each subject underwent two PLA treatments (PLAb and PLAc) and two CHO treatments (CHOb and CHOc). Subjects were informed they were ingesting CHO for all beverage and capsule treatments. The H₂O treatment was used as a control. All treatments other than H₂O were coded and prepared by an individual not directly involved with the study. Treatment contents were revealed to the investigators at the completion of the study. Subjects ingested fluids at five intervals during each trial; at 5km, after each sprint (15, 30, 45km) and at 55km, before the last sprint. Fluids were given to subjects at 4 ± 1°C. Subjects received 284 ± 30 mL of beverage or 24 ± 3 capsules plus 284 ± 30 mL of distilled water at each of the five intervals with all hydration procedures adapted from Hamilton et al. (1991) (i.e. 4 mL of fluid per kg of body mass). For each subject, the amount of CHO power in beverage and the number of capsules consumed with distilled water were carefully calculated to ensure the mixture corresponded to a 6% CHO solution. The exact composition of the CHO and PLA treatments have been described in a companion paper (chapter three) and were prepared by a commercial chemist (Stenlake®, Sydney, NSW, Australia) to ensure PLAb and CHOb matched in colour and flavour.

4.2.3 Pre-testing requirements

Subjects performed all trials at the same time of day and day of the week with each trial separated by 12 ± 7 days. Subjects were required to maintain their usual training routine up to 24 h prior to each treatment, after which they were instructed to abstain from strenuous activity, caffeine, and alcohol consumption. For two days prior to each trial, subjects were required to consume a diet with 60-70% CHO, 1.2-1.7 g of protein/kg of body mass, and 20-
25% of fat, which was prescribed individually by a dietitian. The purpose of this diet was to ensure complete recovery of muscle glycogen stores following the last day of training prior to each treatment. Subjects were not required to fast overnight prior to each treatment and were required to consume the same morning meal for each treatment.

4.2.4 Physiological and metabolic measures

The hydration status of subjects were assessed when they arrived at the laboratory and also post-exercise by urine specific gravity (503 Nippon Optical Warks, Japan). Core temperature was measured using the CoreTemp™ Telemetry System (HQInc. Wireless Sensing System and Design, Palmetto, Florida, U.S.A.). Blood samples were taken from the middle finger at rest, at 5 km intervals throughout each time trial and immediately post exercise for the determination of blood glucose (Accu-check Advantage®, Roche Diagnostics Corporation, Indianapolis, U.S.A.) and blood lactate concentration (Lactate Pro®, ARKRAY, Kyoto, Japan).

4.2.5 Neuromuscular testing apparatus

Neuromuscular testing was performed before and after each of the five time trials. All tests were performed on the dominant knee extensors under isometric conditions using a custom testing device (Master Equipamentos, Belo Horizonte, Brazil). Testing was performed with subjects seated upright with the hip flexed at 90° (0° being full extension) and the knee flexed at 65° (0° being full extension). Once positioned, subjects were secured in the chair via waist and shoulder straps. During all tests, subjects crossed their arms against the chest to ensure that additional forces did not contribute to performance. The lever arm axis of the testing device was aligned with the lateral epicondyle of the femur with the lower leg attached to the lever arm 1 cm above the lateral malleolus of the ankle. Voltage signals from a force cell with
a 100 kg capacity (model 601, Tedea-Huntleigh, Don Mills, Canada) were fed to a host computer and were A/D converted at 16-bit resolution (DAQCard-6024E, National Instruments, Austin TX, U.S.A), low-pass filtered at 5 Hz, and sampled at a rate of 1000 Hz using data acquisition software (DASYLab version 9.0, Dasytec USA Inc, Bedford NH, USA). Force cell specifications indicate it had a linear response to loading (± 0.02%) represented by the equation; f(x) = ax + b. The voltage signal from the force cell while unloaded was determined and multiplied by −1. This value was used in the equation above (variable b) to zero the signal. Subsequently, a series of known loads were placed on the force cell and the corresponding voltages determined. The ratio between the increase in load applied to the force cell and the increase in the voltage signal generated was determined (variable a) and was used to convert force cell signals (variable x) into units of measurement (i.e. newtons; variable f(x)). These procedures were performed using data acquisition software (DASYLab version 9.0, Dasytec USA Inc, Bedford NH, USA). A representative subject positioned ready for neuromuscular testing is presented in Figure 9.
Figure 9. A representative subject positioned ready for neuromuscular testing.
4.2.6 M-wave Amplitude

Electromyography (EMG) signals from the rectus femoris (RF) and vastus medialis (VM) of the dominant leg were captured during maximal evoked testing. EMG signals were detected using single-differential pre-amplified EMG electrodes (bar configuration 1mm x 10mm; interelectrode distance 10mm; bandwidth of 20-450 Hz) (model DE-2.1, Delsys, Boston, MA, USA) linked to an amplifier where signals where acquired at a gain of 100V/V with common mode rejection ratio >90dB (model Bagnoli-4, Delsys, Boston, MA, USA). Electrodes were positioned on the RF and VM according to SENIAM recommendations (Hermens & Freriks, 1997). Skin was first prepared by shaving, abrading of the outer layer of epidermal cells, and removing of oil and dirt using an alcohol swab. For the rectus femoris, the EMG electrode was attached half way between the superior anterior iliac spine and the superior part of the patella. For the vastus medialis, the EMG electrode was attached at the distal 4/5 point between the superior anterior iliac spine and the joint space in front of the anterior border of the medial ligament at the knee. A disposable gel adhesive electrode was used as a reference (#2330, 3M, Red Dot, St. Paul, MN, USA), which was positioned on the acromial process of the right shoulder. Signals were fed to a host computer (DAQCard-6024E, National Instruments, Austin TX, USA), and sampled using EMGWorks Signal Acquisition software version 3.0 (Delsys, Boston, MA, USA.) at a rate of 2000 Hz.

Muscle activation was achieved by percutaneous stimulation of the intramuscular branches of the femoral nerve using two 20 mm diameter disposable foam electrodes (Medi-trace 200 series, Kendall, USA) positioned about the medio-anterior aspect of the upper thigh directly below the inguinal fold and at the ventral portion of the vastus medialis. The current applied to the nerve was delivered by a constant-current stimulator (Digitimer DS7, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) using a single square-wave pulse with a width of 200 µs (400 V with a current of 15-80 mA) linked to a host computer (DAQCard-6024E, National Instruments, Austin TX, USA). Initially, the current was manually applied in
incremental steps until a muscle twitch of moderate amplitude was observed on the computer monitor. Stimulating electrodes positions were then adjusted medially and/or laterally until the site most responsive to stimulation was located. The electrodes were then secured in position and the location marked on the skin for future assessments. The stimulus intensity was gradually increased until a plateau in twitch amplitude was achieved, where the stimulus intensity was increased by 25% to ensure that supramaximal stimulation was delivered. For testing, five pulses, each separated by 10 s, were delivered with the subject at complete rest. This was determined based on the absence of EMG signals from the rectus femoris or vastus medialis and the absence of any load placed on the force cell other than that due to the effect of gravity on the lower leg. For processing, M-wave data for both RF and VM were averaged across all five treatments with the mean analysed for peak to peak amplitude.

4.2.7 Evoked twitch contractile properties

Immediately following M-wave testing, the dominate knee extensors were assessed by evoked twitch properties. Muscle activation was achieved by using the same electrode locations, testing apparatus, and stimulation parameters as previously described for assessing M-wave amplitude. For testing, five pulses, each separated by 10 s, were delivered with the subject at complete rest. This was determined based on the absence of EMG signals from the RF or VM and the absence of any load placed on the force cell other than that due to the effect of gravity on the lower leg. Force data were exported into spreadsheet software and multiplied by lever arm length to express data in units of torque (Nm). Torque data were then corrected for the effect of gravity on the lower leg, which was achieved by determining the average load applied to the force cell during the 50 ms period immediately before stimulation. Evoked torque-time curves were averaged across all treatments with the mean used to determine; 1) peak twitch torque (Pt; defined as the highest isometric torque value achieved during the evoked contraction), 2) time to peak
torque (TPt; defined as the time from torque onset to Pt), 3) half-relaxation time (½RT; defined as the time required for Pt to decline by half), 4) contraction duration (CD; TPt plus ½RT), 5) the rate of torque development (RTD; defined as the mean tangential slope of the twitch torque-time curve between the onset of torque development and Pt), and 6) the rate of relaxation (RR; defined as the mean tangential slope of the twitch torque-time curve between Pt and ½RT). For all evoked twitch contractions, torque onset was defined as the point at which torque data following stimulation increased beyond 2 SD of the mean torque calculated over the 50 ms period immediately before stimulation (Cannon et al., 2008).

4.2.8 Maximal voluntary contraction

The assessment of maximal voluntary isometric torque (MVC) consisted of up to eight attempts where subjects were instructed to attain peak force as fast as possible and continue exerting maximal effort for a period of 5 s. A minimum rest period of 30 seconds separated each attempt and testing continued until the final three attempts had values within ± 5% of each other. Strong verbal encouragement was provided during all voluntary efforts and subjects received continuous visual feedback of performance from a computer monitor. Force data were exported into spreadsheet software and corrected for the effect of gravity on the lower leg offline, which was achieved by determining the average load applied to the force cell during the 1 s period immediately before voluntary muscle contraction while the subject was at complete rest. The average load applied to the force cell during this period was used to offset the performance data obtained during testing. Following this, performance data were multiplied by lever arm length to express data in units of torque (Nm). MVC for each subject was determined as the single highest torque value produced among trials. A minimum of 30 s rest elapsed between attempts.
4.2.9 Voluntary activation

Following the assessment of MVC, the level of voluntary activation of the dominant knee extensors was assessed using the twitch superimposition technique (Saboisky et al., 2003). Muscle activation was achieved by using the same electrode locations, testing apparatus, and stimulation parameters used for assessing evoked twitch contractile properties. Testing consisted of a series of four attempts whereby subjects received a superimposed stimulus during a MVC. For all attempts, subjects were instructed to produce maximal effort as fast as possible and continue exerting maximal effort until instructed to relax, which was typically within 3-4 s. During each attempt, the trigger for stimulation was manually primed ~1 s after initiation of each contraction. Once primed, the stimulus was automatically triggered when software detected a decline in peak force (DASYLab version 9.0, Dasytec USA Inc, Bedford NH, USA). Manually priming the trigger was necessary to prevent premature stimulation prior to the attainment of peak force. When primed, the decline in peak force necessary to automatically trigger the stimulus was <1%. A minimum of 30 s rest elapsed between each attempt. Strong verbal encouragement was provided during all voluntary efforts and subjects received continual visual feedback of performance from a computer monitor.

For analysis, force data were exported into spreadsheet software and corrected for the effect of gravity on the lower leg offline, which was achieved by determining the average load applied to the force cell during the 1 s period immediately before voluntary muscle contraction while the subject was at complete rest. The average load applied to the force cell during this period was used to offset the performance data obtained during testing. Following this, data were multiplied by lever arm length to express data in units of torque (Nm). Superimposed MVC was determined as the peak torque value produced during the 50–150 ms period subsequent to the delivery of the stimulus. Voluntary peak torque during each superimposed contraction prior to stimulation was determined as the mean torque value produced during the 25 ms before stimulus delivery. The level of voluntary activation using
the following equation: \( \text{Voluntary Activation (\%) = \left( \frac{\text{Voluntary peak torque}}{\text{Superimposed torque}} \right) \times 100} \). All attempts were assessed with the attempt yielding the highest level of voluntary activation used for subsequent analysis. The method used to calculate the level of voluntary activation is provided in Figure 8. It should be noted that due to the small decline (<1%) in peak force necessary to automatically trigger the stimulus during the superimposed contractions, the level of voluntary activation may be underestimated. However, this decline is less than the variance observed in the measurement and is unlikely to have any meaningful impact on the data obtained. Figure 10 shows the method used to calculate the level of voluntary activation.
Figure 10. Method used to calculate the level of voluntary activation. A) Superimposed maximal voluntary isometric contraction with the stimulus trigger identified. B) Magnified section from A identifying the stimulus trigger, voluntary peak torque, and superimposed torque. Voluntary activation was calculated as; Voluntary Activation (%) = (Voluntary peak torque / Superimposed torque) × 100.
4.2.10 Statistics

These data are part of a larger investigation. As such, the entire data set for each variable were analysed collectively using a 5 x 1 factorial repeated measures ANOVA with a LSD adjustment for multiple pairwise comparisons. The level of significance was set at $p < 0.05$. All statistical procedures were performed using SPSS™ for MS-Windows version 14.0 (Statistical Package for the Social Sciences, Chicago, IL). An LSD correction for multiple pairwise comparisons was used for analysis as applying a Bonferroni or Sidak correction would require a $p$ value of $< 0.0006$ to identify a statistically significant difference between comparisons. Although adjustments for multiple pairwise comparisons reduce the chance of a Type I error, they inherently increase the risk of a Type II error. We propose that effectively setting the level of significance at $p < 0.0006$ is much too stringent and would actually lead to more errors of data interpretation due to inflation in the number of Type II errors. Support for this comes from Rothman (1990) who argues against adjusting for multiple pairwise comparisons and suggests each result should be interpreted as its own analysis, regardless of the number of comparisons tested.
4.3 RESULTS

4.3.1 Time trial performance and physiological measures

The mean time to complete the time trial across treatments was 137.9 ± 19.2 min and was not significantly different between treatments (p> 0.05). Urine specific gravity pre and post-exercise was >1029 in all cases and was not significantly different within or between treatments (p> 0.05). This indicates that subjects commenced and terminated all trials in a hydrated state. Core temperature and blood glucose values pre and post-exercise for each treatment are displayed in Table 6. Core temperature significantly increased over time in all treatments (p< 0.05); however, the increase in core temperature observed and the final post-exercise core temperature values recorded were not significantly different between treatments. Blood glucose concentration was significantly lower post-exercise in the H₂O, PLA₀, and PLA₀ treatments and was significantly higher post-exercise in both the CHO₀ and CHO₀ treatments compared with all other treatments (p< 0.05); except between the CHO₀ treatment and the H₂O treatment (p= 0.138). This is most likely explained by a delay in the absorption of CHO from the capsules that were ingested at 55 km. Table 7 shows core temperature and blood glucose pre and post-exercise for each treatment.
Table 7: Core temperature and blood glucose pre and post-exercise for each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post-Exercise Core Temperature (°C)</th>
<th>Post-Exercise Blood Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>37.0 ± 0.2</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>PLA₀</td>
<td>37.1 ± 0.2</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>CHO₀</td>
<td>37.0 ± 0.3</td>
<td>5.7 ± 0.8</td>
</tr>
<tr>
<td>PLA₁</td>
<td>36.9 ± 0.4</td>
<td>5.7 ± 0.8</td>
</tr>
<tr>
<td>CHO₁</td>
<td>37.0 ± 0.2</td>
<td>5.9 ± 0.8</td>
</tr>
<tr>
<td>Mean</td>
<td>37.0 ± 0.3</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>38.0 ± 0.5*</td>
<td>4.9 ± 0.6*</td>
</tr>
<tr>
<td>PLA₀</td>
<td>38.1 ± 0.4*</td>
<td>5.0 ± 0.5*</td>
</tr>
<tr>
<td>CHO₀</td>
<td>38.2 ± 0.5*</td>
<td>5.8 ± 0.5#</td>
</tr>
<tr>
<td>PLA₁</td>
<td>38.0 ± 0.5*</td>
<td>4.9 ± 0.3*</td>
</tr>
<tr>
<td>CHO₁</td>
<td>38.2 ± 0.6*</td>
<td>5.7 ± 0.9*</td>
</tr>
<tr>
<td>Mean</td>
<td>38.1 ± 0.5*</td>
<td>5.3 ± 0.7</td>
</tr>
</tbody>
</table>

Pre; pre-exercise, Post; post-exercise, H₂O; water treatment, PLA₀; placebo beverage treatment, CHO₀; carbohydrate beverage treatment, PLA₁; placebo capsule treatment, CHO₁; carbohydrate capsule treatment, Mean; mean value across all treatments. *Indicates significance difference from pre-exercise measure. †Indicates significant difference from H₂O, PLA₀, and PLA₁ treatments post-exercise. +Indicates significant difference from the PLA₀ and PLA₁ treatments post-exercise. Values are presented as mean ± SD.
4.3.2 *Neuromuscular performance*

MVC, the level of voluntary activation, and M-wave amplitude pre and post-exercise for each treatment are presented in Table 8. MVC pre-exercise was comparable between treatments (p > 0.05) and was observed to decreased post-exercise in all treatments to a similar extent (~13%; p < 0.05) and the magnitude of the decrease was comparable between all treatments.
Table 8. Maximal voluntary contraction, the level of voluntary activation, and M-wave amplitude data pre and post-exercise for each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MVC (Nm)</th>
<th>VA (%)</th>
<th>M-wave Amplitude (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RF</td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>178 ± 35</td>
<td>97.2 ± 3.3</td>
<td>5.8 ± 1.0</td>
</tr>
<tr>
<td>PLA₀</td>
<td>172 ± 39</td>
<td>97.3 ± 2.3</td>
<td>5.2 ± 1.8</td>
</tr>
<tr>
<td>CHO₀</td>
<td>168 ± 38</td>
<td>97.8 ± 1.4</td>
<td>6.5 ± 0.8</td>
</tr>
<tr>
<td>PLA₁</td>
<td>169 ± 38</td>
<td>97.4 ± 2.1</td>
<td>5.8 ± 1.1</td>
</tr>
<tr>
<td>CHO₁</td>
<td>180 ± 35</td>
<td>97.3 ± 3.8</td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>173 ± 37</td>
<td>97.4 ± 2.6</td>
<td>6.0 ± 1.1</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>157 ± 42*</td>
<td>95.1 ± 5.2*</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>PLA₀</td>
<td>153 ± 38*</td>
<td>96.0 ± 3.8</td>
<td>5.6 ± 1.7</td>
</tr>
<tr>
<td>CHO₀</td>
<td>150 ± 43*</td>
<td>94.7 ± 8.2</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
<td>PLA₁</td>
<td>155 ± 36*</td>
<td>97.0 ± 3.1</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>CHO₁</td>
<td>152 ± 36*</td>
<td>95.3 ± 7.6</td>
<td>6.2 ± 0.9</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>153 ± 40*</td>
<td>95.6 ± 5.6</td>
<td>6.4 ± 1.0</td>
</tr>
</tbody>
</table>

Pre; pre-exercise, Post; post-exercise, MVC; maximal voluntary isometric torque, VA; voluntary activation, RF; rectus femoris, VM: vastus medialis, MVC, maximal voluntary contraction, VA; level of voluntary activation, H₂O; water treatment, PLA₀; placebo beverage treatment, CHO₀; carbohydrate beverage treatment, PLA₁; placebo capsule treatment, CHO₁; carbohydrate capsule treatment, Mean; mean value across all treatments. *Indicates significance difference from pre-exercise measure (p< 0.05). VA post-exercise was significantly lower compared to pre exercise values (p= 0.014). Values are presented as mean ± SD.
The level of voluntary activation pre-exercise was comparable between all treatments (p > 0.05) and remained unchanged post-exercise (p > 0.05); except for the H$_2$O treatment where a significant decline was observed (–2.1%; p = 0.014). M-wave peak to peak amplitude pre and post-exercise within and between treatments were not different for either RF or VM (p > 0.05).

Evoked twitch contractile properties pre and post-exercise for each treatment are presented in Table 9. No differences between treatments were observed pre-exercise in any evoked twitch properties assessed (p > 0.05). Comparable increases in both Pt and RTD were observed post-exercise for all treatments (16% and 38%, respectively; p < 0.05); except for CHO$_b$ where the increase in Pt post-exercise did not reach significance (p = 0.09). A trend was observed for RR to decrease post-exercise in the CHO$_c$ treatment (p = 0.054); except for the CHO$_c$ treatment where the decrease observed did not reach significance (p = 0.057). Decreases in TPt were observed post-exercise in the PLA$_c$, CHO$_b$ and CHO$_c$ treatments (–11%; p < 0.05). ½RT increased post-exercise in the CHO$_c$, PLA$_b$ and PLA$_c$ treatments (13%; p < 0.05) while a trend was observed for ½RT to increase in the treatment; CHO$_b$ treatment (p > 0.05). CD was similar pre and post-exercise in all treatments (p > 0.05). TPt was longer post-exercise in the CHO$_b$ treatment compared with the CHO$_c$ treatment (p < 0.05) and a trend was observed for TPt to be longer post-exercise in the CHO$_b$ treatment compared with the PLA$_b$ and PLA$_b$ treatments (p = 0.08). Example evoked twitch contractions pre and post-exercise from a representative subject are displayed in Figure 11 and Table 9 shows evoked twitch contractile properties pre and post-exercise for each treatment.
Figure 11. Sample of evoked twitch contractions pre and post-exercise from subject 1, PLA_c treatment. The post-exercise evoked contraction is labelled for the twitch contractile properties assessed: peak twitch torque (A), rate of torque development (B), time to peak torque (C), rate of relaxation (D), half-relaxation time (E), and contraction duration (F). Note the presence of both potentiation (A and B) and fatigue (D and E) in the post-exercise twitch contraction.
Table 9. Evoked twitch contractile properties pre and post-exercise for each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pt (Nm)</th>
<th>RTD (Nm.s⁻¹)</th>
<th>RR (Nm.s⁻¹)</th>
<th>T Pt (ms)</th>
<th>½RT (ms)</th>
<th>CD (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>36.5 ± 9.4</td>
<td>510.9 ± 153.9</td>
<td>176.0 ± 47.4</td>
<td>87.3 ± 9.3</td>
<td>92.6 ± 15.6</td>
<td>179.9 ± 17.3</td>
</tr>
<tr>
<td>PLAᵇ</td>
<td>37.7 ± 11.8</td>
<td>535.9 ± 197.8</td>
<td>167.6 ± 66.7</td>
<td>87.0 ± 7.4</td>
<td>95.2 ± 9.2</td>
<td>173.4 ± 30.6</td>
</tr>
<tr>
<td>CHOᵇ</td>
<td>36.1 ± 9.4</td>
<td>553.0 ± 159.5</td>
<td>191.1 ± 66.2</td>
<td>83.8 ± 9.2</td>
<td>89.6 ± 15.8</td>
<td>173.4 ± 13.9</td>
</tr>
<tr>
<td>PLAᶜ</td>
<td>36.3 ± 9.2</td>
<td>532.2 ± 134.7</td>
<td>168.5 ± 51.2</td>
<td>87.1 ± 8.3</td>
<td>94.2 ± 14.1</td>
<td>174.6 ± 31.2</td>
</tr>
<tr>
<td>CHOᶜ</td>
<td>38.5 ± 11.9</td>
<td>522.7 ± 165.2</td>
<td>192.8 ± 65.0</td>
<td>88.7 ± 4.3</td>
<td>90.9 ± 16.1</td>
<td>170.7 ± 30.0</td>
</tr>
<tr>
<td>Mean</td>
<td>37.1 ± 10.0</td>
<td>530.9 ± 157.3</td>
<td>179.2 ± 58.4</td>
<td>86.8 ± 7.8</td>
<td>92.5 ± 14.0</td>
<td>174.4 ± 26.1</td>
</tr>
<tr>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>44.2 ± 10.7*</td>
<td>708.1 ± 242.8*</td>
<td>169.8 ± 36.9</td>
<td>72.1 ± 8.6</td>
<td>102.7 ± 14.5</td>
<td>174.8 ± 12.9</td>
</tr>
<tr>
<td>PLAᵇ</td>
<td>43.0 ± 11.5*</td>
<td>742.7 ± 216.6*</td>
<td>147.7 ± 41.9</td>
<td>70.9 ± 7.7</td>
<td>109.8 ± 14.8*</td>
<td>174.5 ± 14.8</td>
</tr>
<tr>
<td>CHOᵇ</td>
<td>43.6 ± 10.4</td>
<td>704.1 ± 261.1*</td>
<td>159.6 ± 53.3</td>
<td>75.0 ± 10.9*</td>
<td>107.2 ± 23.7*</td>
<td>182.2 ± 22.0†</td>
</tr>
<tr>
<td>PLAᶜ</td>
<td>42.0 ± 10.6*</td>
<td>737.0 ± 237.1*</td>
<td>150.4 ± 37.7</td>
<td>70.4 ± 8.9*</td>
<td>106.4 ± 12.3*</td>
<td>170.9 ± 17.9</td>
</tr>
<tr>
<td>CHOᶜ</td>
<td>42.7 ± 10.2*</td>
<td>764.5 ± 210.0*</td>
<td>148.6 ± 31.8</td>
<td>68.4 ± 9.9*#</td>
<td>105.6 ± 11.3*</td>
<td>167.2 ± 17.0</td>
</tr>
<tr>
<td>Mean</td>
<td>43.1 ± 10.3*</td>
<td>731.3 ± 225.7*</td>
<td>155.2 ± 40.2</td>
<td>71.4 ± 9.1</td>
<td>106.3 ± 15.4*</td>
<td>173.9 ± 18.1</td>
</tr>
</tbody>
</table>

Pre; pre-exercise, Post; post-exercise, Pt; peak twitch torque, RTD; rate of torque development, RR; rate of relaxation, T Pt; time to peak torque, ½RT; half-relaxation time, CD; contraction duration, H₂O; water treatment, PLAᵇ; placebo beverage treatment, CHOᵇ; carbohydrate beverage treatment, PLAᶜ; placebo capsule treatment, CHOᶜ; carbohydrate capsule treatment, Mean; mean value across all treatments.

*Indicates significance difference from pre-exercise measure. †Indicates significant difference from PLAᶜ and CHOᶜ treatments post-exercise. Values are presented as mean ± SD.
4.4 DISCUSSION

The development of neuromuscular fatigue during prolonged exercise has been the focus of considerable research in recent years (Lepers et al., 2000; Lepers et al., 2001; Nybo & Nielsen, 2001b; Lepers et al., 2002; Millet et al., 2003; Saboisky et al., 2003), yet few studies have examined the influence of CHO ingestion on the central and/or peripheral contributions to decreased performance following such exercise (Nybo, 2003; Stewart et al., 2007; Abbiss et al., 2008). This study applied a robust experimental design to examine the effects of 6% CHO ingestion on the central and/or peripheral contributions to neuromuscular fatigue in non-fasting subjects following prolonged self-paced cycling in the heat. The major findings were that; 1) the reduction in MVC post-exercise was comparable between all treatments; 2) a decline in level of voluntary activation was observed post-exercise for the H_2O treatment only; 3) M-wave amplitude was unchanged post-exercise for all treatments; and 4) evoked twitch properties post-exercise were altered in all treatments demonstrating evidence for both potentiation and fatigue. These data suggest that the development of neuromuscular fatigue following prolonged, self-paced cycling in the heat was primarily related to peripheral factors. Furthermore, CHO supplementation, irrespective of the method of ingestion, did not attenuate the reduction in MVC or influence the central and/or peripheral contributions to fatigue.

The reduction in MVC observed post-exercise is comparable to losses reported in previous studies examining prolonged constant load cycling (Sahlin & Seger, 1995; Booth et al., 1997; Lepers et al., 2000; Lepers et al., 2002; Saboisky et al., 2003). As such, the extent of fatigue observed in the present study appears comparable with previous investigations. Additionally, the failure to observe an attenuated loss in MVC post-exercise with CHO ingestion is also in agreement with a previous study involving combine constant load and self-paced cycling in the heat (Abbiss et al., 2008). However, Stewart et al. (2007) reported reductions in MVC of 39% and 50% at 90-min and at exhaustion, respectively, after cycling.
at a constant load in ambient conditions in the PLA treatment were less marked compared with the CHO treatment. The ergogenic effect of CHO on MVC post-exercise reported by Stewart et al. (2007) may be related to substantial reduction in MVC observed, possibly because subjects were untrained. However, this is yet to be confirmed. Due to the limited availability of data, it remains unclear if, and under what conditions, CHO ingestion during prolonged exercise can attenuate the loss in post-exercise MVC.

A number of studies provide evidence for a decline in the level of voluntary activation of the active muscle group following prolonged cycling in both thermoneutral conditions (Lepers et al., 2001; Lepers et al., 2002) and in the heat (Nybo & Nielsen, 2001a; Saboisky et al., 2003; Abbiss et al., 2008). The results of the present study conflict somewhat with previous findings as the level of voluntary activation was unchanged post-exercise in all PLA and CHO treatments. It is unclear why the level of voluntary activation did not decline with exercise in the present study, especially since exercise was performed in the heat. It has been argued that exercise-induced hyperthermia may be associated with a decline in central drive in an attempt to limit heat storage and prevent cellular catastrophe (Marino, 2004). Although the final core temperatures observed in the present study were moderate compared with the proposed critical core temperature of ~39.5°C (Nielsen & Nybo, 2003), subjects may have subconsciously regulated exercise intensity using an anticipatory feed-forward mechanism to control heat storage (Tucker et al., 2006b). Despite this, however, reductions in the level of voluntary activation following self-paced prolonged exercise in the heat have been observed with final core temperatures similar those reported here (Saboisky et al., 2003; Abbiss et al., 2008). As such, an insufficient rise in core temperature alone cannot explain these results. The possibility that the training status of subjects partially influenced the present results cannot be dismissed. A previous study also failed to observe an effect of CHO ingestion of the level of voluntary activation after constant load cycling in ambient conditions (Stewart et al., 2007). Additionally, Abbiss et al. (2008) reported that although significant reductions in the level of voluntary activation following prolonged cycling in the
heat were apparent following both the CHO and PLA treatments, the decreases observed were comparable between treatments.

Although voluntary activation levels in the present study were unchanged post-exercise in all CHO and PLA treatments, a decline in the level of voluntary activation post-exercise was observed following exercise in the H\textsubscript{2}O treatment. This could be explained by the fact that subjects were less motivated to perform during this treatment as they were aware that water only was being ingested. Alternately, it is possible that the decrease observed may represent a true physiological impairment associated with exercise; however, a placebo effect during the PLA treatments may have been operating that was equal to the benefit provided by CHO ingestion (Clark et al., 2000). Such a phenomenon may also explain why Abbiss et al. (2008) did not observe increased voluntary activation levels post-exercise in the CHO treatment despite a significant decrease in serum free fatty acids and unchanged serum serotonin concentrations. Based on the available results, it appears that any increase in the level of voluntary activation following prolonged exercise associated with CHO supplementation is probably no greater than the benefit attained due to a placebo effect.

It is possible fatigue may reduce M-wave amplitude due to the increased concentration of K\textsuperscript{+} and ammonia (MacLaren et al., 1989), which may impair ionic processes by disrupting Na\textsuperscript{+}-K\textsuperscript{+} gradients. Furthermore, it has been suggested that CHO ingestion may protect muscle membrane excitability following prolonged exercise by providing blood glucose to fuel Na\textsuperscript{+}-K\textsuperscript{+} pump activity (Okamoto et al., 2001). However, decreases in M-wave amplitude following prolonged exercise are inconsistently reported in the literature (Lepers et al., 2001; Lepers et al., 2002; Saboisky et al., 2003); although, CHO ingestion may offer a protective effect from a decline in M-wave amplitude (Green et al., 2007; Stewart et al., 2007). In the present study, M-wave amplitude in both RF and VM were comparable pre and post-exercise in all treatments. This finding is interpreted to mean the effectiveness of neuromuscular transmission and sarcolemma and T-tubule excitability remained unchanged following exercise. Thus, neural signalling to the muscle was probably not compromised
post-exercise. Previous studies that report decreases in M-wave amplitude following prolonged exercise differ markedly with regard to the duration of exercise required to induce such an effect. For example, Stewart et al. (2007) observed a decline in M-wave amplitude from the vastus medialis after 90-min of cycling, while Lepers et al. (2002) noted that M-wave amplitude in the vastus lateralis was not depressed until after 4h of cycling. Hence, the factors contributing to such changes are likely to be complex. Furthermore, studies reporting a decline in M-wave amplitude post-exercise and a protective effect from CHO ingestion all involve untrained subjects performing constant load exercise (Lepers et al., 2002; Stewart et al., 2007). The failure of observe a decrease in M-wave amplitude in the present study may have been related to training history of the subjects involved. As such, it is possible that the expression of peripheral fatigue in trained subjects may not include impaired neuromuscular transmission or sarcolemma action potential propagation (Behm & St-Pierre, 1998; Nielsen & Clausen, 2000). Also, unlike constant load exercise, subjects were able to regulate exercise intensity throughout the duration of the self-paced protocol (Tucker et al., 2004). This may increase opportunities for motor unit substitution (Bawa et al., 2006) and possibly provide greater intermittent recovery for the neuromuscular junction and sarcolemma of active motor units. It should be noted, however, that the inability to detect declines in M-wave amplitude post-exercise may be related to the use of single stimulus protocols. During exercise, motor unit signal pathways are stressed as they may be required to generate 10-20 action potentials per second for an extended period depending on the intensity of work (Adam & De Luca, 2005). As such, the effect of fatigue on M-wave amplitude may best be examined using a low-frequency train of pulses as this may more accurately replicate physiological demands.

Neuromuscular fatigue is associated with impaired contractile function and is often characterised in vivo as a depression in twitch contractile characteristics; specifically, a reduction in peak twitch amplitude and declines in the rate of torque development and relaxation (Lepers et al., 2000; Lepers et al., 2001). The mechanisms contributing to these changes are highly complex; however, the accumulation of H\(^+\) and inorganic phosphate are
significant as they limit Ca\textsuperscript{2+} release from the sarcoplasmic reticulum and reduce myofibrillar Ca\textsuperscript{2+} sensitivity, which ultimately decreases the number of cross-bridges formed and their rate of binding (Allen et al., 1995). However, depressed twitch contractile characteristics following prolonged exercise are not always observed and some investigations demonstrate evidence for twitch potentiation (Millet et al., 2002; Millet et al., 2003). In the present study, it was observed that Pt and RTD were potentiated, while RR and ½RT were depressed following exercise in all treatments. These results provide further evidence that alterations in twitch contractile properties post-exercise are determined by the summation of competing influences from both peripheral muscle fatigue and potentation. Furthermore, the magnitude of the changes observed were comparable between treatments, which suggest that CHO ingestion had no effect on the evoked twitch response post-exercise. Although the preset study cannot identify the mechanisms contributing to the increase in Pt and RTD post-exercise, increased phosphorylation of myosin regulatory light chains during exercise was likely involved, which increases the sensitivity of the contractile apparatus to activation by Ca\textsuperscript{2+} (Klug et al., 1982) and enhances the ability of the cross-bridges to enter a forcing-producing state (Persechini et al., 1985). Given the context, it should be stated that twitch potentiation is greater for endurance trained athletes compared with sedentary individuals (Hamada et al., 2000), which may assist to explain the disparity between previous results.

An important factor to consider when interesting the results between studies examining the influence of CHO ingestion on the development of neuromuscular fatigue is the extent to which blood glucose levels decline with exercise. Much of this is likely to be related to the fasting status of the subjects involved. Available studies have examined non-fasting subjects, which is likely to provide results of practical significance (Burke et al., 2000; Clark et al., 2000; Nassif et al., 2008). However, the results from such studies typically demonstrate that although blood glucose levels at the end of exercise in the PLA treatments are significantly reduced compared with pre-exercise levels, they not are indicative of hypoglycemia (Stewart et al., 2007; Abbiss et al., 2008). Under such conditions, it is not surprising the CHO ingestion has not been reported to attenuate the development of
neuromuscular fatigue, particularly in trained subjects. Therefore, in order to provide a greater understanding of the role of CHO availability on the development of fatigue during prolonged exercise future studies should involve fasting subjects in an attempt to exacerbate the decline in blood glucose associated with exercise.

4.5 CONCLUSION

In the present study, prolonged self-paced cycling in the heat was associated with significant neuromuscular fatigue post-exercise in all treatments, which was demonstrated by reductions in MVC. The loss in MVC observed post-exercise was primarily related to peripheral mechanisms. Evidence for this comes from the decrease observed in MVC despite comparable levels of voluntary activation and maintained M-wave amplitude post-exercise. Additionally, evoked twitch contractile properties provided some evidence for peripheral fatigue post-exercise; however, they also demonstrated evidence for postactivation potentiation, which may have partially masked the influence of peripheral fatigue on the contractile apparatus. Furthermore, CHO ingestion did not attenuate the loss in MVC post-exercise nor did it influence the central and/or peripheral contributions to fatigue. The failure of CHO ingestion to reduce the development of fatigue may be related to either the self-paced nature of the exercise protocol or the non-fasting status of the subjects involved. In this regard, subjects may have subconsciously applied a pacing strategy that subconsciously restricted their power output during exercise relative to the ambient conditions to limit the rise in core temperature (Tucker et al., 2004) and delay the onset of fatigue. Additionally, because subjects consumed a high CHO diet in the hours immediately prior to testing, the decreases in blood glucose observed at the end of the H2O, PLAb, and PLAc treatments were moderate may not have been sufficient to elicit a significant decline in central motor drive or peripheral muscle function. Despite this, however, CHO ingestion may play a role in preventing hypoglycaemia and attenuating the development of neuromuscular
fatigue in non-fasting exercise subjects when higher core temperatures are reached or during longer duration self-paced activities (i.e. >3h).
Chapter 5

STUDY THREE

Sustained submaximal skeletal muscle contraction following prolonged exercise in the heat with and without carbohydrate ingestion
Abstract Study Three

Objective: The purpose of this study was to examine the effect of double blind ingestions of 6% CHO and a placebo (PLA) in a beverage and in capsules with distilled water on parameters of neuromuscular fatigue associated with a sustained submaximal isometric contraction to fatigue following prolonged self-paced cycling in the heat.

Methods: Ten trained male subjects (mean ± SD: 26 ± 3 years) completed 5 x 60 km cycling time trials punctuated with 4 x 1 km sprints in the heat (32 ºC and 50 % relative humidity). In a randomized double blind fashion, subjects ingested either placebo (PLA) in capsules (c) or in a beverage (b) form (PLAc and PLAb) in two trials and 6%CHO in corresponding trials (CHOc and CHOc) in addition to a H2O trial. At the completion of the time trial, peak isometric torque (MVC), the level of voluntary activation (VA), M-wave amplitude, and evoked twitch contractile properties (TCP) were assessed on the dominant knee extensors before and after a sustained isometric contraction at 20% MVC to fatigue.

Results: MVC, the level of voluntary activation, and M-wave amplitude were unchanged following the sustained submaximal isometric contraction in all treatments. However, TCP demonstrated a significant reduction in the rate of relaxation and an increase in contraction duration in all treatments.

Conclusion: The present data suggest that the development of neuromuscular fatigue following prolonged self-paced cycling in the heat assessed by sustained submaximal contraction was primarily related to peripheral factors. Furthermore, it appears that CHO ingestion during prolonged exercise may not provide protective effect on the development of fatigue following such exercise.
5.1 INTRODUCTION

During prolonged exercise, CHO ingestion is thought to assist in maintaining adequate blood glucose levels, thereby delaying the onset of fatigue by limiting the cascade of central and/or peripheral events that occur as a result of hypoglycaemia (Coyle et al., 1983; Davis, 1995; Chin & Allen, 1997; Tsintzas & Williams, 1998; Davis et al., 2000). Specifically, hypoglycaemia is thought to be a contributing factor in peripheral fatigue due to the depletion of muscle glycogen stores (Coyle et al., 1986; Coggan & Coyle, 1988; Davis et al., 1988) and impair neuromuscular transmission and/or muscle membrane excitability (Stewart et al., 2007). Additionally, hypoglycaemia and muscle glycogen depletion may also promote central fatigue due to the progressive shift in substrate utilization, which increases serum free fatty acid concentration (Fernstrom & Fernstrom, 2006; Chen et al., 2008) and ultimately stimulates serotonin synthesis (Davis, 1995; Meeusen et al., 2006).

Although the mechanisms by which CHO ingestion during prolonged exercise may attenuate the development of fatigue are well reported, very few studies have examined the effects of CHO ingestion on the central and/or peripheral contributions to fatigue following prolonged exercise. Stewart et al. (2007) examined the effect of CHO ingestion versus a flavoured placebo (PLA) in untrained, fasting subjects performing moderate intensity constant load cycling in thermoneutral conditions and reported that at 90 min and at fatigue the reduction in maximal voluntary isometric torque (MVC) was less marked while the compound muscle action potential (M-wave) amplitude was greater during the CHO treatment compared with the PLA treatment. In contrast, Abbiss et al. (2008) examined the effect of CHO ingestion versus a flavoured placebo in trained, non-fasting subjects performing 90 mins of moderate intensity constant load cycling followed by a 16.1 km time trial in the heat and reported that the decrease in MVC and the level of activation post-exercise were comparable between treatments. An explanation for the discrepancies between these results are yet to be provided, but may be related to differences in the
exercise protocols (Theurel & Lepers, 2008), the fasting status of the subjects (Schabort et al., 1999), and/or the training history of the subjects (Friedlander et al., 1997; Tsintzas & Williams, 1998).

Available studies examining the effect of CHO ingestion on the central and/or peripheral contributions to fatigue following prolonged exercise have primarily compared changes in neuromuscular parameters before and immediately after exercise between CHO and PLA treatments (Stewart et al., 2007; Abbiss et al., 2008). However, the use of short duration assessments may not provide adequate sensitivity to detect differences in the central and/or peripheral contributions to fatigue between CHO and PLA treatments. Evidence to support this comes from Nybo (2003) who reported that the reduction in MVC and the level of voluntary activation at the onset of a 120 s sustained maximal isometric contraction following prolonged moderate intensity constant load exercise were comparable between CHO and PLA treatments. However, as the sustained contraction continued significant differences were observed between treatments from 60-120 s whereby the reduction in MVC observed was greater for the PLA treatment compared with the CHO treatment and significant reductions in the level of voluntary activation were observed in the PLA treatment only. These findings suggest the ergogenic effect of CHO ingestion during prolonged exercise may be demonstrated through an increased capacity to sustain high levels of MVC and voluntary neural drive post-exercise, rather than the ability to achieve high levels of MVC and voluntary neural drive for a limited period of time (Nybo, 2003). However, similar studies using a sustained submaximal isometric contraction to detect differences in the central and/or peripheral contributions to neuromuscular fatigue following prolonged exercise are yet to be performed.

Therefore, the purpose of this study was to examine the effect of double blind ingestions of 6% CHO and a placebo (PLA) in a beverage and in capsules with distilled water on parameters of neuromuscular fatigue associated with a sustained submaximal isometric contraction to fatigue following prolonged self-paced cycling in the heat. This study is part of a larger investigation and a detailed account of the effect of PLA and CHO ingestion on time
trial performance and muscle recruitment patterns during cycling can be found in chapter three. Additionally, a description of the effect of PLA and CHO ingestion on the central and/or peripheral contributions to neuromuscular fatigue immediately following the cycle time trial can be in chapter 4.

5.2 METHODS

5.2.1 Subject Characteristics

Ten male athletes (nine mountain bikers and one triathlete) volunteered to participate in the investigation. All subjects had an extensive history of regular cycle training for at least three years prior to the study and completed a training distance of 350-400 km per week for the three months prior to data collection. All subjects were released by a physician after completing a health screening questionnaire and a physical examination were required. The physical characteristics of the subjects were as follows; age 26 ± 3 years, mass 64.5 ± 7.7 kg, height 170 ± 9 cm, sum of nine skinfolds 54.2 ± 13.1 mm, peak power output 323 ± 41 W, and maximal oxygen consumption (VO$_{2\text{max}}$) 70.7 ± 8.8 mL/kg/min. Skinfold sites were biceps, triceps, chest, sub axila, subscapular, suprailiac, abdomen, thigh and calf. Subjects performed the progressive VO$_{2\text{max}}$ test using a Flow Trainer (TACX®, T1684, Netherlands) with their own bicycle attached in a thermoneutral environment (22 ± 1 °C) using a previously described protocol (Kay et al., 2001). Prior to testing, subjects attended an information session outlining the nature of the research and possible risks involved and signed a letter of consent. The research was approved by the Ethics in Human Research Committee of the University.
5.2.2 Experimental design and time trial protocol

Using a randomized Latin square experimental design, subjects completed a 60 km time trial punctuated with 4 x 1 km sprints (at 14, 29, 44 and 59 km) on five separate occasions at the same time of day with each treatment separated by 12 ± 7 days. Before and after each time trial, subjects were assessed for M-wave amplitude, evoked twitch contractile properties, maximal voluntary isometric torque (MVC), and the level of voluntary activation. Immediately following the post-exercise neuromuscular assessments, subjects performed a submaximal isometric fatigue protocol that required them to sustain 20% of their pre-exercise MVC for as long as possible. Once the submaximal isometric protocol was terminated, the neuromuscular assessments were re-evaluated.

During each time trial, subjects were instructed and encouraged to complete the 60 km distance as fast as possibly by completing the stage between sprints as quickly as possible and performing a maximal effort for the duration of each sprint. Subjects were permitted to alter gear ratio and cadence and pedal standing up when needed, other than in the sprints where they were required to remain seated. Trials took place in an environmental chamber (Russels® Technical Products, model WMD-1150-5, Holland, MI, U.S.A.) in the heat (32 °C and 50% relative humidity). During each trial, air movement through the chamber was provided by a fan positioned in front of the subject with a wind speed of 3 m/s in addition to the air circulation provided by the chamber. Subjects were given feedback about cadence at all times and distance at each 3 km interval. No feedback about time, blood results or heart rate was available to subjects during any treatments until after the study was completed.

For each of the five time trials, subjects ingested either a placebo (PLA), 6% carbohydrate (CHO), or water (H₂O) in a double-blind fashion. The PLA and CHO treatments were each administered across two trials each using different methods of ingestion; as a beverage (b) or in capsules (c) with distilled water. Therefore, each subject underwent two PLA treatments (PLAb and PLAc) and two CHO treatments (CHOb and CHOc). Subjects were informed they were ingesting CHO for all beverage and capsule treatments. The H₂O
treatment was used as a control. All treatments other than H$_2$O were coded and prepared by an individual not directly involved with the study. Treatment contents were revealed to the investigators at the completion of the study. Subjects ingested fluids at five intervals during each treatment; at 5km, after each sprint (15, 30, 45km) and at 55km, before the last sprint. Fluids were given to subjects at 4 $\pm$ 1 ºC. Subjects received 284 $\pm$ 30 mL of beverage or 24 $\pm$ 3 capsules plus 284 $\pm$ 30 mL of distilled water at each of the five intervals with all hydration procedures adapted from Hamilton et al. (1991) (i.e. 4 mL of fluid per kg of body mass). For each subject, the amount of CHO power in beverage and the number of capsules consumed with distilled water were carefully calculated to ensure the mixture corresponded to a 6% CHO solution. The exact composition of the CHO and PLA treatments have been described in chapter 3 and were prepared by a commercial chemist (Stenlake®, Sydney, NSW, Australia) to ensure PLA$_b$ and CHO$_b$ matched in taste, colour and flavour.

5.2.3 Pre-testing requirements

Subjects were required to maintain their usual training routine up to 24 h prior to each treatment, after which they were instructed to abstain from strenuous activity, caffeine, and alcohol consumption. For two days prior to each treatment, subjects were required to consume a diet with 60-70% CHO, 1.2-1.7 g of protein/kg of body mass, and 20-25% of fat, which was prescribed individually by a dietitian. The purpose of this diet was to ensure complete recovery of muscle glycogen stores following the last day of training prior to each treatment. Subjects were not required to fast overnight prior to each treatment and were required to consume the same morning meal for each trial.
5.2.4 Physiological and metabolic measures

The hydration status of subjects was assessed when they arrived at the laboratory and post-exercise by urine specific gravity (503 Nippon Optical Warks, Japan). Core temperature was measured using the CoreTemp™ Telemetry System (HQInc. Wireless Sensing System and Design, Palmetto, Florida, U.S.A.). Blood samples were taken from the middle finger at rest, at 5 km intervals throughout each time trial and immediately post-exercise for the determination of blood glucose (Accu-check Advantage®, Roche Diagnostics Corporation, and Indianapolis, USA).

5.2.5 Neuromuscular testing apparatus

All neuromuscular assessments were performed on the dominant knee extensors under isometric conditions using a custom testing device (Master Equipamentos, Belo Horizonte, Brazil). Testing was performed with subjects seated upright with the hip flexed at 90° (0° being full extension) and the knee flexed at 65° (0° being full extension). Once positioned, subjects were secured in the chair via waist and shoulder straps. During all tests, subjects crossed their arms against the chest to ensure that additional forces did not contribute to performance. The lever arm axis of the testing device was aligned with the lateral epicondyle of the femur with the lower leg attached to the lever arm 1 cm above the lateral malleolus of the ankle. Voltage signals from a force cell with a 100 kg capacity (model 601, Tedea-Huntleigh, Don Mills, Canada) were fed to a host computer and were A/D converted at 16-bit resolution (DAQCard-6024E, National Instruments, Austin TX, U.S.A), low-pass filtered at 5 Hz, and sampled at a rate of 1000 Hz using data acquisition software (DASYLab version 9.0, Dasytec USA Inc, Bedford NH, USA). Force cell specifications indicate it had a linear response to loading (± 0.02%) represented by the equation; \( f(x) = ax + b \). The voltage signal from the force cell while unloaded was determined and multiplied by −1.
This value was used in the equation above (variable b) to zero the signal. Subsequently, a series of known loads were placed on the force cell and the corresponding voltages determined. The ratio between the increase in load applied to the force cell and the increase in the voltage signal generated was determined (variable a) and was used to convert force cell signals (variable x) into units of measurement (i.e. newtons; variable f(x)). These procedures were performed using a data acquisition software (DASYLab version 9.0, Dasytec USA Inc, Bedford NH, USA).

5.2.6 Electromyography

Electromyography (EMG) signals from the vastus medialis (VM) and rectus femoris (RF) were captured during maximal evoked testing (M-waves) and EMG signals from the VM, RF and vastus lateralis (VL) were captured during the submaximal isometric fatigue protocol (voluntary EMG). EMG signals were detected using single-differential pre-amplified EMG electrodes (bar configuration 1mm x 10mm; interelectrode distance 10mm; bandwidth of 20-450 Hz) (model DE-2.1, Delsys, Boston, MA, USA) linked to an amplifier where signals where acquired at a gain of 100V/V for M-waves or 1000V/V for voluntary EMG with a common mode rejection ratio >90dB (model Bagnoli-4, Delsys, Boston, MA, USA). Electrodes were positioned on the muscles according to SENIAM recommendations (Hermens & Freriks, 1997). Skin was first prepared by shaving, abrading of the outer layer of epidermal cells, and removing of oil and dirt using an alcohol swab. For the vastus medialis, the recording electrode was attached at the distal 4/5 point along the line between the anterior superior iliac spine and the joint space in front of the anterior border of the medial ligament at the knee. For the rectus femoris, the recording electrode was attached half way along the line between the anterior superior iliac spine and the superior border of the patella. For the vastus lateralis, the recording electrode was attached at the distal 2/3 point along the line between the anterior superior iliac spine to the lateral border of the patella. A disposable
gel adhesive electrode was used as a reference (#2330, 3M, Red Dot, St. Paul, MN, USA), which was positioned on the acromial process of the right shoulder. Signals were fed to a host computer (DAQCard-6024E, National Instruments, Austin TX, USA) and sampled at a rate of 2000 Hz for M-wave amplitude using EMGWorks Signal Acquisition software (version 3.0, Delsys, Boston, MA, USA) and 1000 Hz for voluntary EMG during the submaximal fatigue protocol using DASYLab (version 9.0, Dasytec USA Inc, Bedford NH, USA).

For M-waves, muscle activation was achieved by percutaneous stimulation of the intramuscular branches of the femoral nerve using two 20 mm diameter disposable foam electrodes (Medi-trace 200 series, Kendall, USA) positioned about the medio-anterior aspect of the upper thigh directly below the inguinal fold and at the ventral portion of the vastus medialis. The current applied to the nerve was delivered by a constant-current stimulator (Digitimer DS7, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) using a single square-wave pulse with a width of 200 µs (400 V with a current of 15-80 mA) linked to a host computer (DAQCard-6024E, National Instruments, Austin TX, USA). Initially, the current was manually applied in incremental steps until a muscle twitch of moderate amplitude was observed on the computer monitor. Stimulating electrodes positions were then adjusted medially and/or laterally until the site most responsive to stimulation was located. The electrodes were then secured in position and the location marked on the skin for future assessments. The stimulus intensity was gradually increased until a plateau in twitch amplitude was achieved, where the stimulus intensity was increased by 25% to ensure that supramaximal stimulation was delivered. For testing, five pulses, each separated by 10 s, were delivered with the subject at complete rest. This was determined based on the absence of EMG signals from the rectus femoris or vastus medialis and the absence of any load placed on the force cell other than that due to the effect of gravity on the lower leg. For processing, M-wave data for both RF and VM were averaged across all five treatments with the mean analysed for peak to peak amplitude.

Voluntary EMG signals were sampled throughout the duration of the submaximal contraction. For processing, EMG signals were: 1) bandpass filtered with a low corner
frequency of 20 Hz and a high corner frequency of 450 Hz using a second order Butterworth filter; 2) high-pass filtered at 15 Hz using a second order high-pass Butterworth filter to remove movement artefact 3) full wave rectified; then 3) low-pass filtered at 5 Hz to smooth the data. These procedures were performed using Labview software (version 8.0, National Instruments, Austin TX, USA). For analysis, EMG data were divided into six 5 s intervals relative to the time to fatigue. Interval 1 was obtained at the start of the contraction immediately subsequent to the attainment of the target torque (0% time to fatigue), interval 2 was obtained at 20% time to fatigue, interval 3 was obtained at 40% time to fatigue, interval 4 was obtained at 60% time to fatigue, interval 5 was obtained at 80% time to fatigue, and interval 6 was obtained during the final 5 s of contraction immediately prior to fatigue. Processed EMG data from each interval were integrated (iEMG) and normalised against the data obtained during interval 1. Thus, changes in iEMG over time were calculated as a percentage of interval 1 using the equation; \[ \Delta \text{iEMG} \% = \left( \frac{\text{Interval X}}{\text{Interval 1}} \right) \times 100 \], where interval X is the interval examined (i.e. intervals 2-6). These procedures were performed using spreadsheet software for MS-Windows (Excel 2007™, Redmond, WA, USA).

**5.2.7 Evoked twitch contractile properties**

Immediately following M-wave testing, the dominate knee extensors were assessed by evoked twitch properties. Muscle activation was achieved by using the same electrode locations, testing apparatus, and stimulation parameters as previously described for assessing M-wave amplitude. For testing, five pulses, each separated by 10 s, were delivered with the subject at complete rest. This was determined based on the absence of EMG signals from the RF or VM and the absence of any load placed on the force cell other than that due to the effect of gravity on the lower leg. Force data were exported into spreadsheet software and multiplied by lever arm length to express data in units of torque (Nm). Torque data were then corrected for the effect of gravity on the lower leg, which was
achieved by determining the average load applied to the force cell during the 50 ms period immediately before stimulation. Evoked torque-time curves were averaged across all trials with the mean used to determine; 1) peak twitch torque (Pt; defined as the highest isometric torque value achieved during the evoked contraction), 2) time to peak torque (TPt; defined as the time from torque onset to Pt), 3) half-relaxation time (½RT; defined as the time required for Pt to decline by half), 4) contraction duration (CD; TPt plus ½RT), 5) the rate of torque development (RTD; defined as the mean tangential slope of the twitch torque-time curve between the onset of torque development and Pt), and 6) the rate of relaxation (RR; defined as the mean tangential slope of the twitch torque-time curve between Pt and ½RT). For all evoked twitch contractions, torque onset was defined as the point at which torque data following stimulation increased beyond 2 SD of the mean torque calculated over the 50 ms period immediately before stimulation (Cannon et al., 2008).

5.2.8 Maximal voluntary contraction

The assessment of maximal voluntary isometric torque (MVC) consisted of up to eight attempts where subjects were instructed to attain peak force as fast as possible and continue exerting maximal effort for a period of 5 s. A minimum rest period of 30 seconds separated each attempt and testing continued until the final three attempts had values within ± 5% of each other. Strong verbal encouragement was provided during all voluntary efforts and subjects received continuous visual feedback of performance from a computer monitor. Force data were exported into spreadsheet software and corrected for the effect of gravity on the lower leg offline, which was achieved by determining the average load applied to the force cell during the 1 s period immediately before voluntary muscle contraction while the subject was at complete rest. The average load applied to the force cell during this period was used to offset the performance data obtained during testing. Following this, performance data were multiplied by lever arm length to express data in units of torque (Nm). MVC for
each subject was determined as the single highest torque value produced among attempts. A minimum of 30 s rest elapsed between attempts. Strong verbal encouragement was provided during all voluntary efforts and subjects received continuous visual feedback from a computer monitor.

5.2.9 Voluntary activation

Following the assessment of MVC, the level of voluntary activation of dominant knee extensors was assessed using the twitch superimposition technique (Saboisky et al., 2003). Muscle activation was achieved by using the same electrode locations, testing apparatus, and stimulation parameters used for assessing evoked twitch contractile properties. Testing consisted of a series of four attempts whereby subjects received a superimposed stimulus during a MVC. For all attempts, subjects were instructed to produce maximal effort as fast as possible and continue exerting maximal effort until instructed to relax, which was typically within 3-4 s. During each attempt, the trigger for stimulation was manually primed ~1 s after initiation of each contraction. Once primed, the stimulus was automatically triggered when software detected a decline in peak force (DASYLab version 9.0, Dasytec USA Inc, Bedford NH, USA). Manually priming the trigger was necessary to prevent premature stimulation prior to the attainment of peak force. When primed, the decline in peak force necessary to automatically trigger the stimulus was <1%. A minimum of 30 s rest elapsed between each attempt. Strong verbal encouragement was provided during all voluntary efforts and subjects received continual visual feedback of performance from a computer monitor.

For analysis, force data were exported into spreadsheet software and corrected for the effect of gravity on the lower leg offline, which was achieved by determining the average load applied to the force cell during the 1 s period immediately before voluntary muscle contraction while the subject was at complete rest. The average load applied to the force cell during this period was used to offset the performance data obtained during testing. Following
this, data were multiplied by lever arm length to express data in units of torque (Nm). Superimposed MVC was determined as the peak torque value produced during the 50–150 ms period subsequent to the delivery of the stimulus. Voluntary peak torque during each superimposed contraction prior to stimulation was determined as the mean torque value produced during the 25 ms before stimulus delivery. The level of voluntary activation using the following equation: 

\[ \text{Voluntary Activation} \% = \left( \frac{\text{Voluntary peak torque}}{\text{Superimposed torque}} \right) \times 100. \]

All attempts were assessed with the attempt yielding the highest level of voluntary activation used for subsequent analysis. It should be noted that due to the small decline (<1%) in peak force necessary to automatically trigger the stimulus during the superimposed contractions, the level of voluntary activation may be underestimated. However, this decline is less than the variance observed in the measurement and is unlikely to have any meaningful impact on the data obtained.

**5.2.10 Submaximal fatigue test**

Immediately following the post-exercise neuromuscular assessments, subjects performed a submaximal fatigue test involving the dominant knee using the neuromuscular testing apparatus previously described. For this test subjects were required to sustain an isometric contraction at an intensity of 20% of their pre-exercise MVC until: 1) torque output varied ± 15% of the target torque required for a period of ≥3 s or 2) the test was terminated by the subject due to voluntary fatigue. It should be noted that the first criterion was never met and all subjects terminated the testing due to voluntary fatigue. Subjects received strong verbal encouragement throughout all submaximal fatigue tests and were provided with continuous visual feedback of performance from a computer monitor. Performance was determined as the time to voluntary fatigue once the target torque value was attained, which was typically within 2-3 s after the initiating contraction.
5.2.11  Statistics

These data are part of a larger investigation. As such, the entire data set for each variable were analysed collectively using a 5 x 1 factorial repeated measures ANOVA with a LSD adjustment for multiple pairwise comparisons. The level of significance was set at p< 0.05. All statistical procedures were performed using SPSS™ for MS-Windows version 14.0 (Statistical Package for the Social Sciences, Chicago, IL).

An LSD correction for multiple pairwise comparisons was used for analysis as applying a Bonferroni or Sidak correction would require a p value of < 0.0005 to identify a statistically significant difference between comparisons. Although adjustments for multiple pairwise comparisons reduce the chance of a Type I error, they inherently increase the risk of a Type II error. We propose that effectively setting the level of significance at p< 0.0005 is much too stringent and would actually lead to more errors of data interpretation due to inflation in the number of Type II errors. Support for this comes from Rothman (1990) who argues against adjusting for multiple pairwise comparisons and suggests each result should be interpreted as its own analysis, regardless of the number of comparisons tested.

5.3 RESULTS

5.3.1 Time trial performance and physiological measures

The mean time the complete the time trial across treatments was 137.9 ± 19.2 min and was not significantly different between treatments (p> 0.05; see chapter three). Urine specific gravity pre and post-exercise was >1029 in all cases and was not significantly different within or between treatments (p> 0.05). This indicates that subjects commenced and terminated all trials in a hydrated state. Core temperature significantly increased over time in all treatments (p< 0.05); however, the increase in core temperature was not significantly different between treatments with the final post-exercise core temperature values ranging
from 37.0 – 38.3°C across all subjects and treatments (p< 0.05). Blood glucose levels were comparable pre-exercise between all treatments and ranged between 4.3-6.6 mmol/L across all subjects and treatments (p< 0.05). Blood glucose was significantly lower post-exercise in the H₂O, PLAₐ, and PLAₖ treatments and was significantly higher post-exercise in both the CHOₐ and CHOₖ treatments compared with all other treatments (p< 0.05); except between the CHOₖ and the H₂O treatments (p= 0.138). This is most likely explained by a delay in the absorption of CHO from the capsules that were ingested at 55 km.

5.3.2 Submaximal fatigue test

The time to fatigue during the sustained submaximal contraction was 110.8 ± 76.2 s for the H₂O treatment, 118.7 ± 101.6 s for the PLAₐ treatment, 107.3 ± 72.7 for the CHOₐ treatment, 103.2 ± 63.9 for the PLAₖ treatment, and 96.4 ± 51.7 for the CHOₖ treatment and were not significantly different between treatments (p> 0.05). An example torque-time curve and EMG-time curve from a sustained submaximal contraction from a representative subject is provided in Figure 12.
Figure 12. Sample of torque-time curve and EMG-time curve from the submaximal fatigue test from subject 8, PLAc treatment.
The target torque during the sustained submaximal contraction was 20% of the pre-exercise maximal isometric torque. The limits of maximum variation in torque output during the sustained contraction of ±15% are identified.

The change in iEMG during the submaximal fatigue test for each treatment is presented in Figure 13. The iEMG was significantly higher for intervals 2 through to interval 6 compared with interval 1 in all treatments (p< 0.05); except for the CHO treatment where an increase in iEMG was not observed until interval 6 (p< 0.05). iEMG was significantly higher for intervals 3 through to interval 6 compared with interval 2 in the H2O and the PLA treatments (p< 0.05). During interval 6, iEMG was significantly higher for the H2O treatment compared with the PLA treatment (p< 0.05).
Figure 13. Change in EMG amplitude over time during the sustained submaximal isometric contraction for each treatment. aIndicates significant difference from interval 1 in the H₂O, PLA₀, PLA₀, and CHO₀ treatments (p< 0.05). bIndicates significant difference from interval 1 in the CHO₀ treatment (p< 0.05). cIndicates significant difference from interval 2 in the H₂O and the PLA₀ treatments (p< 0.05). dIndicates significant difference between the H₂O and the PLA₀ treatments (p< 0.05). Values presented as the mean ± SD.
MVC, the level of voluntary activation, and M-wave amplitude post-exercise and immediately following the sustained submaximal contraction for each treatment are presented in Table 10. No significant differences were observed in MVC, the level of voluntary activation, or M-wave amplitude before or after the submaximal fatigue test in any treatment (p> 0.05).
Table 10. Maximal voluntary isometric torque, the level of voluntary activation, and M-wave amplitude before and after the submaximal fatigue test for each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MVC (Nm)</th>
<th>VA (%)</th>
<th>M-wave Amplitude (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RF</td>
<td>VM</td>
</tr>
<tr>
<td><strong>Before</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>157 ± 42</td>
<td>95.1 ± 5.2</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>PLA&lt;sub&gt;b&lt;/sub&gt;</td>
<td>153 ± 38</td>
<td>96.0 ± 3.8</td>
<td>5.6 ± 1.7</td>
</tr>
<tr>
<td>CHO&lt;sub&gt;b&lt;/sub&gt;</td>
<td>150 ± 43</td>
<td>94.7 ± 8.2</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
<td>PLA&lt;sub&gt;c&lt;/sub&gt;</td>
<td>155 ± 36</td>
<td>97.0 ± 3.1</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>CHO&lt;sub&gt;c&lt;/sub&gt;</td>
<td>152 ± 40</td>
<td>95.3 ± 7.6</td>
<td>6.2 ± 0.9</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>153 ± 40</td>
<td>95.6 ± 5.6</td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td><strong>After</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>157 ± 42</td>
<td>96.1 ± 3.4</td>
<td>6.9 ± 1.0</td>
</tr>
<tr>
<td>PLA&lt;sub&gt;b&lt;/sub&gt;</td>
<td>151 ± 42</td>
<td>95.7 ± 4.1</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td>CHO&lt;sub&gt;b&lt;/sub&gt;</td>
<td>152 ± 39</td>
<td>96.5 ± 3.2</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>PLA&lt;sub&gt;c&lt;/sub&gt;</td>
<td>152 ± 31</td>
<td>96.8 ± 3.0</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>CHO&lt;sub&gt;c&lt;/sub&gt;</td>
<td>152 ± 39</td>
<td>94.6 ± 8.2</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>153 ± 36</td>
<td>96.0 ± 4.7</td>
<td>6.6 ± 0.9</td>
</tr>
</tbody>
</table>

Before, before the submaximal fatigue test; After, after the submaximal fatigue test; MVC; maximal voluntary isometric torque, VA; the level of voluntary activation, RF; rectus femoris, VM: vastus medialis, MVC, maximal voluntary contraction, VA; level of voluntary activation, H₂O; water treatment, PLA<sub>b</sub>; placebo beverage treatment, CHO<sub>b</sub>; carbohydrate beverage treatment, PLA<sub>c</sub>; placebo capsule treatment, CHO<sub>c</sub>; carbohydrate capsule treatment, Mean; mean value across all subjects and treatments. Values presented as mean ± SD.
Evoked twitch contractile properties for pre and post the fatigue test are presented in Table 11. After the submaximal fatigue test Pt increased in the CHO treatment and decreased in the PLA treatment (p< 0.05). RR and CD were significantly lower after the submaximal fatigue test in all treatments (p< 0.05). ½RT was significantly longer after the submaximal fatigue test in the H2O, PLA, CHO treatments (p< 0.05).
Table 11. Evoked twitch contractile properties before and after the submaximal fatigue test for each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pt (Nm)</th>
<th>RTD (Nm.s⁻¹)</th>
<th>RR (Nm.s⁻¹)</th>
<th>TPt (ms)</th>
<th>½RT (ms)</th>
<th>CD (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>44.2 ± 10.7</td>
<td>708.1 ± 242.8</td>
<td>169.8 ± 36.9</td>
<td>72.1 ± 8.6</td>
<td>102.7 ± 14.5</td>
<td>174.8 ± 12.9</td>
</tr>
<tr>
<td>PLA_b</td>
<td>43.0 ± 11.5</td>
<td>742.7 ± 216.6</td>
<td>147.7 ± 41.9</td>
<td>70.9 ± 7.7</td>
<td>109.8 ± 14.8</td>
<td>174.5 ± 14.8</td>
</tr>
<tr>
<td>CHO_b</td>
<td>43.6 ± 10.4</td>
<td>704.1 ± 261.1</td>
<td>159.6 ± 53.3</td>
<td>75.0 ± 10.9</td>
<td>107.2 ± 23.7</td>
<td>182.2 ± 22.0</td>
</tr>
<tr>
<td>PLA_c</td>
<td>42.0 ± 10.6</td>
<td>737.0 ± 237.1</td>
<td>150.4 ± 37.7</td>
<td>70.4 ± 8.9</td>
<td>106.4 ± 12.3</td>
<td>170.9 ± 17.9</td>
</tr>
<tr>
<td>CHO_c</td>
<td>42.7 ± 10.2</td>
<td>764.5 ± 210.0</td>
<td>148.6 ± 31.8</td>
<td>68.4 ± 9.9</td>
<td>105.6 ± 11.3</td>
<td>167.2 ± 17.0</td>
</tr>
<tr>
<td>Mean</td>
<td>43.1 ± 10.3</td>
<td>731.3 ± 225.7</td>
<td>155.2 ± 40.2</td>
<td>71.4 ± 9.1</td>
<td>106.3 ± 15.4</td>
<td>173.9 ± 18.1</td>
</tr>
<tr>
<td><strong>After</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>44.5 ± 9.9</td>
<td>746.2 ± 238.9</td>
<td>130.5 ± 32.5</td>
<td>75.9 ± 21.3</td>
<td>125.4 ± 25.1</td>
<td>201.3 ± 25.2</td>
</tr>
<tr>
<td>PLA_b</td>
<td>45.5 ± 11.4*</td>
<td>738.5 ± 260.3</td>
<td>122.1 ± 52.6*</td>
<td>79.9 ± 23.9</td>
<td>121.5 ± 13.9</td>
<td>201.4 ± 20.0*</td>
</tr>
<tr>
<td>CHO_b</td>
<td>45.4 ± 11.0</td>
<td>675.0 ± 184.7</td>
<td>130.5 ± 36.4*</td>
<td>85.2 ± 25.0</td>
<td>123.9 ± 26.5</td>
<td>209.1 ± 20.1*</td>
</tr>
<tr>
<td>PLA_c</td>
<td>43.5 ± 11.8</td>
<td>763.6 ± 223.9</td>
<td>126.3 ± 33.0*</td>
<td>69.5 ± 7.6</td>
<td>131.4 ± 16.3*</td>
<td>200.9 ± 15.7*</td>
</tr>
<tr>
<td>CHO_c</td>
<td>46.1 ± 10.9*</td>
<td>783.3 ± 242.3</td>
<td>128.7 ± 33.5*</td>
<td>73.1 ± 9.8</td>
<td>129.4 ± 15.5*</td>
<td>202.6 ± 22.0*</td>
</tr>
<tr>
<td>Mean</td>
<td>45.0 ± 10.6</td>
<td>741.3 ± 224.8</td>
<td>127.6 ± 36.9*</td>
<td>76.7 ± 19.0</td>
<td>125.1 ± 19.6</td>
<td>203.1 ± 20.2*</td>
</tr>
</tbody>
</table>

Before, before the submaximal fatigue test; After, after the submaximal fatigue test, Pt; peak twitch torque, RTD, rate of torque development, TPt; time to peak torque, RR; rate of relaxation, ½RT; half-relaxation time, CD; contraction duration, RF; rectus femoris, VM: vastus medialis, MVC; maximal voluntary contraction, VA; level of voluntary activation, H₂O; water treatment, PLA_b; placebo beverage treatment, CHO_b; carbohydrate beverage treatment, PLA_c; placebo capsule treatment, CHO_c; carbohydrate capsule treatment, Mean; mean value across all treatments. *Indicates significance difference from the pre-submaximal fatigue test measurement. Values are presented as mean ± SD.
This study compared the effects of a 6% CHO beverage and an equivalent 6% CHO mixture taken as capsules with distilled water versus corresponding PLA treatments and a \( \text{H}_2\text{O} \) treatment on parameters of neuromuscular fatigue before and after a sustained submaximal contraction to fatigue in well trained, non-fasting subjects following prolonged self-paced cycling in the heat.

It was observed that 1) the time to fatigue during the sustained submaximal contraction was not significantly different between treatments; 2) iEMG significantly increased by the end of the sustained submaximal contraction in all treatments and was greater for the \( \text{H}_2\text{O} \) treatment compared with the PLA treatment immediately prior to fatigue; 3) no significant differences were observed in MVC, the level of voluntary activation, or M-wave amplitude after the submaximal contraction; and 4) evoked twitch properties displayed evidence for fatigue after the sustained submaximal contraction in all treatments and also evidence for postactivation potentiation in the PLA and CHO treatments. These findings demonstrate that CHO ingestion during a 60 km self-paced cycling time trial in the heat did not influence the development of neuromuscular fatigue or potentiation associated with a sustained submaximal isometric contraction performed immediately following exercise.

The mean time to fatigue during sustained submaximal isometric contraction observed in the present study of \(~110\) s was somewhat shorter than the time to fatigue reported in previous studies using similar submaximal protocols in subjects who did not perform exercise prior to the sustained contraction (Bilodeau et al., 2001; Hunter et al., 2002; Hunter & Enoka, 2003; Rochette et al., 2003). For example, Rochette et al. (2003) reported that the time to fatigue during a sustained isometric contraction of the knee extensors at 20% MVC was \(~300\) s across three separate trials. However, this is to be expected given that the subjects in the present study had already completed a 60 km cycling time trial. The neuromuscular mechanisms contributing to the inability to sustain a target torque during
submaximal contractions are complex, but appear to primarily involve central factors (Place et al., 2005; Eichelberger & Bilodeau, 2007). However, regardless of the precise mechanisms involved, the present data indicate that CHO ingestion during prolonged self-paced exercise does not enhance the time to fatigue during a subsequent sustained submaximal contraction.

It is well documented that sustained submaximal isometric contractions are associated with a progressive increase in EMG activity (Fuglevand et al., 1993; Ebenbichler et al., 1998; Rochette et al., 2003). The increase in EMG most likely reflects the recruitment of additional motor units, modulation of motor unit discharge rates, and possibly increased motor unit synchronization (Carpentier et al., 2001; Gandevia, 2001; Kleine et al., 2001) in an attempt to maintain the target torque. In agreement with previous studies, a progressive increase in iEMG during the submaximal contraction was also observed in all treatments in the present study, which equated to an increase of ~50% during the final 5 s of the contraction compared with initial 5 s after the target torque was attained, which is similar to the increase in root-mean-square EMG previously observed following a similar fatigue protocol in non-exercised subjects (Rochette et al., 2003). The comparable increase in iEMG observed during the stages of the submaximal contraction prior to fatigue during both the CHO treatments and both PLA treatments and the H2O treatment suggests that CHO ingestion during the prolonged self-paced exercise had no influence on the pattern of muscle recruitment during the sustained submaximal isometric contraction. Because accelerated fatigue is associated with an increased rate of rise in iEMG during a sustained submaximal contraction (Maluf & Enoka, 2005), this finding supports the time to fatigue data suggesting that neuromuscular mechanisms contributing to fatigue during the sustained submaximal task were comparable between the CHO, PLA and H2O treatments.

The interesting finding is that MVC values before and after the sustained submaximal contraction were not significantly different in any treatment. This result is in contrast to previous studies examining time to fatigue using a submaximal isometric contraction with subjects that did not perform prolonged exercise prior to initiating contraction, which typically
report reductions in MVC of ~30-45% immediately following test termination (Hunter & Enoka, 2003; Rochette et al., 2003). The maintenance of MVC in the present study may be related to the relatively short duration of contraction (~50%) compared with previous investigations using similar submaximal fatigue tests (Hunter & Enoka, 2003; Rochette et al., 2003). Additionally, motor unit substitution and rotation has been observed during submaximal fatigue tasks (Enoka et al., 1996; Bawa et al., 2006), which would limit fatigue on a single population of motor units. Furthermore, CHO ingestion did not provide an ergogenic effect on MVC after the sustained submaximal contraction. However, this finding is in contrast with the results of Nybo (2003) who reported that although substantial reductions in MVC after performing a sustained maximal isometric contraction for 120 s following prolonged constant load exercise were observed, an attenuated reduction was observed during the CHO treatment. Unlike the PLA treatment, the attenuated reduction in MVC observed by Nybo (2003) during the CHO treatment was mediated by a decline in neural drive (see subsequent section).

Although an objective criterion was used to determine when to terminate the submaximal fatigue test, all subjects in all treatments terminated testing due to voluntary exhaustion. As such, voluntary exhaustion during the sustained submaximal contraction in the present study was not associated with additional fatigue to that observed immediately following the prolonged self-paced time trial. Therefore, it would appear that the termination of sustained submaximal contraction was most likely related to a loss of motivation to continue exercise, probably due to the additional discomfort associated with the sustained submaximal task. In this regard, subjects indicated an RPE of 15-19/20 across all treatments at the completion of the time trial, which although was not reassessed following the sustained submaximal task, would have likely increased due to the additional sensory discomfort associated with maintaining the isometric contractions (Demura et al., 2008). This finding provides partial support for the theory of St Clair-Gibson et al. (2003) which states that the perception of discomfort is a contributing factor to the development of fatigue; rather than being a result of the regulation process. However, it should be stated that although a
reduction in MVC was not observed after the sustained submaximal contraction, MVC values did not demonstrate any evidence of recovery following the completion of the 60km time trial. Thus, the loss of MVC associated with the prolonged exercise was maintained during the sustained submaximal contraction.

A number of studies examining sustained submaximal isometric contractions using a similar fatigue protocol as the present study have report moderate to large reductions in M-wave amplitude at the onset of exhaustion (Fuglevand et al., 1993; Place et al., 2005). The decline in M-wave amplitude following sustained submaximal activity is most likely related to the inability to restore transmembrane concentration gradients associated with Na\(^+\) and K\(^+\) along the sarcolemma and T-tubule system (Clausen, 2003) due to an increased concentration of K\(^+\) and ammonia (MacLaren et al., 1989), which restricts neuromuscular propagation. Although many studies examining sustained submaximal contractions report declines in M-wave amplitude, such changes are not universally reported following prolonged dynamic exercise (Lepers et al., 2001; Lepers et al., 2002; Saboisky et al., 2003). However, recent studies indicate that CHO ingestion during prolonged dynamic exercise may assist to maintain M-wave amplitude and Na\(^+\)-K\(^+\)-ATPase activity (Green et al., 2007; Stewart et al., 2007). In the present study, in addition to completing a cycling time trial, subjects also performed a submaximal fatigue test to exhaustion. Yet despite the additional strain placed on the muscle membrane due to the submaximal fatigue test, M-wave amplitude remained unchanged following exercise. The failure to observe a decrease in M-wave in the present study may seem surprising given the duration and volume of exercise completed by the subjects in this study. However, a number of physiological mechanisms may assist to protect muscle membrane and T-tubule system excitability during prolonged submaximal exercise in vivo, including; 1) motor unit rotation to share the electrical work across different pools of motor units; 2) decreased motor unit discharge frequencies; 3) motor unit activation at optimal discharge rates for force output; 4) increased Na\(^+\)-K\(^+\) pump activity to maintain adequate ionic gradients; and 5) progressive inactivation of Na\(^+\) channels so that the influx of K\(^+\) and Cl\(^-\) flux required for repolarization is reduced (c.f. Allen et al., 2008).
Declines in the level of voluntary activation have been reported in a number of studies examining the central contributions to fatigue during submaximal contractions in rested subjects (Place et al., 2005; Eichelberger & Bilodeau, 2007; Taylor & Gandevia, 2008). In most of these studies, subjects progressively increase voluntary effort to recruit additional motor units as previously active muscle fibers fatigue (Bigland-Ritchie et al., 1986; Adam & De Luca, 2005). At this moment, despite maximal effort, superimposed motor nerve stimulation typically demonstrates incomplete neural drive to the muscle (Loscher et al., 1996). Additionally, Nybo (2003) reported a decline in the level of voluntary activation after 60 s during a 120 s sustained MVC following prolonged constant load cycling in the PLA treatment, but not during the CHO treatment. In contrast to these results, the level of voluntary activation at test termination in the present investigation was not significantly reduced. It remains unknown why a decline in the level of voluntary activation was not observed during the present study, especially given the extended duration of self-paced activity followed by the submaximal fatigue test. Unlike Nybo (2003) where subjects in the PLA treatment demonstrated hypoglycaemic towards the end of exercise, the present study did not require subjects to fast prior to exercise and therefore blood glucose levels remained euglycaemic, which likely lead to the adequate supply of fuel to the central nervous system structures. Alternatively, it is possible that subjects terminated the submaximal fatigue test prematurely due to a loss of motivation because of the discomfort associated with the sustained maximal isometric contraction.

Although a reduction in MVC was not observed in the present study following the sustained submaximal contraction, the rate of muscle relaxation was significantly slowed. This is demonstrated by the significant decrease in RR and CD in all treatments, in addition to the increase in ½RT in the H2O, PLAc and CHOc treatments after the submaximal fatigue test. The mechanisms responsible for muscle relaxation are complex and involve a variety of processes; specifically, the re-uptake of Ca²⁺ by the sarcoplasmic reticulum, Ca²⁺ dissociation from troponin, and the cessation of cross-bridge cycling (Allen et al., 2008). These processes are highly sensitive to H⁺, ADP, and P₅, whereby the concentrations of each
change with fatigue (Westerblad et al., 1997). In contrast to concentric muscle actions (Allen et al., 1995), a slower rate of relaxation may benefit performance during sustained isometric contractions by allowing tetanic fusion to occur at lower stimulation frequencies, thus attenuating the decline in muscle force when the motor neuron discharge rates decrease (Bigland-Ritchie et al., 1979). Thus, the slowing of muscle relaxation may assist to explain why a reduction in MVC was not observed after the sustained fatigue test. Additionally, an increase in Pt was observed after the submaximal fatigue test in the PLA_b and CHO_c treatments. This postactivation potentiation is frequently observed during submaximal exercise and is related to the phosphorylation of the myosin regulatory light chain (Manning & Stull, 1982; Moore & Stull, 1984), which has been reported to increase muscle force at submaximal, but not saturating, Ca^{2+} concentrations (Persechini et al., 1985).

5.5 CONCLUSION

It was observed in the present study that the development of neuromuscular fatigue following prolonged self-paced cycling in the heat was primarily associated with peripheral factors when assessed using a sustained low-intensity submaximal isometric contraction. Furthermore, the present data also demonstrate that CHO ingestion during prolonged self-paced cycling did not attenuate development of fatigue. These data should be interpreted in the given context where the subjects involved were well trained and were not fasting prior to exercise. As such, it appears under such conditions that the development of neuromuscular fatigue is primarily related to peripheral factors. However, it is possible that decreased voluntary effort may be present due to a loss of motivation to continue exercising, rather than an exercise-induced decline in central drive.
Sustained submaximal skeletal muscle contraction following prolonged exercise in the heat with and without carbohydrate ingestion

6.1 MAJOR FINDINGS

The main finding of this thesis is that CHO ingestion does not improve prolonged (~ 2 h) time trial performance in the heat in fed highly trained subjects. CHO ingestion also did not succeed in improving maximal voluntary contraction and submaximal isometric performance. That is, subsequent maximal voluntary contraction and sustained submaximal isometric performance are independent of the CHO ingestion during prolonged self-paced exercise in the heat. Even though much research has been completed on the effect of CHO ingestion on exercise performance, the ergogenic effect of CHO is still questionable as it evidenced by the varied results from many different studies. Fatigue is a complex physiological and psychological response and its mechanisms are of peripheral and central in origin; as such this thesis adds to the literature in its evaluation of CHO ingestion in fed highly trained subjects during exercise in the heat. It is clear from most studies that CHO ingestion maintains blood glucose, but not necessarily improves exercise performance particularly when a proper placebo was used during a self-paced exercise in a Latin Square design study.

As more studies show the role of the CNS in controlling the body homeostasis and determining the point of absolute fatigue, it is still not clear if CHO ingestion affects the CNS, as more invasive studies are not ethically approved in humans and need to be tested in animal models. However, limitations might be present when transferring results from studies undertaken in animal models.
This thesis confirms that exercise in the heat affects CNS function, but CHO ingestion does not attenuate this effect or improves performance. As the body attempts to maintain homeostasis a number of changes are made by the CNS to minimize the effect of the heat production and storage as a result of the exercise to allow exercise to continue without any danger to the organism. Adjustments such as sweating to assist body cooling, reduction in exercise intensity which is accompanied by reductions in heat production, heart rate, power, cadence, speed and muscle recruitment, show that the CNS is in command by integrating signals from the periphery so that a reduction in intensity takes place. Therefore, the CNS acts as a protective control of the body, not letting the individual override the CNS command but at the same time allowing the task to be completed as best as possible without any damage.

This thesis showed a reduction in voluntary activation when subjects ingested water but not when CHO were thought to be ingested even though when subjects were actually ingesting a PLA. This is strong evidence that even though caution had been taken to minimize the possible PLA effect, this effect is present as subjects showed a reduced voluntary activation only in the water treatment; the one treatment in which they knew there was no CHO content. The fact that no CHO were being ingested might have reduced the subjects motivation as it might influence the conscious part of the CNS to give its best as the psychological effect of not ingesting CHO might have been in effect. This indicates that it is clear that even though all has been done to minimize the PLA effect; the effect is still present. When subjects ingested PLA, no difference in time trial performance and voluntary activation was observed compared to the CHO treatments, independently of the method of ingestion. For this reason, this thesis has contributed to our understanding in showing that there is a PLA effect when CHO ingestion studies are conducted, independently of all procedures designed to minimize this effect. These findings suggest that CHO might improve performance but through a psychological pathway and not necessarily a physiological one as previously thought.
6.2 LIMITATIONS

The use of muscle stimulation can be considered a limitation in the study. Although it was a procedure approved by the Human Ethics Committee, the technique can be quite uncomfortable for some people. That is one of the reasons twitch responses might not have been as expected, as higher values were observed in post-exercise measurements when compared to pre-exercise measurements. Even though subjects were attempting to consciously relax while waiting for the muscle stimulation, they still could not be completely relaxed in order to obtain definitive twitch measurements.

Even though the high number of capsules ingested by subjects every ingestion during testing did not seem to affect the result of the present study, one should try to decrease as much as possible the amount of capsules being ingested by subjects. – The duration of each day of testing was also a limitation as trials could take around 7 hours to be completed from arrival in the lab to departure, and possibly increasing the psychological stress on subjects.

It is also important to note that the sample size was similar to studies which have previously used CHO; (range n = 8 - 12). The number of participants in the present study was 10 (n = 10 per intervention). Therefore, although the sample size was well within the sample of previous studies, some caution should be used in drawing conclusions from data as the individual cell sizes were reduced and thus chances of a Type could result.

6.3 SPECIFIC CONCLUSIONS FROM THIS THESIS ARE:

1. CHO ingestion does not improve 60 km self-paced cycling time trial in the heat;
2. CHO ingestion does not change maximal voluntary contraction after self-paced cycling in the heat;
3. CHO ingestion does not improve the time subjects are able to hold a submaximal isometric contraction after self-paced exercise in the heat;
4. Blood glucose is increased during exercise with CHO ingestion independent of the method of ingestion; beverage or capsule;

5. CHO ingestion had no effect on pacing strategies used by subjects to complete the 60 km self-paced time trial in the heat;

6. Muscle recruitment is altered in the final stages of the 60 km self-paced time trial (~36 km) so homeostasis is not compromised;

7. A PLA effect is present when subjects ingest water, knowing that the drink has no energy content as evidenced by a reduction in voluntary activation

6.4 RESEARCH QUESTIONS ADDRESSED:

1. (a) Will the effect of ingesting either a 6% CHO beverage or an equivalent 6% CHO capsule versus corresponding placebo during prolonged endurance exercise (60km time trials) in the heat when subjects are fed produce better performance?

   No. Our data demonstrate that cycling performance as indicated by time to complete the 60km time trial was not significantly different between treatments. Therefore, in the present study CHO ingestion irrespective of the method of consumption had no effect on the cycling performance compared with a corresponding placebo.

   (b) Further, will subjects alter their pacing strategy (speed, cadence and power output) according to the type of ingestion and if so will the alteration in pacing strategy be associated with alterations in skeletal muscle recruitment?

   No. Our data demonstrate that the pacing strategy employed and muscle recruitment patterns during the 60km time trial were not significantly different
between treatments. Although muscle recruitment was altered in the final stages of the 60 km self-paced time trial (~36 km) the changes observed were comparable between all treatments. Therefore, in the present study CHO ingestion irrespective of the method of consumption had no effect on pacing strategies or muscle recruitment patterns compared with a corresponding placebo.

2. (a) Will the effects of either a 6% CHO beverage or an equivalent 6% CHO mixture taken as capsules versus corresponding placebo treatments and water alter parameters of neuromuscular fatigue in non-fasting participants following prolonged self-paced cycling in the heat?

No. Our data demonstrate that independent of the form CHO were ingestion it did not affect neuromuscular fatigue in non-fasting participants following prolonged self-paced cycling in the heat.

(b) Specifically, will M-wave amplitude, evoked twitch contractile properties, MVC, and the level of voluntary activation be altered following prolonged exercise in the heat with respect to CHO ingestion?

No. Our data demonstrate that changes in M-wave amplitude, evoked twitch contractile properties, and MVC post-exercise were comparable between all treatments. A placebo effect was present when subjects ingest water as evidenced by a reduction in voluntary activation post-exercise. However, no significant differences in voluntary activation post-exercise were observed between the placebo or CHO treatments. Therefore, in the present study CHO ingestion irrespective of the method of consumption had no effect on the development of neuromuscular fatigue compared with a corresponding placebo.
3. Will double blind ingestions of 6% CHO in beverage and capsule form versus a placebo alter the neuromuscular parameters associated with sustained submaximal contractions to fatigue following prolonged exercise in the heat?

No. CHO ingestion did not improve the time subjects were able to hold a submaximal isometric contraction after self-paced exercise in the heat. Furthermore, parameters of neuromuscular fatigue, specifically M-wave amplitude, evoked twitch contractile properties, MVC and the level of voluntary activation, following the sustained submaximal isometric contraction were comparable between all treatments. Therefore, in the present study CHO ingestion irrespective of the method of consumption had no effect on the development of neuromuscular fatigue associated with a sustained submaximal isometric contraction compared with a corresponding placebo.

6.5 DIRECTIONS FOR FUTURE RESEARCH:

1. Studies that evaluate CHO ingestion should give preference to methods that do not allow subjects to know the content of what is being ingested. Studies on the effect of suggestion should be rigorously conducted in order to further understand the placebo effect during exercise under given conditions.

2. A Latin square design should be used to minimize other factors which might affect the results and the main objective of the study;

3. Trained subjects should be used when studying the effect of CHO ingestion on performance, as they will mostly benefit from the findings from these studies;
4. Research examining the effect of CHO ingestion on self-paced prolonged exercise when subjects are fed should be conducted in order to determine if the fed status has an effect on the measurements of fatigue. Subjects should be normally fed as a pre-race meal before treatments, as this is closer to reality on a race day;

5. Capsules and infusion should be methods used when complete matching of PLA and CHO beverages is not possible;

6. The use of techniques to evaluate the role of central fatigue, such as interpolated twitch and EMG should be included in studies to add other variables that might be able to elucidate which mechanisms affect performance, peripheral or/and central mechanisms.

7. Studies examining the effects of CHO supplementation on neuromuscular responses during prolonged exercise would enable a more thorough understanding of the role of energy supply and usage during prolonged exercise.
APPENDIX A

CONSENT FORM

Charles Sturt University
School of Human Movement Studies
Panorama Avenue – Bathurst NSW 2795 Australia
E-mail: tricamilanassif@yahoo.com.br

CHARLES STURT UNIVERSITY – CONSENT FORM
ETHICS IN HUMAN RESEARCH COMMITTEE – (PROTOCOL # 2005/064)

RESEARCH TITLE

The Effects of Carbohydrates Ingestion on Fatigue Parameters during Exercise in the Heat

JUSTIFICATION AND RESEARCH OBJECTIVES

The mechanisms responsible of fatigue during prolonged exercise are not well established. Recently, some researchers have focused their studies on a hypothesis which associated fatigue with the central nervous system. Considering this hypothesis, researchers use the ingestion of carbohydrates as a possible nutritional strategy capable of delaying central fatigue during prolonged exercise. Therefore, the conditions of this experiment, such as, the exercise mode and the environmental conditions can influence or not the development of central fatigue. This research intends to add to a more detailed understanding of central fatigue and in which conditions it manifests. For this, we will evaluate the effects of carbohydrates on fatigue parameters during exercise in the heat.

RESEARCH PROCEDURES

Previously to the experimental conditions, you will go through a couple of tests (medical, physical and nutritional) with the aim of characterizing and selecting the participants.

As a cyclist, triathlete or mountain biker, you will realize five experimental trials, one with water ingestion and the other four with the ingestion of carbohydrates during exercise. In two of these four trials carbohydrates will be ingested in a liquid form, similar to carbohydrate beverages sold in stores. In the other two trials, gelatine capsules containing carbohydrate powder will be ingested with proper amount of water. The exercise will be cycling, on your own bike, sixty kilometres in the short time possible. Every fourteen kilometres, sprints of 1km will be required. The exercise will be in an environmental chamber capable of maintaining a dry temperature of 32°C and 50% of relative humidity.

During exercise a variety of variables will be measured using safe and reliable methods. All variables are described below.

- Total Time of Exercise (TTE): corresponds the total time you will take to cycle the 60 km in each experimental trial.

- Blood Glucose Concentration (GLU): it will be measured during rest, every 5kms and after each sprint, using a portable device.

- Heart Rate (HR): it will be measured during rest, every 2kms and at the end of exercise, using a heart rate monitor.
- Blood Lactate Concentration (LAC): it will be measured during rest, every 5 kms and after each sprint, using a portable device.

- Rate of Perceived Exertion (RPE): it will be evaluated every 5 kms, before and after each sprint and at the kms 7.5, 21.5, 36.5 e 51.5, using the Borg (1982).

- Power, Cadence and Speed: it will be registered every kms and showed by the cyclo computer attached to the trainer.

- Core Temperature (T\textsubscript{Core}): it will be measured using a thermal sensor, inside a capsule, which will be ingested approximately 12 hours before the exercise starts, so that the capsule reaches the intestines by the time of the exercise. The reading of the T\textsubscript{Core} will be done using a data register system (CorTemp™ Data Recorder), and it will be registered during rest and every 2 km of exercise.

- Skin Temperature: sensors will be placed on four parts of your body (arm, chest, thigh and calf) so temperatures can be monitored during rest, every 2 km and at the end of exercise.

- Urine Specific Gravity (USG): it will be measures before and after exercise to verify the hydration status in each experimental trial.

- Sweat Rate (SR): total sweat (TS) will be calculated by the difference in body mass before and after exercise, considering everything you will ingest and the amount of sweat left in your cycling shorts, socks, cycling shoes and all materials your that will be attached to you during exercise. A SR will be calculated dividing your TS by the time between the weightings and your body surface area.

Variables related to neuromuscular function

Surface electrodes will be positioned on the quadriceps muscle, where shaving the area might be required. The electric stimulation current will be applied in progressive intensities, during short time intervals, respecting in all moments the subject’s subjective tolerance. These variables will be measured: maximal voluntary contraction, muscle fatigue, electromyography, maximal muscle fibres tension and voluntary muscular activation.

POSSIBLE DISCOMFORTS AND RISKS

You might experience muscle pain, later after exercise or not, tiredness which should disappear within two to five days of each trial. Soreness can appear in the area where the venous puncture was done, disappearing in a week time maximal. General risks on physical activities should be considered, such as muscle skeletal injuries, trauma in general and heart attacks. However, you will exercise in laboratory conditions, strictly controlled, with careful procedures and technically proper executed. Besides this, you will notice, on the following days after each experimental trial with carbohydrate capsules, differences in the colour of your faeces, which will be similar to the colour used on the gelatine capsules ingested. This is normal, since the gelatine the capsules are made is not absorbed by the body being eliminated later. You also might feel some discomfort when receiving the electrical stimulation. However, your tolerance with the discomfort will always be respected so that the procedure gets done in the best way possible, with intervals between measurements so it is done within your own comfort.
EXPECTED BENEFITS

As a benefit in participating in this study you will have the opportunity to learn how your body adjusts itself in the heat and how it reacts when carbohydrates are ingested. You will receive a copy of the results of your physical evaluation, important information for your training method. At the end of the study, the researchers will interpret and present all the results related to the performance on the experimental trials, if it is your interest. Furthermore, this research will help researchers generate knowledge about the influence of carbohydrates on performance, relating it to fatigue and the central nervous system.

ALTERNATIVE EXISTING METHODS

Traditionally, studies have used beverages as a method of supplying carbohydrates during exercise. In this study we will use capsules as well, as an alternative method. Therefore, we will be able to verify if there are differences in the variables measured when you ingest beverage or capsules containing carbohydrates.

FOLLOW UP AND ASSISTANCE

You will be cared by experienced researchers during all stages of the study. During the experimental trials, with the objective of guarantying your physical integrity, researchers will follow a careful criterion to interrupt exercise, according to recommendations internationally recognized. The appearance of one of the criterion alone is enough to interrupt exercise during the experimental trials. They are:

- Core temperature ≥ 39.5°C;
- Perceived exertion of 20 (Borg scale);
- You asking to interrupt exercise;
- You describing or the researchers observing any symptoms such as, dizziness, mental confusion, lack of coordination, paleness, nausea, cold and humid skin, cyanosis, etc.

If by any means any problem occurs, an interdisciplinary team, composed of Physical Education, Physiotherapy and Medicine will be present. In case of emergency, triple zero (000) will be called.

GUARANTEE OF ELUCIDATION AND RIGHTS

You have all the right to have all your questions elucidated that might come up before and during the study. Any questions, contact the responsible researchers: Dr. Emerson Silami Garcia, Ms. Camila Nassif Leonel or Aline R. Gomes, (tel: (31) 34992350). You will have the right to refuse participating or withdraw your consent, in any stage of the study, without penalty and losses to you. You should also understand that the researchers might decide to exclude you from the study for scientific reasons, which you will be clearly informed.

PRIVACY

All information about you is confidential. Your identity will never be revealed publicly by any means and only the researchers involved will be able to access this information that will be used for research purposes.

EVENTUAL EXPENSES AND/OR REEMBURSEMENTS

As you will be using your own bicycle, a budget has been put aside for maintaining your equipment, in case any problem occurs by inadequate use of the bicycle by researchers during the experimental trials. This budget comes from the School of Human
Movement Studies (Charles Sturt University), Australian institution participating in this research.

Except from what has been said above, there is no kind of remuneration of payment for possible medical expenses for you, subject. All specific expenses related to the study are responsibility of the Exercise Physiology Laboratory (EEFFTO/UFMG) and the Human Performance Laboratory (School of Human Movement Studies/Charles Sturt University).

CONSENT

I agree with all that has been exposed above and voluntarily accept participating in the study “The Effects of Carbohydrates Ingestion on Fatigue Parameters during Exercise in the Heat” which will take place at the Exercise Physiology Laboratory of the School of Physical Education, Physiotherapy and Occupational Therapy of the Federal University of Minas Gerais. The results of this research will be used to elaborate a master’s dissertation and a PhD thesis, besides scientific initiation abstract.

Belo Horizonte _____ of ___________de 2007

Subject’s Signature: __________________________________________

Witness’ Signature: __________________________________________

We declare for any means that we have explained the aims of this study to the subject, considering the limitations of our scientific knowledge.

________________________________
Ms. Camila Nassif Leonel
PhD Candidate / Researcher (CSU)

________________________________
Prof. Dr. Frank Marino
Supervisor (CSU – Bathurst / Australia)

This study was approved by the Ethics in Human Research Committee of Charles Sturt University (Protocol Number 2005/064). Any considerations or complaints, contact Julie Hicks – Executive Officer of the Ethics in Human Research Committee (CSU/Bathurst). Phone number +61 (02) 6338 4628; email: ethics@csu.edu.au.
APPENDIX B

TRIAL INSTRUCTIONS

Charles Sturt University
School of Human Movement Studies
Panorama Avenue – Bathurst NSW 2795 Australia
E-mail: tricamilanassif@yahoo.com.br

“THE EFFECTS OF CARBOHYDRATES INGESTION ON FATIGUE PARAMETERS DURING EXERCISE IN
THE HEAT”

Responsible Researcher: Camila Nassif Leonel
Supervisor: Prof. Dr. Frank E. Marino
Co-Supervisor: Dr. Jack Cannon

IMPORTANT INSTRUCTIONS FOR PARTICIPATING IN THE STUDY

1) Initial Instructions

- Your trial is scheduled to happen on the day ______________ at _______________. Any matter that might occur making it impossible for you to come, please, inform the responsible researcher as soon as possible.

- These instructions were put together to inform you, subject, about all procedures you will have to follow prior to data collection. You must follow these instructions rigorously! If for any reason you are not able to follow it, you should inform the responsible researcher as soon as possible before data collection happens. By not following one of these instructions can lead to loss of results and a need to repeat the trial which the instruction was not followed. Read carefully the list below and if by any chance you have any questions, feel free to ask the responsible researcher for explanation.

- Any discomfort in any stage of the study, inform the responsible researcher immediately. If you notice any out of normal symptom in your body the days that proceed the trials, inform the responsible researcher.
2) General instructions for all tests (VO2max, and 60km Time trials)

- **ATTENTION!** During the period that you will be participating in this study, actually, during a month and a half period, do not submit yourself to a magnetic resonance, since during each trial you will ingest a core temperature pill, what does not allow this kind of test to be undertaken.

- Do not participate in physical activity and/or training the day prior to the trials.
- For your comfort, bring your cycling shoes, your bike and riding shorts which will be used during exercise.
- Do not ingest foods rich in caffeine (coffee, teas, soft drinks and chocolate) and alcoholic beverages during your breakfast before each trial.
- We recommend you bringing shower items in case you would like to shower after the trials. The lab is equipped with a toilet and shower to subjects’ convenience.

3) Specific Instructions for the 60km time trials

- In your last day of training which will happen two days prior to each trial, give preference to training in the morning.
- The responsible researcher will meet you the night before each trial. She will hand you the core temperature capsule that should be ingested after the last meal of the day prior to the trial (dinner), according to the diet prescribed by the nutritionist.
- You should follow the diet prescribed by the nutritionist for the two days prior to each trial.
- Hydrate yourself the day prior to each trial similarly to a pre competition day. Follow the diet prescribed by the nutritionist which is rich in carbohydrates.
- You will be required to drink 500 ml of water 2 hrs prior to each trial. Therefore, if your trial is to start at 9:00 am, the water should be ingested at 7 am. To make sure the right amount (500 ml) will be ingested, you will use a cup that will be given to you by the researchers.
- After breakfast, you will not be allowed to ingest and food or liquids.
- In the chart below, write what you have ingested for breakfast prior to your first trial. You should repeat this breakfast on the other following trial days (four trials). Below the chart, write the time of start and finish of your breakfast.

<table>
<thead>
<tr>
<th>Food Ingested</th>
<th>Amount</th>
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<tbody>
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</table>

Start: _______________    Finish: _______________

- A snack will be provided at the end of each trial so you can leave the lab fed and give sequence to your other activities for the rest of the day.
APPENDIX C

MEDICAL QUESTIONNAIRE

"THE EFFECTS OF CARBOHYDRATES INGESTION ON FATIGUE PARAMETERS DURING EXERCISE IN THE HEAT"

Responsible Researcher: Camila Nassif Leonel
Supervisor: Prof. Dr. Frank E. Marino
Co-Supervisor: Dr. Jack Cannon

MEDICAL QUESTIONNAIRE

Instructions:

- All answers to this questionnaire are confidential.
- Only the responsible physician and the researchers will have access to it.
- This questionnaire must be answered and returned in a sealed envelope.

Name: ________________________________________________________________
Date of Birth: ____/____/____ Age: ______ City of Birth: ______________
Address: ___________________________________________________________________
Phone Number: ___________________________________
Marital Status: ___________________________________
Profession: ___________________________________
Higher Level of Scholarly: _________________________________
In case of an emergency, contact: _______________________________________
CLINICAL QUESTIONNAIRE N°01

Do you have any complains about your health at the moment?

(If positive, describe what you feel, for how long you have been feeling it and what you have been doing to ease the problem.)

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

1) When was your last visit to a physician? For which reason?
2) Have you had or have you any disease or wound after your last medical examination?
3) Have you been admitted to hospital? For which reason?
4) Have you gone through surgery of any kind? Which and when?
5) Are you taking any medication at the moment? When?
6) Have you ever taken any kind of food supplement or vitamin to help you gain or lose weight?
7) And to enhance your performance?
8) Do you have allergies that medical treatment is needed? (pollen, medication, food, insects)
9) Have you had eruptions like urticaria during or after exercise?
10) Have you ever felt sick during or after exercising in the heat?
11) Have you ever fainted during or after exercise?
12) Have you felt dizzy during or after exercise?
13) Have you ever had chest pain during or after exercise?
14) Do you get tired faster than your friends when you exercise?
15) Have you had palpitations, or felt your heart accelerate, or out of pattern heart rate?
16) Have you ever had your blood pressure measured? What was the result?
17) Have you ever had your blood cholesterol measured? What was the result?
18) Have you ever had your blood glucose measured? What was the result?
19) Have any physician told you that you have any problem with your heart?
20) Have any member of your family or relative died of heart problems or died suddenly before they turned 50 years old? Who?
21) Have any physician prohibited you or limited your participation in sports?
22) Did you have any infection in the past month?
23) Do you have any skin problems at the moment (eruptions, acne, fungus, blisters, itching, wart)?
24) Have you ever had any cuts in the head?
25) Have you ever been hit in the head, fell unconscious or lost your memory?
26) Have you had a convulsion?
27) Do you have headaches frequently and very strong ones?
28) Have you felt numbness, tingling on arms, hands, legs or feet?
29) Have you felt sting, burning or painful retraction of a nerve?
30) Have you used or do you use alcohol? How frequent?
31) Do you smoke or have smoked in the past? How many cigarettes a day?
32) Do you cough, shriek or have difficulties breathing during and after exercise?
33) Do you have asthma?
34) Have you ever used inhalator?
35) Have you ever used or do you use correction pieces (knee collar, neck collars, orthopaedic shoes, teeth protectors, hearing impaired device)?
36) Do you have any problems in your eyes or vision?
37) Do you wear glasses, contact lents or ocular protector?
38) Is your weight stable?
39) Would you like to weight more or less than what you weight now?
40) Do you do any diet to control your weight?
41) Do you feel nervous, overwhelmed or stressed?
42) Have you been vaccinated against:
   Tetanus:  
   Missles:  
   Hepatitis B:  
   Mumps:  
43) Have you ever had a torsion, distension or swollen after a sport accident?
44) Have you fractured any bone or sprained any joint?
45) Have you ever had any problem of pain or swollen muscles, tendons, bones or joints? If so, described the part of the body.

I declare that the answers above are answered correctly and as complete as possible.

________________________________________________________________________

Subject's Signature

Date: ____/____/____

Observations of the responsible physician:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

Date: ____/____/____

________________________________________________________________________

Signature and Stamp of Responsible Physician
CLINICAL QUESTIONNAIRE N° 02

1) Important Observations about the Core Temperature Pill

So you can ingest the core temperature pill with safety, please, answer the questions below.

a) Do you weight less than 36.28 kg?

b) Do you have any or have suspected of any known obstructive disease of the gastrointestinal tract, including but not limited to diverticulitis and inflammatory bowel disease?

c) Do you have or have a history of disorders or impairment of the gag reflex?

d) Have you undergone any gastrointestinal surgery? When?

e) Do you have felinization of the esophageus?

f) Do you believe you might undergo Nuclear Magnetic Resonance (NMR) or MRI scanning during the period that you will be using the CorTemp™ Disposable Temperature Sensor in this study?

g) Do you suffer from hypo motility disorders of the gastrointestinal tract including but not limited to Ileus?

h) Do you have a cardiac pacemaker or other implanted electro medical device?

ATENTION!

During the period that you will be participating in this study, actually, during a month and a half period, do not submit yourself to a magnetic resonance, since during each trial you will ingest a core temperature pill, what does not allow this kind of test to be undertaken.

I declare that the questions above were answered as clear and correct as possible and that I am aware of being cautious using the temperature capsule during the study, as described above during the study.

________________________________________
Subject’s Signature

Date: _____/_____/_____
APPENDIX D

NUTRITIONAL ASSESSMENT

Charles Sturt University
School of Human Movement Studies
Panorama Avenue – Bathurst NSW 2795 Australia
E-mail: tricamilanassif@yahoo.com.br

“THE EFFECTS OF CARBOHYDRATES INGESTION ON FATIGUE PARAMETERS DURING EXERCISE IN THE HEAT”

Responsible Researcher: Camila Nassif Leonel
Supervisor: Prof. Dr. Frank E. Marino
Co-Supervisor: Dr. Jack Cannon

NUTRITIONAL ASSESSMENT

Subject: ________________________________________________
Date: ____/____/____
Date of Birth: _____/_____/_____   Age: ______________
Height: _________   Body Mass: _________   %BF: _____________
Visual Perception of body composition: ____________________________

Questionnaire

1) Actual Daily Activities: study and/or work? Details and schedules.
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
2) Usual time of waking up and going to sleep:

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________


3) Physical Training Program (time, duration, intensity and sport):

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________


4) Food habits (types of food preferred, amount, and milk (full or skim), sugar, olive oil, sweets, chocolate and size of glasses used for fluid ingestion during meals):

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________
5) Chart of times and meals

<table>
<thead>
<tr>
<th>Time</th>
<th>Meal</th>
<th>Foods/Amount</th>
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6) Makes use of any energetic beverages or supplement? Details (amounts, what time of the day its ingested, brand, etc.).

__________________________________________________________________________
__________________________________________________________________________
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__________________________________________________________________________

7) Other important observations:

__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

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APPENDIX E

DIET PRESCRIPTION

Example of a Diet Received for Each Subject

Diet Plan

• Name:

• Objective: Muscle Glycogen recuperation for trials

• Caloric Content: 6800 Kcal

• Nutritionist:

Last Day of Training before Trials

8:30 - Breakfast

2 carbohydrate portions - 2 french breads...

1 portion of protein - 6 knife points of cheese spread (1 soup spoon full)...

1 portion of fruit - see list

1 portion of milk - 1glass 250mL skim milk...

Coffee with sugar

During Training session

4 measures of maltodextrin

2 portions of fruit - 2 bananas...

2 small gel sachets

2 cereal bars
After training session
1 litre of juice with sugar

13:00/14:00 - Lunch
8 carbohydrate portions - 8 big serving spoons of rice...
4 portions of protein - 4 big serving spoons of beans...
2 portions of protein - 2 pieces of chicken...
Vegetables A and B free amounts

16:00- Mid Afternoon
2 carbohydrate portions - 2 french breads...
1 portion of protein - 6 knife points of cheese spread (1 soup spoon full)...
1 portion of fruit - see list
1 portion of milk - 1 glass 250mL skim milk...
Coffee with sugar

19:00- Snack
2 carbohydrate portions - 2 french breads...
3 portions of protein - 3 slices of cheese...
500ml of juice with sugar
1 portion of fruit - see list

21:30- Dinner
The same as lunch or 25 pieces of Japanese food (give preferences to the ones that have rice)
300ml of juice
Diet Plan

- Name:
- Objective: Muscle Glycogen recuperation for trials
- Caloric Content: 6100 Kcal
- Nutritionist:

Rest Day before Trial

8:30- Breakfast

2 carbohydrate portions - 2 french breads...
1 portion of protein - 6 knife points of cheese spread (1 soup spoon full)...
1 portion of fruit - see list
1 portion of milk - 1 glass 250mL skim milk...
Coffee with sugar

11:00- Mid Morning

2 portions of fruit - 2 bananas...
2 cereal bars

13:00/14:00 - Lunch

8 carbohydrate portions - 8 big serving spoons of rice...
4 portions of protein - 4 big serving spoons of beans...
2 portions of protein - 2 pieces of chicken...
Vegetables A and B free amounts
16:00- Mid Afternoon

2 carbohydrate portions - 2 french breads...
1 portion of protein - 6 knife points of cheese spread (1 soup spoon full)...
1 portion of fruit - see list
1 portion of milk - 1 glass 250mL skim milk…

Coffee with sugar

19:00- Snack

2 carbohydrate portions - 2 french breads...
3 portions of protein - 3 slices of cheese...
500ml of juice with sugar
1 portion of fruit - see list

21:30- Dinner

The same as lunch or 25 pieces of japanese food (give preferences to the ones that have rice

300ml of juice
**Diet Plan**

- Name:
- Objective: Muscle Glycogen recuperation for trials
- Caloric Content: 6500Kcal
- Nutritionist:

**Trial Day**

**6:30- Breakfast**

2 carbohydrate portions - 2 *french breads*...

1 portion of protein - 6 *knife points of cheese spread (1 soup spoon full)*...

1 portion of fruit - see list

1 portion of milk - 1*glass 250mL skim milk*...

**Snack 40 min to 1 hour before start of Exercise**

2 portions of fruit - 2 *bananas*...

2 cereal bars

**13:00/14:00 - Lunch**

8 carbohydrate portions - 8 *big serving spoons of rice*...

4 portions of protein - 4 *big serving spoons of beans*...

2 portions of protein - 2 *pieces of chicken*...

Vegetables A and B free amounts

**16:00- Mid Afternoon**

2 carbohydrate portions - 2 *french breads*...
1 portion of protein - 6 knife points of cheese spread (1 soup spoon full)...
1 portion of fruit- see list
1 portion of milk- 1glass 250mL skim milk…
Coffee with sugar

19:00- Snack
2 carbohydrate portions -2 french breads...
3 portions of protein - 3 slices of cheese...
500ml of juice with sugar
1 portion of fruit- see list

21:30- Dinner
The same as lunch or 25 pieces of Japanese food (give preferences to the ones that have rice or 1 foot long subway sandwich
300ml of juice

OBSERVATIONS:

Your meals are balanced quite well, most importantly the amount of carbohydrates....

If you feel like eating more, it is not a problem! You can have more carbs and fruits!!!

Good Luck!!!

Distribution according to ACSM: 60 to 70 % of carbohydrates

1,2 to 1,8 g / prot / Kg of body mass

20 to 25 % of fat


Soares, D. D. (1993). Efeitos da elevação da temperatura interna sobre o tempo total de exercício, a percepção subjetiva do esforço e as respostas termorregulatórias durante o...
exercício submáximo realizado em ambiente termoneutro. Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.


Até o fim...
Não; não pares,
E graça divina começar bem,
gração maior manter o ritmo...
na caminhada certa.
Mas, a graça das graças é não desistir.
Podendo ou não podendo
caindo embora aos pedaços,
Chegar até o fim.

Dom Hélder Câmara

 Until the end...
 No, don’t give up,
 It is a blessing to start well,
 higher blessing to keep the pace...
 on the right track.
 But, the blessing of the blessings is not give up.
 May or may not,
 while breaking up into pieces,
 Get to the finish.

Dom Hélder Câmara