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Methods to optimise substrate utilisation during endurance performance.

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Methods to optimise substrate utilisation during endurance performance.

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1. INTRODUCTION

1.1. Limitations to endurance performance

Endurance events often take humans to the very edge of their capacity to perform both physically and mentally. The basic limitation to performance when the levers just cannot produce any more force or move any quicker can be traced back to oxygen kinetics in relation to a specific bout of work asked of the body, the accumulation of subsequent by products prematurely resulting in fatigue, and ultimately the depletion of fuel sources.

To produce a movement, i.e. running, muscles contract and change the anatomical positioning of the levers. Muscle contractions however require ‘currency’, in the form of adenosine tri-phosphate (ATP), to carry out a contraction. Hydrolysis of ATP provides energy and ‘recyclable’ products, adenosine di-phosphate (ADP) and phosphate (Pi), for subsequent energy production (see equation 1.1.1.).

Equation 1.1.1. Simplified overview; Hydrolysis of ATP

\[
\text{ATP + H}_2\text{O} \rightleftharpoons \text{ADP + Pi (+ ENERGY )}
\]

Reverse process; In presence of O\(_2\) = aerobic metabolism/ oxidative phosphorylation

In absence of O\(_2\) = anaerobic metabolism

Free ATP is however a limited resource and must be synthesised once the free ATP is reduced. The quickest pathways of ATP production are through anaerobic (without oxygen) metabolism of phosphocreatine (PCr) and glycolysis. The ATP-PCr cycle provides a rapidly accessible source of phosphate however will supplement the muscles demand for high intensity energy requirements for only seconds. Anaerobic glycolysis oxidises simple forms of carbohydrates which can sustain high intensity exercise; however this pathway is not efficient in ATP production and becomes exhausted after thirty seconds to two minutes. The by products of anaerobic glycolysis cause fatigue which negate the extended duration of this pathway. Lactic acid is the principle end product, and the associated increase in hydrogen ion
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Concentration inhibits the reduction of glycogen to glucose-1-phosphate (G-1-P) and fructose-1-phosphate (to F-1-P) and thus slowing down the glycolytic and energy production pathways.

The third pathway utilised during exercise is aerobic metabolism. This takes place in the mitochondria in the presence of oxygen and can be sustained for hours on end with substrate availability being a limiting factor to this pathway. This process of aerobic glycolysis produces ATP at a rate about two thirds that of the glycolytic pathway (Maughan and Gleeson, 2004) however this process is more abundant in net ATP yield (38/ 39 ATP.mol\(^{-1}\)) and sustainable for longer duration events. The demand for energy requirement from the muscle during an endurance event must conform to the rate limitation of this pathway. Endurance events are therefore conducted at lower intensities which allow for sufficient ATP production to match rates of utilisation in the muscle, as 99% of the energy requirements in events lasting longer than two hours come from aerobic sources (Burke, 2004). To maintain aerobic energy production sufficient fuel must be provided for these pathways which require oxidation of either carbohydrate or fat. The rate of fat oxidation to produce energy is only one third that of aerobic carbohydrate metabolism (Maughan and Gleeson, 2004). However dependant on the intensity and duration of event athletes can be faced by a limitation of endogenous carbohydrate availability, glycogen, to fulfil the ‘cost’ of completing the event without having to utilise oxidising fat stores. There is a limited supply of between 400 - 500g of endogenous carbohydrate in the human body mainly stored as glycogen in the liver (~100g/ 20%) and muscle (~400g/ 80%) with minor circulating levels (<2%) in the blood as glucose. This store can potentially account for up to 6400 – 8000 kJ of energy, however a potential limitation arises when the estimated consumption of a recreational athlete completing the marathon is closer to 11000kJ (Williams et al, 1984). This in turn forces the body to turn to fat oxidation, which produces ATP at a slower rate but is more abundant in the body (> 400, 000 kJ (Latta., 2003 even lean runners have fuel to go for >600 miles)), to complete the event.

In endurance events such as the marathon there is an experience spoken about which athletes deem as a definitive point in which their performance declines, at around 24–32 km. This experience is described as ‘hitting the wall’ which Martin et al (2003) define as ‘basically running out of energy’. Raporoport (2010) supports that ‘hitting the wall’ is not a lack of stored energy, rather a depletion of endogenous and exogenous glycogen stores. The stage at which a given athlete will ‘hit the wall’ can be predicted from using leg muscle mass and
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Bases on the estimation used since the 60s (Margaria et al., 1963), that running will expend energy at a rate of \( \sim 4.2\text{kJ.kg}^{-1}.\text{km}^{-1} \) the average athlete (70 kg man) can expect to consume around 9000 kJ of endogenous stores before total ‘hitting the wall’. This will therefore prove an energy deficit based on the estimated 11,000 kJ used on average to complete the distance (42.195km), yet substantially within the hundreds of thousands of kJ of energy the human body stores. As energy metabolism is dependant on running intensity typically \( \sim 85\% \text{VO}_{2}^{\text{max}} \) for a marathon (Romijn et al., 1993) Raporoport (2010) suggests that at this intensity the fractional utilisation of the athlete will be \( \sim 75\% \) carbohydrate and \( \sim 25\% \) fat. This would therefore support that ‘hitting the wall’ will coincide with a massive exhaustion towards total depletion of carbohydrate stores. When ‘hitting the wall’ the athlete is experiencing severe fatiguing symptoms, yet is in the presence of such abundant fat stores, suggests the need for a fuelling strategy is of great importance to prevent carbohydrate stores to reaching this critical level and limiting subsequent performance.

1.2 Strategies to overcome limitations

As carbohydrate stores are limited, and not sufficient for the completion of many endurance events, it is necessary to maximise the stores of carbohydrate and minimise carbohydrate usage to maximise performance in such events.

Manipulation of diet and training regime in the lead up to a race in an attempt to increase the stored levels of carbohydrate have produced clear results of a direct improvement in endurance performance. The method by which an increase in muscle glycogen content is achieved has become less extreme but it is clear that increasing carbohydrate availability is directly associated to improved performance during an endurance event.

1.2.1. Glycogen supercompensation strategies

Consuming an exogenous supply of carbohydrate is the most common method to delay glycogen depletion and is used by almost any athlete planning to seriously compete in an endurance event. There are however other methods used by athletes to super-compensate
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glycogen stores before an event through manipulation of pre-race diet (MacLean et al., 1992; Chryssanthopoulos et al., 1994; Burke et al 2000; Sherman et al 1981; Goforth et al., 1997; Walker et al., 2000) and/or training regime (Ahlborg et al., 1967, Basseau et al., 2003, Goforth et al., 2003; Hawley et al., 2008).

The classical studies by Bergstrom and Hultman in the mid- late 1960s (1966, ’66, ’67) still provide the basis of our knowledge in that “muscle glycogen fatigue explains fatigue in fed subjects who exercise to exhaustion” as it is noted that within subjects the greater the muscle glycogen store prior to exercising the greater the capacity this individual has to maintain a specific work load. This pioneering work investigated a seven day protocol (Ahlborg et al., 1967) to supercompensate endogenous stores of carbohydrate. Athletes had a 3-4 day period of hard training, known as the depletion phase, on a diet low in carbohydrate. This was followed by a 3 - 4 day period of exercise tapering and a diet high in carbohydrate, and on completion significantly increased cycling time to exhaustion.

This method, though viable, proved a complex option and work by Sherman (et al., 1981) investigated a method for diet induced glycogen loading using a three day protocol consuming a diet high in carbohydrate. This protocol investigated the benefits of the second stage of the protocol pioneered by Bergstrom and Hultman, tapering an exercise protocol and consuming a diet high in carbohydrate. This protocol was able to show an increase glycogen stores in the muscle by up to 160-200 mmol.kg⁻¹ wet weight supercompensating glycogen stores by almost 100% when compared to normal resting values of athletes 80-120 mmol.kg⁻¹ wet weight (Ahlborg et al., 1967; Bergstrom et al., 1967). An increase of this magnitude in the muscles needs no explanation to find the increase in endurance capacity noted when these super-compensated athletes take part in endurance events (Chryssanthopoulos et al., 1994).

More recently however this protocol has been revised again to allow for a shortened period of supercompensation. Basseau (et al., 2002) principally investigated a three day glycogen loading protocol were eight endurance athletes consumed 10 g.kg⁻¹ body mass per day. Muscle biopsies were taken after one and three days of the carbohydrate loading. After only one day of loading with a high carbohydrate diet resting levels of glycogen significantly rose to ~180 mmol.kg⁻¹ wet weight from baseline values of ~90 mmol.kg⁻¹ wet weight. Glycogen
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Concentration thereafter remained stable questioning the need to consume a diet so high in carbohydrate for any longer than twenty-four hours.

Rauch et al (2005) demonstrates this on 8 well trained cyclists. On analysis of a double blind crossover study exercising athletes carbohydrate loaded (3 day protocol) and on their normal diet it was noted that although a higher power output was able to be sustained when prior carbohydrate loading had taken place the end muscle glycogen values were relatively similar between the trails for each individual but absolutely different between the individuals. Increasing stores of carbohydrate whether it be endogenously prior to competing or exogenously during competition are accepted as being beneficial to performance (Ahlborg et al., 1967, Sherman et al., 1982, Burke, 2007, Coyle et al., 2007, Jeukendrup et al., 2009, Jeukendrup et al 2010), however with the length of endurance events such as a marathon the requirement for energy cannot alone be met by carbohydrate supplementation and/or supercompensation. Glycogen sparing has also been considered through the up regulation of fat oxidation. Protocols providing a dietary adaptation to up regulate fat oxidation have been successful but their application in terms of performance and health are not definitive. Such protocols are carried out over 5 days consuming low levels of carbohydrate (<2.5 g.kg$^{-1}$) and a high amount of fat (~60-70% of total energy intake). Concern has been voiced against this protocol by Burke and Kiens (2006) to the prolonged (>4weeks) exposure to this protocol on training outcomes and health risks. Therefore strategies aim to offset or prevent glycogen stores from reaching their pre-determined marker for protection. This lead into investigations to either utilise alternative or supplementary products to provide assistance to offset glycogen depletion and ‘hitting the wall’.

The consumption of a diet high in carbohydrate has been accepted for over forty years to shown a super compensation of stored muscle glycogen (Karlsson and Saltin, 1971, Goforth et al 1997, Sherman et al 1981). This super compensation also correlates positively with an improvement in performance in submaximal exercise lasting over 90 minutes (Chryssanthopoulous et al 1994). However the exact route by which performance is enhanced is still researched and definitive explanation is yet to be found as described below in a study by a group of Dutch researchers (Stellingwerf et al 2007).
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During endurance events it is widely accepted that consumption of exogenous carbohydrates in a timely fashion will increase performance above placebo through maintaining euglycemia and prolonging the time to ‘hitting the wall’ (Jeukendrup et al 2007, Jeukendrup et al 2010, Coyle 2004). Stellingwerf (et al., 2007) demonstrated that the consumption of exogenous carbohydrate reduces dependence on endogenous glycogen during the early stages of prolonged exercise however this adaptation did not effect intramuscular tri-glyceride (IMTG) oxidation rates (Stellingwerff et al, 2007). Ten endurance trained cyclists were exercised for three hours twice at 63 ± 4% O₂ uptake. One trial exercised with a glucose infusion (0.7g CHO.kg.hour⁻¹) and second trial consuming water only. Glucose tracers estimated that during the glucose infusion trial glycogen use was reduced during the first hour of exercise in both type I (39 ±19%) and type II (57± 22%) muscle fibres. Therefore if this increased dependence on endogenous carbohydrate can be mediated through the consumption of exogenous carbohydrate during the race then this will increase the amount of stored glycogen for use during the latter stages of prolonged exercise. Weltan (et al 1998) however demonstrated that consumption of exogenous carbohydrate solution at regular intervals is sufficient to maintain euglycemia and reduce the reliance of glycogen as a substrate. Thirteen endurance cyclists performed a glycogen depleting protocol by which they cycled for performed a VO₂max test then rested for 20 minutes before cycling for 90 minutes at 70% VO₂max including 5 minute intervals at 90% VO₂max every 20 minutes. The following day diet was restricted (only 16% CHO) for one group to impair glycogen repletion, and this group was the low glycogen content group. The normal glycogen content group had no restrictions on diet. Subjects then cycled at 70% VO₂ max for 145 minutes with a glucose infusion to maintain blood glucose at around 4.5 mmol.l⁻¹. Both groups began exercise with different levels of muscle glycogen, however they completed the exercise on similar levels of muscle glycogen content. Weltan therefore believes the actual mechanism behind the sparing of glycogen requires further research but is likely to be linked to a neural or hormonal factors distant from the muscle. All subjects finished at similar levels of muscle glycogen however began on varied levels of muscle glycogen therefore there may be an intramuscular controlling mechanism which determines the rate of muscle glycogen oxidation preventing muscle glycogen from reaching critical levels.

Now however it is acceptable to consume carbohydrate on the day to optimise performance capacity. There are guidelines in place for consumption of exogenous carbohydrate to prevent
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hypoglycaemia and improve endurance performance (ACSM, 2007). With more research into the metabolism of carbohydrate during exercise there is now a shift towards increasing these limits towards 90g.hour\(^{-1}\) (Hawley et al., 2009) through consuming glucose and fructose discussed later in detail.

1.2.2. Mode of delivery

One important decision that must be made prior to the event is what mode of carbohydrate the athlete will consume. Carbohydrate supplements are available in three main forms, solid, gel and liquid. Making the right choice of supplement, or combination, is dependant on the duration of the race, the mode of exercise, and the environment in which the event will take place.

An effective and widespread method for delivery of carbohydrate during prolonged exercise was through consuming carbohydrate dissolved in liquid to provide a 6% solution containing monosaccharide polymer glucose (Angus et al 2000). These drinks also contain electrolytes to address another potential limitation of dehydration through fluid loss through sweating however the principal aim of the supplement is to deliver high levels of carbohydrate. More recently it has been shown that the most effective method for delivery carbohydrate is a combination of glucose (6%) and another monosaccharide sugar fructose (3%) to provide a 9% carbohydrate solution (Jeukendrup et al 2009, Jeukendrup et al., 2010, Jetjens et al., 2004). This has the potential to deliver 50% more carbohydrate to the working muscles (Jetjens et al, 2004) as the two different forms of carbohydrate are able to be delivered synergistically on two different transporters (Jetjens et al, 2005).

To address the potential issue of gastro-intestinal distress caused by a stomach full of liquid athletes can consume carbohydrate in the form of either solid or gel based products (ACSM, 2007). There is a limited research base directly comparing all modes of carbohydrate delivery against running performance. However, of note, Campbell et al (2008) recently investigated all three modes of carbohydrate delivery during a cycling protocol. Sixteen subjects performed the protocol on four separate occasions consuming either sports beans, sports drinks, carbohydrate gel and water as a placebo. Subjects cycled for 80 minutes at 75% of VO\(_{2}\)peak before completing a 10 km time trial. There was a significant improvement in 10 km time trial
Methods to optimise substrate utilisation during endurance performance. Performance on all three carbohydrate loading protocols compared to water. However there was no difference between the mode of supplementation and all mode of carbohydrate supplementation were able to maintain blood glucose.

The use of solid carbohydrate as a supplement is seen as a less effective method for delivering carbohydrate at fast rates in an endurance protocol of both running and cycling (Peters et al, 1995). Thirty-two male triathletes completed the three hour endurance protocol cycling for 51 and 43 minutes (first and third) and running twice for 43 minutes (second and fourth) all at 75% VO$_2$max (85% of max HR). In this instance the solid food consumed was of more complex carbohydrates, white bread, marmalade, and bananas which put this group at a disadvantage due to the process involved in breaking down these foods before absorption. The solid has the additional process of chewing before it can be swallowed and again requires adequate fluid to aid absorption and in certain instances prevent the onset of dehydration. However Pfeiffer (et al., 2010) has shown how a solid form of carbohydrate is just as effective as a liquid carbohydrate solution in eight well-trained cyclists working at 50% WR$_{max}$ for 180 minutes. There was no difference in peak carbohydrate oxidation (BAR 1.25 ± 0.15 g.min$^{-1}$ and DRINK 1.34 ± 0.27 g.min$^{-1}$) or in the rate of carbohydrate oxidation during the last 2 hours of exercise (BAR 2.26 ± 0.31, LIQUID 2.22 ± 0.43 g.min$^{-1}$).

For running the gel based product provides an effective compromise between the GI distress felt through consuming large amounts of liquid and the delayed transit time of consuming solid carbohydrate. Consuming a gel based product will also maintain blood glucose levels (Patteron and Gray, 2007, Campbell et al., 2008) and provide carbohydrate at the same rate as supplementation of liquid carbohydrate alone (Jeukendrup et al., 2010). Consuming a gel based carbohydrate product also does not require consumption of excess amounts of fluid to deliver the carbohydrate. However it is still advised to consume 0.4-0.8litres.hour$^{-1}$ of water improves transit whilst also providing adequate fluid to prevent dehydration when taking part in prolonged exercise (ACSM, 2007). The same simple carbohydrates as included in the fluid carbohydrate mixtures prove just as effective as in the gel formation. Pfeiffer et al (2010) studied eight trained cyclists over three separate trails at 50% WR$_{max}$ (59 ± 4% VO$_2$max) for 180 minutes whilst consuming gel carbohydrate, fluid carbohydrate, and a placebo (water). Both carbohydrate feeding trials provided glucose and fructose at a 2:1 ratio, and delivered 108 g.hour$^{-1}$ carbohydrate, and consumed 867 ml.hour$^{-1}$ of fluid. There was no significant
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difference in the rate at which the gel or liquid supplements were oxidised with both products providing similar peak exogenous carbohydrate oxidation rates.

1.2.3. Mechanism of carbohydrate absorption

The principal monosaccharide sugar used in exogenous carbohydrate supplementation is glucose. Glucose is absorbed in the small intestine on the cotransporter SGLT1 (see fig 1.2.3.1). The cotransporter SGLT1 is thought to be saturated for transport of glucose at consumption rates of greater than 1.2g.min\(^{-1}\) as even when high rates of glucose are administered the rate of carbohydrate oxidation does not linearly increase and can in fact be adversely affected (Hawley 2008, Jetjens et al., 2005, Jeukendrup et al., 2006, Jeukendrup, 2010). The SGLT1 cotransporter works by active transport on a Na\(^+\) dependant diffusion mechanism. When the concentration of glucose is raised in the intestinal lumen the active diffusion of glucose occurs. The protein molecule responsible for the transportation of glucose from the intestine into the blood is GLUT2. This transporter maintains glucose homeostasis and is also stimulated by muscle contraction causing transport of glucose into the muscle to enter the energy production pathways. To maintain the optimum efficiency of this pathway homeostasis of blood glucose concentration must be maintained. This allows for active transport of glucose into the skeletal muscle on a separate transport, GLUT4. This pathway allows for the delivery of around 80 grams of glucose to enter the energy production pathways per hour when the gut is full to maintain gastric emptying constant at the optimum rate (Hawley 2008, Jeukendrup., 2010).
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The protein responsible for the transport of fructose is GLUT5. This molecule works on a separate pathway and allows the synergistic transportation of fructose from the small intestine, another monosaccharide sugar, to be taken up in the skeletal muscle for oxidation. Being able to transport these simple forms of carbohydrate on different pathways increases the amount of energy available through the consumption of exogenous carbohydrates, when the glucose pathway becomes saturated. This is an effective way to increase the rate of carbohydrate oxidation as potentially up to another 30 grams of carbohydrate per hour can be utilised in this way. Providing carbohydrate in a 2:1 ratio of glucose to fructose has been to show to increase carbohydrate oxidation rates to $> 1.4 \text{ grams.min}^{-1}$ (review see Jeukendup 2010).

1.2.4. Current guidelines

There are guidelines and recommendations in place that have been validated to assure the safety and wellbeing of athletes from recreational to elite standard. These guidelines are
Methods to optimise substrate utilisation during endurance performance. published by the American College of Sports Medicine (ACSM) in 2007 and their application is to endurance performance (lasting > 1 hour).

ACSM include in their recommendations for prolonged exercise to supplement with 30-60g.hour$^{-1}$ of exogenous carbohydrate to maintain euglycemia and sustain exercise performance (Coyle et al 1993, Coyle 2004). The range of 30 - 60g.hour$^{-1}$ is relevant in which it can provide exogenous forms of carbohydrate to supplement the bodies own stores of glycogen whilst the amount allows for the concentration within the gut to be rapidly absorbed and transported to the working muscle. This consumption of carbohydrate in turn provides over 1000 kJ towards the deficit incurred by the exercise itself. As previously described this can offset the demand for glycogen stores early in the event to be available for the latter stages for use as an energy source.

The ACSM position stand also advises of a recommended consumption of fluid, either as part of a carbohydrate supplementation strategy or as an addition to one. This recommendation is to consume 0.4-0.8 litres.hour$^{-1}$ of fluid to prevent dehydration (Noakes, 2003). These values were based on the sweat rates representative of the general populations weight (50 kg, 70 kg, and 90 kg) and running speeds (8.5 km.hr$^{-1}$, 10 km.hr$^{-1}$, 12.5 km.hr$^{-1}$, and 15 km.hr$^{-1}$) to prevent dehydration. The amount of fluid optimum level of fluid an athlete should consume will vary on body weight and running speed, and the predicted loss of body water. For the purposes of putting an arbitrary value on dehydration this physiological event is noted as a loss of 2 - 3% of total body weight. Dehydration is deemed detrimental to performance and its onset in certain endurance events lasting longer than 3 hours it is not by any means unachievable if sufficient preventative measures are not in place (Brooks et al., 2000).

If the carbohydrate being consumed is in solution the solution should be of no greater than 8% carbohydrate, possibly slightly less as above this value impairment of gastric emptying has been noticed. If the gastric contents exceed 8% glucose, and in this instance the gut is hyper-concentrated, fluid may diffuse into the gut to aid absorption lowering blood volume and increasing the chances of dehydration. Likewise a hypo-concentration in a cool environment will not saturate the transportation pathways and the muscles will fatigue earlier. (Hawley 2008, Jetjens et al., 2005, Jeukendrup et al., 2006, Jeukendrup, 2010, Prieffer et al., 2010 a, Prieffer et al., 2010 b )
1.2.5. Potential supplements and effects

Caffeine is a commonly consumed product and is currently recognized as having ergogenic properties in relation to endurance exercise (Docherty and Smith, 2005). Docherty and Smith show that on a meta analysis of the effects of caffeine do indeed lead to an improvement in performance however they do relate almost one third of this improvement to decrease in perceived exertion of the task being performed and are still unable to provide a definitive physiological explanation. Initially Ivy (et al., 1979) portrayed that caffeine was responsible for increasing lipolysis and decreasing reliance on glycogen. Subjects performed isokinetic cycling for two hours on three separate occasion consuming carbohydrate and 500mg of caffeine, carbohydrate only or placebo. Work done was 7.4% greater during the carbohydrate and caffeine trial compared to placebo and 5.3% greater than the carbohydrate only trial. Fat oxidation peaked after one hour and was maintained during the final hour of the trial containing caffeine was significantly enhance for the final hour compared to the other trials. This was reiterated by Powers and Dodd (1985) when caffeine was shown to have properties in the mobilisation of and thus potential to up regulate the oxidation of fatty acids and potentially provide sparing the working muscles requirement for glycogen. Kovacs (et al 1998) went on to investigate these properties against endurance performance and found an increase in one hour cycling performance when consuming caffeine and carbohydrate above consuming carbohydrate alone. However this paper does not provide any metabolic measurements and only provides speculation to the properties of caffeine as an ergogenic aid to performance. More recently this explanation has been linked to a mixture of both cognitive and physiological function (Hogervost et al., 2008). When endurance cyclists consumed either 44.9g of carbohydrate and 100mg of caffeine, isocalorific carbohydrate, and flavoured water on three separate occasions. Subjects cycled at 60% VO$_{2\text{max}}$ for 150 minutes then after a five minute rest cycle to exhaustion at 75% VO$_{2\text{max}}$. When consuming the caffeine and carbohydrate subjects cognitive function was improved in the ‘Stroop’ and ‘Rapid visual information processing task’ after constant load exercise and ride to exhaustion. Physiologically subjects increased time to exhaustion compared to carbohydrate alone and placebo. Regardless of the exact mechanism behind caffeine and exercise performance there is extensive support for caffeine as an ergogenic supplement for physical performance (Costill et al., 1978; Graham & Spriet, 1991; Wiles et al., 1992; Lindinger et al., 1993; Graham & Spriet,
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1995; MacIntosh & Wright, 1995; Cole et al., 1996; Jackman et al., 1996; Bell et al., 1998; Doherty, 1998; Graham et al., 1998; Van Soeren & Graham, 1998; Anderson et al., 2000; Bruce et al., 2000; Bell & McLellan, 2002; Bell et al., 2002a, b; Cox et al., 2002; Conway et al., 2003; Doherty et al., 2004).

More recently there have been investigations into the effects of the active catechin in green tea, epiogallate catechin gallate (EGCG) showing increase in weight loss and weight management in humans (Hursel et al., 2009). EGCG began to be investigated in humans as it was shown to increase fat oxidation, improve exercise performance and prevent obesity in mice (Murase et al., 2002, 2005, 2006; Shimotoyodome et al., 2005). This improvement in endurance capacity is of particular note as if transferrable can potentially transform an athlete’s ability to regulate substrate during endurance events were substrate is limited. EGCG acts by directly inhibiting the enzyme catechol 0-methyltransferase (Lu et al., 2003) and allowing consumption to provide a regulatory effect on sympathetic activation and lipolysis (Venables et al., 2008). Venables has shown how consumption of EGCG increases fat metabolism during exercise of which there is little evidence for humans. The little research to date is not definitive on a dose response of consumption of EGCG and its effect during endurance events or if the benefit in performance comes through supplementation during a period of training. Therefore a more controlled setting to analyse its effect in response to exercise in humans would prove beneficial in understanding the role this compound has during exercise.
2.1. INTRODUCTION

Over the last twenty years the marathon has become a relatively common endurance challenge with over 500 organised marathons spanning sixty-five countries and seven continents annually (Marathon-world). A marathon is a 42.2 kilometre footrace in which energy expenditure is around 10500 – 14000 kJ dependant on body mass and running economy but not necessarily speed.

To enter a state were the body allows total depletion of its glycogen stores would itself be rare as before the body enters this state it is hypothesised that a safety mechanism is triggered to preserve the glycogen stores and increase the metabolism of less efficient sources of fuel such as fatty acids (Weltan et al, 1998). This is apparent by the decrease in sustainable work rate achieved by the working muscles as glycogen stores begin to run low which translates to a decrease in overall speed (Costill, 1973). Therefore to negate the possibility of full depletion of glycogen stores it has been shown that supplementing with exogenous carbohydrate can improve endurance performance (Ahlborg et al., 1967, Sherman et al., 1982, Burke, 2007, Coyle, 1983, Coyle et al., 2007, Jeukendrup et al., 2009, Jeukendrup et al 2010).

A marathon is an endurance event which requires a continuous supply of ATP to fuel the work of the muscles. The production of ATP is dependant on factors such as substrate availability, and oxygen demand as previously described. This continuous energy requirement of the working muscles places great demand on the energy production pathways but poses the threat of hyperthermia and or hypothermia (Coyle, 2004). To attenuate a rise in core temperature there must be a balance between the requirement for vasodilation as a cooling mechanism and the requirements for oxygenated blood of the working muscles. The initial response to exercise is to maintain blood flow to the working muscles therefore to cool the body’s temperature an increased sweat rate is noticed. Increasing fluid leaving the body then requires osmotic balance to be subsequently maintained and as such to maintain fluid balance the American College of Sports Medicine, 2007 guidelines for fluid replacement during prolonged exercise (duration > 1 hour), is 0.4 – 0.8 litres.hour\(^{-1}\) in a cool environment. This allows for
Methods to optimise substrate utilisation during endurance performance. Maintenance of body water and aids gastric emptying. This is where sports drinks manufacturers have for years marketed products to simultaneously overcome the energy deficit and replace fluid athletes consume solutions containing carbohydrate (and salts) of 6-8% to keep in line with the research by Coyle (1992, 2004).

This is a valid and effective way to consume exogenous carbohydrate however carbohydrate supplementation is available in varied forms and consuming surplus fluid in a cool environment primarily to offset the energy deficit during a marathon can incur gastrointestinal (GI) distress and potentially negatively affect performance. To overcome this potential GI distress during a foot race such as a marathon there are two subsequently common methods for supplementing with carbohydrate through either supplementing with a solid or a gel form of carbohydrate which can prove just as effective when consumed with sufficient fluid to offset glycogen depletion (Peiffer et al., 2010).

The benefits of consuming exogenous carbohydrate to improve endurance performance are well recognised, however what is not as conclusive is the benefit of supplements that can enhance endurance performance further. Of the nutritional interventions investigated thought to have an ergogenic effect on endurance performance caffeine provided most positive results in improving endurance performance (Burke, 2007). Its effect has been linked to the increase in adrenaline release, fatty acid metabolism from adipose tissue (Powers and Dodd, 1985) and thus utilising other fuel sources other than glycogen earlier in the race and providing a ‘glycogen sparing effect’. Graham and Spriet (1995) were able to show improvements in performance but these did not coincide with increases in lypolysis. However more recently after extensive reviews more recently it thought that the most potent effect of caffeine is to compete with the adenosine directly at the receptor sites (Sokmen et al., 2008, Spriet and Gibala., 2004) or as a mixture of cognitive and physiological properties (Hogervost et al., 2008). The amount of caffeine required to show improvements in performance is between 3 - 6mg.kg\(^{-1}\) of body mass (Graham et al., 1991). Subsequent studies have found improvements in performance with values towards the higher end of this recommendation, 5mg.kg.hr\(^{-1}\) (Yeo et al., 2005), however there are studies showing that there is no added benefit of consuming 4.5mg.kg\(^{-1}\) over and above 3.2mg.kg\(^{-1}\) (Kovacs et al., 1998).
Methods to optimise substrate utilisation during endurance performance. For this study, there were two main research aims. It is hypothesised that more carbohydrate could be consumed in the gel form, and that improving carbohydrate availability will improve time for completion of the marathon. The first aim of this study was to compare different modes of carbohydrate delivery and its effect on marathon performance.

It is also hypothesised that the addition of sufficient amounts of caffeine to a gel form of carbohydrate will provide an ergogenic effect and a subsequent improvement in performance above gel carbohydrate consumption alone. The second aim was to investigate if the addition of caffeine to a carbohydrate gel had a performance benefit over and above the delivery of a carbohydrate only gel.

The final aim of this study was to compare the performance of athletes who are able to consume (a) carbohydrate and (b) fluid commensurate with the current recommendations for a marathon.
2.2. METHODS

2.2.1. Does mode of exogenous carbohydrate consumption affect marathon performance?

Two hundred and sixteen subjects competing in the Dublin Marathon in 2008 volunteered to take part in the study and each subject was randomly separated into one of four groups, control (CON, n=50) solid carbohydrate supplementation (SOLID, n=53) gel carbohydrate supplementation (GEL, n=56) and gel carbohydrate containing caffeine (CAFF, n=57). Once informed consent (Appendix I) was provided by each subject the relevant form of supplements were distributed to the subject. The supplementation provided was in 20g sachets (either gel or solid) and based on meeting the upper end of the recommendations, 60g CHO.hour$^{-1}$, set out for carbohydrate supplementation during prolonged exercise by ACSM (2007). Each subject was provided with a supplement belt (HIGH 5) to carry the supplements around the course. The CON group were asked to complete the marathon using the nutritional strategy as they had planned. Subjects provided a urine sample which was measured for osmolality (Micro osmometer 3300, USA) and were weighed (Secca 770, Germany) in race kit immediately before and on completion of the marathon. On completion of the marathon blood glucose was measured (Accu check aviva), from a fingertip blood sample, and subjects’ completed a recall food and fluid intake diary (Appendix II) for the marathon race.

2.2.2. Carbohydrate Supplements

Carbohydrate supplements were in the form of either 20 gram gel or solid packets. These gels are currently marketed and available for purchase. The solid bars composition are currently marketed however in a larger size (55g) but were specifically constructed in 20 gram packages for the purposes of this research study. The contents of the supplements are detailed in appendix III The gels containing caffeine were not labelled to have such contents to keep the subjects blinded from what group they were in. However it was impossible to blind subjects to which mode of carbohydrate they were supplementing with however the packaging was labelled blank so as to hide the composition of the supplements from the subjects.
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Event

This marathon was run in October 2008 in Dublin, Ireland. The temperature for racing was ~10 degrees Celsius.

2.3 Statistics
A chi squared test was used to check the data for normality and then a one way analysis of variance was carried out to quantify weight loss, blood glucose, actual performance, and relative performance (based on a percentage of the subject’s predicted time), carbohydrate oxidation rate and overall fluid consumption while a two-way analysis of variance used to analyze the urine osmolality to compare groups pre and post race. A Tukey post-hoc test was used to analyse any difference found between the groups.
2.4 RESULTS

All groups were able to meet the recommendation for exogenous carbohydrate and fluid consumption during prolonged exercise. There was significantly more carbohydrate consumed by the groups principally consuming a gel form of carbohydrate (GEL 54 ± 21g.hour\(^{-1}\), CAFF 56 ± 24g.hour\(^{-1}\)) than solid forms of carbohydrate (CON 34 ± 20 g.hour\(^{-1}\) SOLID 36 ± 17 g.hour\(^{-1}\)) (Figure 2.4.1). Fluid intake was commensurate with the lower end of the recommendation for endurance performance 0.4 – 0.8 litres.hour\(^{-1}\) (Figure 4.2.2. CON 0.4 ± 0.2 litres.hour\(^{-1}\) SOLID 0.4 ± 0.3 litres.hour\(^{-1}\) GEL 0.5 ± 0.4 litres.hour\(^{-1}\), CAFF 0.6 ± 0.3 litres.hour\(^{-1}\)). There was a weight loss in all groups that would signify dehydration (> 2%) however this could not be deemed to be detrimental to performance and was not different between the groups (Figure 2.4.4.). Although there was a significant increase in carbohydrate consumption during the marathon in the groups consuming carbohydrate gels there was no difference in post marathon blood glucose (Figure 2.4.3.) With no difference in fluid consumption, weight loss, or post marathon blood glucose and a significant increase in exogenous carbohydrate consumption it was surprising to find that increase in exogenous carbohydrate could not improve time for completion of the marathon or marathon time against predicted performance. It was also interesting to notice that euglycemia was maintained in all groups regardless of the rate of carbohydrate consumption suggesting that blood glucose is reliant on an internal mechanism protecting a critical reduction in blood glucose.

All values reported are mean ± sem.
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Figure 2.4.1. Rate of consumption of different forms of exogenous carbohydrate during a marathon race. The rate of carbohydrate consumed is detailed for each subject and the guidelines for carbohydrate consumption (ACSM, 2007) are represented by broken red lines.

On average the groups met the current recommendations for consumption of carbohydrate during prolonged exercise, CON 34 ± 20 g.hour\(^{-1}\) SOLID 36 ± 17 g.hour\(^{-1}\) GEL 54 ± 21 g.hour\(^{-1}\), CAFF 56 ± 24 g.hour\(^{-1}\) (ACSM, 2007) however significantly more carbohydrate was able to be consumed in the carbohydrate only gel, and caffeinated carbohydrate gel forms compared to solid carbohydrate and a control group (p<0.05).
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Figure 2.4.2 Rate of fluid consumption during a marathon race. On average the groups met the current recommendations for fluid consumption during prolonged exercise in a cool environment (ACSM, 2007), CON 0.4 ± 0.2 litres.hour⁻¹ SOLID 0.4 ± 0.3 litres.hour⁻¹ GEL 0.5 ± 0.4 litres.hour⁻¹, CAFF 0.6 ± 0.3 litres.hour⁻¹ (p>0.05).

Figure 2.4.3. Post marathon blood glucose concentration on completion of the marathon

Euglycemia was maintained on average in all groups and there was no difference between post-marathon blood glucose and neither group could be determined as being hypoglycaemic.
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(2.5 mmol.litre\(^{-1}\)) on completion of the marathon (CON – 5.4 ± 1.4 mmol.litre\(^{-1}\), SOLID – 5 ± 0.9 mmol.litre\(^{-1}\), GEL – 5.7 ± 1.4 mmol.litre\(^{-1}\) CAFF – 5.6 ± 1.6mmol.litre\(^{-1}\)). There was no significant difference between the groups (p>0.05).

Figure 2.4.4. Weight loss after participation in a cool marathon.

Regardless of meeting with the fluid intake guidelines for consumption during prolonged exercise in a cool environment, the mean response from each groups showed loss of body weight greater than 2% (CON 2.6 ± 2.2% SOLID 2.2 ± 3.7% GEL 2.6 ± 3.8% CAFF 2.1 ± 3.5%) which signifies dehydration (ACSM, 2007). There was no significant difference in the amount of weight lost between the groups (p>0.05).
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(a)

(b)

Figure 2.4.5.(a),(b). Marathon performance.

There was no significant difference between any of the groups’ time for completion of the marathon CON: $227.3 \pm 35.8$ mins, SOLID: $238.6 \pm 46.1$ mins, GEL: $227.43 \pm 45.5$ mins CAFF $237.76 \pm 6.17$ mins (p>0.05) (a) or when ultimate performance was compared to finish time predicted prior to the marathon (b) $102 \pm 7\%$ CON, $103 \pm 11\%$ SOLID, $103 \pm 11\%$ GEL and CAFF $101 \pm 9\%$. 
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(a)

(b)

(c)
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Figure 2.4.5.1. (a), (b), (c), (d). Marathon performance vs meeting current guidelines for carbohydrate and fluid intake.

There was no significant difference to (a) actual marathon finishing times (LOW 220 ± 9 mins, MET 230 ± 5 mins, HIGH 223 +/- 6 mins (p>0.05)) or (b) marathon performance relative to prediction (LOW 96 ± 4%, MET 101 ± 1%, HIGH 97 ± 2%, (p>0.05)) if athlete fell short of (LOW), met (MET), or exceeded (HIGH) the current recommendations for carbohydrate consumption (30 – 60g.hour⁻¹) during endurance performance.
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Exceeding the current recommendations (0.4 – 0.8L.hour\(^{-1}\)) for fluid consumption was seen to significantly improve the ultimate marathon performance above the group who consumed fluid commensurate with the current recommendations (\(a\)) (LOW 231 ± 5mins, MET 236 ± 5mins, HIGH 212 ± 9mins, \(p=0.047\)). There was however no difference between LOW or MET. Exceeding the fluid intake recommendations was did not significantly improve performance relative to predicted performance (\(p=0.059\)), however this group had the shortest mean time relative to predicted performance (LOW 100 ± 1%, MET 102 ± 1%, HIGH 96 ± 3%) and the results show a tendency to reducing the time the athlete had perceived prior to the race.

Does the addition of caffeine to carbohydrate improve marathon performance?

The CAFF group on average consumed 3.1g.kg\(^{-1}\) which is commensurate with the lower end of the recommendations for caffeine consumption with a view to improve performance (Graham et al., 1991). Regardless of the addition of carbohydrate commensurate with rates known to improve performance (Graham and Spriet., 1995) there was no improvement when adding caffeine to a gel based carbohydrate product (Figure 2.4.7.). There was also no diuretic effect of caffeine seen as weight loss (Figure 2.4.4.) and urine osmolality (Figure 2.4.6.) was similar between the groups.

![Figure 2.4.6. Comparison of hydration status before and after the marathon.](image-url)
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There was no significant difference in hydration status for either group before or after the marathon (pre - CHO 519 ± 42 CAFF 583 ± 38 p=0.51, post - CHO 491 ± 30 CAFF 499 ± 35 p>0.05). There was also no significant difference in the change in urine concentration between the groups.
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2.5. DISCUSSION

2.5.1. Method of Delivery

When athletes were provided with sufficient carbohydrate to meet with the highest end of the ACSM (2007) recommendations for carbohydrate supplementation during endurance performance, 60g.hr⁻¹, significantly more carbohydrate in gel form (p<0.0001) was consumed compared to solid carbohydrate and a ‘placebo’ group (Figure 4.2.1.). This finding is not in keeping with the work of Pfieffer (2010) however all groups consumed carbohydrate at a rate within the current recommendations, 30 – 60g.hour⁻¹, however this did not translate into a performance improvement in a real world environment. It was also noticed that regardless of the mode of carbohydrate consumption that consuming carbohydrate commensurate with the reconsumption did not improve performance over and above groups failing to meet or exceeding these guidelines (Figure 2.4.5.1). Each group completed the course within one per cent of their estimated time for completion and no there was no difference between any of the groups (Figure 2.4.5.(b). It was hypothesised that increasing exogenous carbohydrate consuming during the course would assist in offsetting the rate of glycogen depletion such a challenge poses allowing the athletes to produce an optimum performance. Therefore the large difference between the upper and lower limits of the recommendations for carbohydrate intake over a prolonged test such as the Dublin Marathon are justified in this case as even with a significantly increased ingestion of gel forms of carbohydrate there is no translation into a benefit in ‘real world’ performance. Average completion time for the three groups for is 231 minutes (3hours 51minutes) (CON: 227.3 ± 35.8 mins, SOLID: 238.6 ± 46.1 mins, GEL: 227.43 ± 45.5 mins CAFF 237.76 ± 6.17mins) and based on the current recommendation there potentially could be a difference in carbohydrate consumption of around 116grams (or ~460kcal). Simply adhering to the lower end of the recommendations may be enough, accompanied with the slower pace (thus utilising a certain amount of fatty acids and protein) to offset the effects of early glycogen depletion. It is therefore acceptable for the ‘average athlete’, tested in this study, to simply plan to meet the lower end of the current recommendations for carbohydrate intake.

Subsequently there were no difference in any of the performance variables measured, which was as expected from the marathon performance. All groups had similar blood glucose levels.
on completion of the course, showing no signs of hypoglycemia, and hydration status was similar both before and on completion of the marathon accompanied with no significant difference in the delta osmolality between the groups. This necessarily does not prove that the groups were in their own physiological optimum conditions for completing the marathon it does however provide data to show that no one group was at any advantage or disadvantage when we draw conclusions on the significance, or lack of, through investigating varied methods of carbohydrate delivery to marathon performance. With the limitations of a field study of this magnitude it has to be accepted that with the selection criteria set out for inclusion in the study that all subjects would have prepared adequately for this race, as all subjects were required to have completed at least two previous marathons with a view to giving an accurate estimation of marathon time prior to competing in the race.

There was an elevated increase in the amount of weight lost during the marathon by all groups. The hypohydration faced is to an extent that deems all groups as dehydrated (>2% reduction in total body weight) on completion of the marathon which certain groups would provide as a limitation or negative impact to performance (Coyle, 2004). All groups were able to consume fluid at a rate commensurate with the lower end of the recommendations for fluid intake of 0.4litres.hour^-1 (ACSM, 2007) to offset this potential dehydration, and thus negatively affect performance. However simply meeting with this recommendation was not sufficient to negate eventual ‘dehydration’ and all groups lost a larger than desirable amount of weight and this cannot be ruled out to have had an affect, potentially negative, on performance. Dehydration as defined by the fluid intake review guidelines as a loss of body water greater than two per cent is surpassed by all groups participating in the study however only minimally. This leaves the author sceptical to draw any definite conclusions on the effect that surpassing this ‘marker’ of performance is having on the results. The first point of note is that regardless of all groups surpassing this marker, no matter how minimally, there is no difference between the groups. Therefore regardless of maintaining all other physiological parameters associated with endurance performance if dehydration onsets this alone is sufficient to negate performance over and above all other physiological parameters. Secondly it is noted that the two per cent loss in body water is a critical value with research conducted upon this ‘threshold’(Coyle, 2004), however Cheuvront et al (2005) cite a three per cent loss in body water as the marker for dehydration and none of the groups reach values close to this ‘threshold’. With any ‘threshold’ there must be a clear indication of response either side of
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this marker, if it exists in such a clear manner. However with such a varied response in the literature to any clear definition of the point of onset of dehydration it cannot be concluded that this marker is definitively passed by the athletes taking part in this study.

What was however noted was that when the mode of delivery was disregarded exceeding the current recommendations for fluid consumption (0.4 – 0.8L.hour\(^{-1}\)) improved performance significantly above athletes who met with the current recommendations (Figure 2.4.5.1.(c)). Exceeding the recommendations did not prove statistically significant for improving relative performance however there was a tendency towards this also (p=0.059). This was surprising to note that even in cooler conditions (~10 degrees celsius) that fluid consumption can be so important and that above carbohydrate consumption. This data may reflect athletes who consumed high rates of fluid consumption had prepared to be working at a greater intensity thus running a quick marathon time but not a time far enough from what they had previously predicted before the race. This data does though provide some important information for real life variables measured during a marathon performance of a large group of athletes.

2.5.2. Does the addition of caffeine improve marathon performance?

The same physiological measurements as previous were compared in the CAFF and GEL groups and again there was no significant difference between the groups in any area. Both groups meet the higher end of the recommendations for carbohydrate intake however there was no significant difference in performance when caffeine was added to the carbohydrate gel. The CAFF group also completed the course within one per cent of their predicted time for completion of the marathon. In this case caffeine did not produce an ergogenic effect as there was no added benefit to ‘real world’ performance. The caffeine consumed was over the duration of the marathon however and the actual caffeine levels of the groups cannot be guaranteed to be maintained at the recommended levels throughout the course but are representative of consumption during the course as a whole. Although over the course of the race caffeine consumption matched the levels consumed to show benefits to performance as noted by Kovacs, et al, (1998) of 3.2mg.kg\(^{-1}\) and there was no benefit to performance. Caffeine as a supplement has diuretic properties which have been shown to be negated during exercise (ref). There is no difference again however in hydration state or delta urine osmolality.
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in the CAFF group showing no signs of a diuretic effect that caffeine is known to have without exercise. Therefore it is not surprising that there was no difference maintenance of body weight between the GEL and CAFF group (CAFF 2.1 ± 3.5%, CHO 2.6 ± 3.8% loss in body weight).

2.5.3. Conclusion

Current recommendations for carbohydrate consumption are achievable in a ‘real-world’ environment. Consuming carbohydrate in the gel form was the most effective method for delivering carbohydrate as high rates (p<0.001). Delivering fluid at a rate commensurate with the current recommendations for prolonged exercise in a cool environment (ACSM, 2007) was not sufficient to prevent dehydration. The addition of caffeine to a carbohydrate gel had no additive effect on marathon performance.
3. Does chronic green tea extract supplementation improve endurance performance?

3.1 INTRODUCTION

Prolonged endurance exercise (> 1 hour) will result in substantial depletion of endogenous carbohydrate stores (contained within the liver and muscle) thus limiting performance in the current bout of exercise and also challenging performance in the next exercise bout (whether that is a second training session on the same day or another race the following day, e.g. a stage race such as the Tour de France) (Wagenmakers, 1991). Thus the ability to switch to oxidation of fats at an early stage and spare glycogen is of great interest to the endurance athlete.

As previously described, the principal fuel utilised during high intensity performance is carbohydrate, and the availability of such fuel is of huge importance to the endurance athlete. However carbohydrate is not the only fuel which the body will use during performance, with fat being stored in greater amounts and providing a greater yield of energy per gram than carbohydrate. If athletes can spare carbohydrate stores, namely glycogen, by switching to a greater rate of fat oxidation earlier then the sparing of this high intensity fuel could be of great benefit for the later stages of a race. One potential method of promoting fat oxidation and thus sparing of glycogen stores is through certain dietary supplements. It has been shown that the active ingredients in green tea extract, the polyphenols (mainly the catechins), increase the rate of fat oxidation by 17% in humans working for only 30 minutes at 60% VO$_{2\text{max}}$ (Venables et al 2008). It is not yet known what effect these polyphenols will have on performance of longer durations and higher intensities or on the adaptation to endurance training. Preloading on a high fat diet during training to subsequently increase fat oxidation has been shown not to improve performance (Yeo et al., 2008). However, no study to date has considered the effect of these polyphenols on fat oxidation during performance or has investigated what effect supplementing with this known promoter of fat oxidation during training will have. It is also not clear whether athletes already predisposed to oxidising fats during training will have a greater or lesser effect from glycogen sparing supplements (Goedecke, unpublished observation).
Methods to optimise substrate utilisation during endurance performance. Therefore this study aims to investigate if supplementing with green tea extract during training will enable subjects to switch on fat oxidation earlier and at a greater rate and ultimately increase exercise performance more than the same training alone.
3.2 METHODS

3.2.1 Pilot Study

3.2.1.1 Introduction
There is a lack of research of the effects of Green Tea Extract (GTE) or more specifically its active ingredient epigallocatechin gallate (EGCG) on human subjects during exercise. Therefore to determine the concentrations and quantities of EGCG that produced the optimum increase of fat oxidation and therefore would be provided for a chronic period of supplementation during a period of training a short pilot study (n=5) was conducted.

Various combinations of quantities and concentrations of EGCG were studied to provide the most effective potency for chronic supplementation. One of the few studies investigating the effects of EGCG during exercise is Hargreaves et al., (2008) were subjects consumed 300 mg of GTE of which 60% active catechins EGCG were present. This study provided a starting base for finding a suitable concentration for use during a training protocol. The following day sub-maximal exercise was performed at 50% VO₂max and energy expenditure was measure in the following 24 hours. This study investigated the effects of short term (24 hours) supplementation of EGCG and the effects of energy expenditure during the course of a 24 hour period post consumption of EGCG and compared to a post placebo. Hargreaves et al. (2008) used a 300 mg supplement containing 60% EGCG. This supplement found an increase in EE of 7% over the course of 24 hours and its effects were directly linked to the supplementation of EGCG. This provided foundation for our pilot investigation into the effects of varied quantities and concentrations of EGCG ability to produce elevated rates of fat oxidation in humans.

3.2.1.2 Methods
Five subjects participated in this pilot investigation and was designed as a random order single blind placebo controlled study in which the supplement was consumed in a degradable capsule to mask the contents. Subjects consumed three capsules within the 24 hours prior to arriving in the lab on each occasion at regular intervals (~7 hours apart). The last capsule containing supplement or placebo was taken a minimum of three hours prior to the test commencing to allow sufficient time for the contents of the capsule to reach the blood stream. In the case were only one capsule was consumed this was taken 3 – 4 hours prior to commencing the test.
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Subjects entered the lab after consuming a capsule containing catechins or placebo as previously described. Each subject changed into exercising kit and had a five minute resting expired air sample collected in a Douglas bag breathing through a two way non-re-breathing valve. Subjects then commenced exercise on a cycle ergometer working at 50% $WR_{\text{max}}$ determined from a ramp protocol performed in the week before testing commenced. The $WR_{\text{max}}$ test was a ramp protocol on a lode ergometer using a 25W.minute$^{-1}$ increment until volitional exhaustion. The last work rate sustained during this test was used as $WR_{\text{max}}$.

Subjects cycled for 30 minutes at 50% $WR_{\text{max}}$ on a lode cycle ergometer continually breathing through a two-way non-rebreathing valve supported by a ‘head mask’. During exercise six, two-minute expired air samples were collected at five minute intervals, during minutes 3-5, 8-10, 13-15, 18-20, 23-25, 28-30. The collected air samples were analysed using a servomex gas analyser for $O_2$, $CO_2$, Temperature and Volume. These values were computed into an excel spreadsheet (example in appendix IV) to calculate $VO_2$, $VCO_2$, fat oxidation, carbohydrate oxidation and RER.

Conditions compared were;

- 3 x 300mg of Sucrose (placebo)
- 3 x 300mg of 60% GTE
- 3 x 300mg of 70% GTE
- 3 x 300mg of 80% GTE
- 3 x 300mg of 90% GTE
- 1 x 300mg of 70% GTE
- 3 x 300mg of 80% GTE + 6mg.kg$^{-1}$ caffeine
- 3 x 300mg of 80% GTE + 50% gurana
- 3 x 400mg of 60% GTE

No more than two experiments were carried out in any one week with a minimum of two clear days between tests to allow washout of any acute dose of active catechin. The dose was taken within 24 hours of the exercise with the last capsule being consumed three hours before commencing exercise.
3.2.1.3 RESULTS

There was not a linear response to consumption of GTE, containing increased EGCG concentration, on the rate of fat oxidation during sub-maximal exercise. As the concentration of EGCG in GTE increased there was an increase in fat oxidation however this was not the case when the highest concentration of EGCG (90%) was consumed. At 90% EGCG (0.233 ± 0.019 g.min⁻¹) there was no increase in fat oxidation above placebo (0.165 ± 0.041 g.min⁻¹). The highest rate of fat oxidation was noticed when consuming 80% EGCG (0.329 ± 0.051 g.min⁻¹), the only condition studies in which there was a significant increase in rate of fat oxidation above placebo (p < 0.05). The response to consumption of GTE with varied concentrations of EGCG is detailed below in figure 3.2.1.3.1. Introducing caffeine and guarana to the most potent concentration (80% EGCG) of GTE was shown to negatively affect EGCG increasing the rate of fat oxidation during exercise. Increasing the amount of one of the lower concentrations 60% EGCG to 400mg in line with the amount of active ingredient consumed in the higher concentrations 80% EGCG more than doubled the rate of fat oxidation for the same concentration of active ingredient (0.293 ± 0.199g.min⁻¹). However this was not significant above placebo (p = 0.08), and this was also the case for 70% EGCG (0.300 ± 0.050 g.min⁻¹) which although raised was not significant above placebo (p = 0.11).
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Figure 3.2.1.3.1 Effect of consumption of varied concentration of GTE over a 24-hour period on fat oxidation (g.min\(^{-1}\)) during submaximal exercise.

3.2.1.4 DISCUSSION

The optimum catechin enriched supplement for increasing fat oxidation during sub-maximal exercise was 3 x 300 mg of 80% GTE. There was a significant increase in the mean fat oxidation for a 30 minute sub-maximal cycle exercise compared to placebo. This product was however less cost effective to produce (at ~£100/kg) than using 70% GTE (at ~ £40/kg) and not significantly different. Even accounting for reducing the capsules to only one per day and increasing the dose this study was with a view to production of a marketable product and had to be as cost effective as possible. With such a small subject population (n = 5) it was important to not negate an increase in fat oxidation if it did not prove statistically significant. In almost all conditions supplementing with only GTE there was an increase in mean fat oxidation, except in trials supplementing with 3 x 300 mg of 60% GTE. This condition actually resulted in three out of the five subjects oxidising less fat than in the placebo trial and provided a decrease in fat oxidation for the trial. However when 3 x 400 mg of 60% GTE was provided the increase in fat oxidation from placebo almost reached significance (p=0.082) against placebo. Therefore we believed that increasing the active ingredient towards the
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amount contained in the 80% GTE would be adequate to induce an increased rate of fat oxidation.

Substances in the current literature thought to produce an increase in fat oxidation was tested. From the preliminary conditions tested (varied concentrations of basic EGCG in GTE) the most significant increase in fat oxidation was 80% GTE to which the maximum recommended amount of caffeine (6mg.kg\(^{-1}\)) was added to potentially induce a synergistic effect with GTE. However this did not provide any synergistic effect and subsequently negatively affected the response previously noted from supplementing with 80% GTE. The rate of fat oxidation was very similar to that of placebo and therefore the affect that 80% EGCG had on fat oxidation had been lost with caffeine either negating or reducing the effects of EGCG on submaximal exercise.

With guarana also been documented as a catalyst for up regulating fat oxidation this was tested to the same concentrations as used in caffeine, with its active properties closely linked (Berube-Parent et al., 2005). This supplementation however did not provide any evidence for potential additional benefits in up-regulating fat oxidation during sub maximal exercise and provided the largest decrease from placebo and all but one of the subjects decreased there mean fat oxidation for the sub maximal exercise.

These conditions provided 300 mg of GTE supplement 3 x 24 hours prior to exercise with the last capsules being consumed 4 hours before exercise. It was then hypothesised that if only one capsule of GTE was provided with the aim to deliver 300 mg of the active EGCG i.e. 430 mg at 70% GTE then we could produce the same benefit during exercise and through a chronic supplementation, 4-week training, the same long term benefits would be noticed. Also this would simplify subject adherence to the study reducing the amount of capsules to be consumed to consume only one capsule per day opposed to three.

3.2.1.5 CONCLUSION

It was proposed that with small subject numbers in our pilot study that improvements noticed from increasing the dose of GTE and that there was little drop when the supplemnet was consumed on an acute basis that a compromise from the data in the pilot study could be made.
With an aim to make a marketable product from the findings of the subsequent research this pilot study precedes, a 430mg GTE supplement containing 70% active catechin was chosen due to financial reasons. Although 80% EGCG produced optimum conditions for increasing fat oxidation the difference between the mean values achieved for fat oxidation and the cost involved in supplementing with this product compared to the 70% EGCG product we chose was deemed sufficient. It was believed that by increasing the dose to 430 mg that the same benefit noticed in increasing the 60% supplement in the pilot study would be achieved.

3.2.2 Supplemented training protocol

There was a 15-week (minimum, dependant on wash-out period) protocol (displayed in figure 3.1.2.1 below), consisting of three ‘types’ of visit to the lab on nine different occasions. In week one subjects’ had familiarisation visit in which the subjects became familiar with the lab setting, ergometer (Computrainer(R) turbo trainer), and equipment that would be used on each visit to the laboratory. For this subjects completed a 100 km time trial using the same equipment they would use in subsequent trials. In week two subjects returned to the lab on two separate days. The first visit comprised of submaximal measurements of gas analysis, body fat, and resting insulin levels. The second visit in the same week comprised of a performance measurement, in the form of a 100km time trial on the same course as in week one. Week two was known as ‘pre-testing’. This was followed by a supplemented 4 week period of training, on either green tea extract (GTE) or placebo (PLA), in weeks three to six. In week seven following the supplemented period of training subjects returned to the lab to repeat, in the same order, the two visits in week two of submaxical measurements and a measurement of performance. There was then a minimum of two weeks of ‘washout’ were the subjects were free to train as much or as little as they wished with no supplementation. On week ten the protocol from weeks two through to seven were then repeated in the same order this time training would be supplemented on the different supplement.

Before the cycling work took place during each visit it was preceded by a 5minute warm-up at 100W and the ergometer was calibrated.
Methods to optimise substrate utilisation during endurance performance.

Figure 3.2.2.1. Schematic of chronic EGCG supplementation protocol.

3.2.3 Time trail
The familiarisation and performance measures consisted of a 100km time trial using two laps of the pre loaded course ‘Loop 50km’ in the computrainer 3D software. On each visit subjects’ were provided with a 500ml of carbohydrate solution (9% carbohydrate - 6% glucose and 3%fructose) ten minutes before they began the warm up to allow sufficient time to allow for gastric emptying until the next fluid provision. Subjects’ were subsequently provided with sufficient carbohydrate solution (250 ml of 9%carbohydrate solution) every fifteen minutes from starting the time trail throughout the test until completion of the 100km course.

3.2.4 Participants
Seventeen endurance trained nationally competing cyclists were recruited through advertisements and recruitment evenings at local cycling clubs. Of that twelve subjects were eligible and completed a familiarisation for this study. From this only four subjects completed both training periods and associated pre and post testing, with a fifth subject dropping out on the last lap of the post testing time trial. The significant drop out is detailed in figure 3.4.1.1. below.

For the purposes of the all measurements apart from the 100 km TT subject characteristis is based on n = 5 (age = 26.2 ± 6.6 years, weight = 70.2 ± 7.2 kg, height = 1.79 ± 0.04 cm)
Methods to optimise substrate utilisation during endurance performance.

3.2.5 Familiarisation

Subjects came in to the lab on week one and allowed to ask any questions before completing a consent form (appendix V) and a medical history questionnaire (appendix VI). Subjects’ were then familiarised with the lab and the workings of the ergometer which they use on every visit to the laboratory. A standard laboratory tyre was fitted to the rear wheel of the subjects bike and the bike was clamped into place. The subject was given a 5 minute warm up and the ergometer was calibrated. The average power for this time trial was calculated by the ergometer software and 90% of this power was used for the subsequent submaximal measurements as a steady state environment.

3.2.6 Submaximal measurements

Submaximal measurements took place on weeks two, seven, ten and fifteen. Subjects were fasted for at least four hours prior to visiting the lab. When subjects arrived at the lab they were asked to void their bladder and get changed into exercising kit before being weighed. Subjects sat rested in the semi supine position for 5 minutes. Ten minutes of expired air was collected in two five minute periods into two Douglas bags, minutes 0-5 and 5-10, breathing through a non-rebreathing valve (Hans Rudolf) and nasal airflow restricted using a nose clip.

The subject then had a measurement of percentage body fat using a BodPod\textsuperscript{(R)}. For this measurement the subject had height measured on a laboratory stadiometer. Subjects’ height was input in to the BodPod computer system software prior to entering the BodPod. The BodPod was calibrated for displacement of air and weight before every use. A minimum of two measurements were taken for each visit to ensure reliability.

The subject was then given a five minute warm at 100 W and the ergometer was calibrated. Subjects arrived to the lab having fasted in the four hours prior to exercising this allowed measurements in the exercising ‘unfed’ state to be made. Subjects then began to exercise at 90% of the average power sustained during the 100 km familiarisation from the previous week for thirty minutes. Three two minute expired air samples were collected at minutes 8-10, 18-20 and 28-30. Subjects preceded these periods of gas collection by breathing through the two way non-rebreathing valve for an additional two minutes prior to the sample being take i.e. minutes
Methods to optimise substrate utilisation during endurance performance.

6-8, 16-18, and 26-28, and nasal airflow was stopped restricted a nose clip for the four minute period of each measurement. This additional two minutes prior to the sample being taken allowed the subject to return to a normal breathing pattern after putting in the mouthpiece and nose clip.

After thirty minutes of exercising subjects came of the ergometer and were given 500ml of a carbohydrate solution (9% carbohydrate - 6% glucose, 3% fructose) and asked to consume the contents. Each subject was given ten minutes to do this within and at minute forty began exercising at the same work rate again. The next set of measurements would now be in fed state. Subjects again followed the same procedure as in the unfed state and cycled for thirty minutes with three expired air samples collected at the similar time points, 48-50, 58-60 and 68-70 minutes, using the same procedure for collecting the samples.

3.2.7 Performance measurements

Performance measurements were taken on weeks two, seven, ten and fifteen. Subjects arrived to the lab and there bikes were fitted with a Computrainer lab tyre and clamped in place to the Computrainer turbo trainer ergometer. The subject changed into race kit and was weighed. The subject was asked to consume 500ml of a carbohydrate solution (9% carbohydrate) 10 minutes prior to beginning exercise as in the familiarisation. Subjects' weight was input into the Computrainer software and two laps of the ‘loop 50km’ course was loaded. The subject had a five minute warm-up period followed by a calibration of the ergometer against the rear wheel of the subjects’ bike fitted with a standard laboratory tyre. The test commenced and the time trial (TT) begun. Every fifteen minutes the subject was provided with the same amount of carbohydrate solution (9% carbohydrate) as they consumed in the familiarisation to consume before the next provision of carbohydrate solution, i.e. fifteen minutes to consume the contents of each bottle provided. The time for completion of the 100km course was noted.

3.2.8 Statistics

A repeated measures ANOVA was used to compare the responses to both conditions during submaximal exercise. A paired t-test was chosen to compare the change in body fat and TT performance. To find a significant difference similar to that in the pilot study in the conditions measured we would have required a minimum of twelve subjects which as described we were
Methods to optimise substrate utilisation during endurance performance. Unable to achieve. Although there was small subjects numbers to complete the protocol statistics were carried out for the purpose of evaluation of any possible trends.
3.3 RESULTS

3.3.1 Metabolic parameters

The parameters measured unsurprisingly showed no statistical significance on such low subjects’ numbers. The values for VO$_2$ (figure 3.3.1.1.), VCO$_2$ (figure 3.3.1.2.), RER (figure 3.3.1.3.), fat oxidation (figure 3.3.1.4.), and carbohydrate oxidation (figure 3.3.1.5.), were similar at each time point pre- and post both periods off supplementation. The significant increase in fat oxidation after supplementation with GTE in the pilot study could not be replicated after chronic supplementation (4 – weeks). There was also no noticeable rise in carbohydrate oxidation or fat oxidation after consumption of the carbohydrate drink in any of the conditions, possibly suggesting that two separate visits may have been beneficial to analyse both the fed and fasted states for a full hour to further test the affect of the supplement. However with a lengthy protocol already in place this was not a viable option.

Although no differences were seen within the subjects’ fat oxidation for two subjects are displayed below (Figure 3.1.1.6) to provide an idea of the response within each subject.

![Figure 3.3.1.1 O$_2$ uptake during submaximal constant load exercise](image-url)
Methods to optimise substrate utilisation during endurance performance.

Figure 3.3.1.2 CO₂ production during constant load submaximal exercise

Figure 3.3.1.3 RER during constant load submaximal exercise
Methods to optimise substrate utilisation during endurance performance.

Figure 3.3.1.4 Fat oxidation during constant load submaximal exercise
Methods to optimise substrate utilisation during endurance performance.

![Carbohydrate oxidation during constant load submaximal exercise](image)

Figure 3.3.1.5 Carbohydrate oxidation during constant load submaximal exercise.
3.3.2. Performance measurements

There was no difference (p>0.05) in the measurements or change in measurements of body fat (figure 3.3.2.1.) or 100 km TT cycling performance (figure 3.3.2.2) during or between the two periods of supplementation. It must be stressed that no values showed statistically significant. With such small subjects numbers it is believed to be fair that a comment may be made on the trend shown by the periods of supplementation for developing the future of this research. The lowest values for body fat (11.88 ± 2.6%) and time of completion of 100km TT (11063 ± 617 secs) were found after supplementing on green tea extract. With differences so small it would require at least a ten-fold increase in completed subjects. However with this in mind it should also be noted that the largest change over a four week training period was seen in the placebo...
Methods to optimise substrate utilisation during endurance performance. Group for both performance measurements. Caution should therefore be taken from any conclusions taken from ‘trends’ and not statistically significant data.

Figure 3.3.2.1 Mean body fat before and after each period of training.
Methods to optimise substrate utilisation during endurance performance.

Figure 3.3.2.2. Time trial performance before and after each period of training.
3.4 DISCUSSION

3.4.1 Subject numbers
This investigation ended with only 4 subjects completing all aspects of the protocol, with a fifth subject completing all but the last lap of the final 100km time trial. This was unfortunate however this was a small piece of the high drop out rate noticed during the investigation and does not provide sufficient weight to the amount of lab time and effort which went into incomplete subjects and subject recruitment. A schematic of subject drop-out demonstrates this below (figure 3.4.1.1).

![Figure 3.4.1.1 Subject drop-out chart](image)

Figure 3.4.1.1 Subject drop-out chart
Methods to optimise substrate utilisation during endurance performance.

This flow chart represents the drop-out rate of subjects during this 15-week minimum protocol. Subject retention was a huge problem especially when the familiarisation alone took on average around five hours of lab time. It was apparent subjects were making a huge time commitment however given that we were only recruiting current competitive cyclists we believed that our protocol would provide an excellent opportunity to monitor their training and performance with power monitors for each training session provided. This group of athletes already train on average of over ten hours per week and it was felt that with only five 100km time trials (one of which being a familiarisation) over the fifteen weeks that participation in this study would not have an impact on training load.

3.4.2 Effects of EGCG supplementation

This investigation alone cannot suggest that EGCG consumption improves endurance performance. The data provided by the pilot study into consumption of varied acute concentrations of ECGC and submaximal exertion were not translated into any chronic increase in submaximal or performance variables. This could suggest that any benefits the catechins may provide can be through acute ingestion opposed to chronic consumption. Currently the opinion is for GTE to be used to improve cardiovascular risk factors such as reducing body weight, body fat, and improving glucose homeostasis (Thielecke, 2009). Although in relation to chronic consumption in athletes we are unable to determine that it can improve endurance performance there is potential for future research as even small improvements to an endurance athlete can be massive.

This study was an adventurous attempt to convey a scientific opinion directly into a ‘real life’ situation. This was ‘adventurous’ as without a large base of current literature on human exercise whilst consuming EGCG we attempted to make our performance measure as close to ‘real life’ as possible. The 100km TT was chosen as the performance measure and was due to the calorific cost that can be expected from an event such as this and every attempt was made to mimic a race situation in a laboratory environment. This is an event by which subjects should be sufficiently glycogen deplete that regardless of consuming an optimum amount of endogenous carbohydrate an efficient fat oxidation mechanism is of great importance. With such little research on the topic it may have been more reasonable to elaborate on the dose-effect relationship the pilot study looked into and develop the protocol from there for future
Methods to optimise substrate utilisation during endurance performance. This would have allowed a greater understanding of the response to exercise whilst consuming EGCG and allowed for a more specific protocol.
Methods to optimise substrate utilisation during endurance performance.

4. DISCUSSION

4.1. Optimising carbohydrate oxidation during endurance performance

The benefits of carbohydrate consumption have been demonstrated since the late 1960s (Bergstrom and Hultman, 1967) and as such the continual representation of the ergogenic properties of carbohydrates have lead to its consumption being an integral part of endurance performance and the lead up to such events. What is not as well known is the mode of carbohydrate which is most effective for delivering carbohydrate at high rates during endurance performance. With carbohydrate available in three main forms a decision as to the most effective method for delivering carbohydrate at high rates. Solid forms of carbohydrate require fluid to aid transport into the gut and also require chewing. Fluid carbohydrate consumption required to maintain optimum gastric emptying is associated with gastrointestinal distress in running due to the up and down motion, however is an effective method for delivering carbohydrate at high rates (Jeukendrup et al., 2009, Coyle, 2004). More recently with the introduction of hyper-concentrated gel based carbohydrate products have been shown to maintain euglycemia when compared to liquid carbohydrate (Campbell et al., 2008). Thus consumption of gel based carbohydrate is a viable option for endogenous consumption of carbohydrate without affecting performance. We were able to produce results that in a real life situation regardless of mode or the rate of carbohydrate consumption there was no benefit to performance. There was however an ability to consume carbohydrate at higher rates when consuming gel based carbohydrate compared to solid carbohydrate and a placebo (were the athlete chose the amount and mode of substrate consumed during the event). Gel based carbohydrate was able to be effectively delivered at high rates commensurate with the upper levels of the guidelines (ACSM, 2007) for carbohydrate consumption during endurance exercise.

What was interesting to note that once the groups were merged and broken down into rates of fluid consumption marathon performance was improved when athletes’ exceeded the recommendations of 0.8L/hour$^{-1}$. This improvement was noticed during a marathon ran at a cool temperature (~10 degrees celcius). It was seen that even at ten degrees celcius fluid consumption is an important factor to consider when preparing for an endurance event. Not just for rate of carbohydrate consumption, which is often focused on, but the addition of fluid to offset dehydration and aid with gastric emptying.
Methods to optimise substrate utilisation during endurance performance.

Over the last twenty years caffeine has been investigated for its ergogenic properties related to improving endurance performance lasting longer than 30-60 minutes (Graham and Spriet, 1991, Graham and Spriet, 1995, Doherty et al., 2004). Kovacs et al (1998) have shown improvements in one hour cycling performance when consuming a small dose of caffeine with carbohydrate above consuming carbohydrate alone. However when caffeine was added in the same dose to our gel supplements such clear conclusions cannot be drawn on this supplement. This may be due to limitations of a field study and the reliance on accurate recall of consumption from the athletes taking part. Although each group recruited over fifty subjects no significant improvement in performance was noted compared to the carbohydrate only gel. Again however we demonstrated that a gel based product was able to deliver carbohydrate at high rates.

More recently the consumption of polyphenols, namely EGCG, have been shown to increase fat oxidation during submaximal exercise (Venables et al., 2007) and increase energy expenditure over a twenty-four hour period (Hardgreaves et al., 2008). Endurance events are performed at a submaximal intensity close to anaerobic threshold. Such an intensity will utilise both carbohydrate and fat oxidation and if consuming EGCG can alter the ratio of substrate utilisation towards fat oxidation then less carbohydrate is required. With carbohydrate a limited store within the body and the amount of endogenous carbohydrate able to be consumed sparing this fuel would prove valuable in events challenging the limits of carbohydrate utilisation. It is hypothesised that if EGCG can permit an adaptation to fat oxidation this adaptation could be transferred into an increased performance capacity.

With little research directly into the effects of EGCG on human exercise the short pilot study was able to provide further information on the dose-response of this supplement during submaximal exercise. Once the concentration and dose of EGCG found to elicit a cost effective increase in fat oxidation was tested it was hypothesised that the acute benefits noted during submaximal exercise would translate into a chronic adaptation if consumed daily and provide an adaptation to increase fat oxidation not only during exercise (Thieleke, 2009). A chronic increase in fat oxidation would be expected to reduce body fat. Reducing body fat would make performance more economical especially for endurance athletes. However for the more sedentary population this could have even more important results in improving health.
Methods to optimise substrate utilisation during endurance performance.
As obesity levels rise due to diets containing higher fat contents surplus fuel has to be stored. The use of EGCG for maintaining weight loss (Hursel et al., 2009) could prove greater benefits until the role of EGCG during exercise is fully understood.

4.2. Conclusion

In conclusion carbohydrate still leads the way in ergogenic aids for endurance performance. Gel based products are able to deliver carbohydrate at higher rates when compared to solid products during a foot marathon. A suitable aid to sparing glycogen cannot be determined from this study however more work has to go into the effects of EGCG and fat oxidation and the optimum concentration and dose required to promote its effects.

4.3. Directions of future research

Research into optimum exogenous carbohydrate consumption methods and concentrations has been thoroughly investigated now for years and has more recently developed to take both event and intensity into consideration when planning supplementation for an endurance event. Carbohydrate is a widely consumed ergogenic aid and will continue to be so as its benefits are proven as a ‘legal’ way to improve performance.

However EGCG has shown that if optimum consumption is attained then fat oxidation is altered. For this reason EGCG will have a role in the future of endurance performance. EGCG has been shown in the pilot study to increase fat oxidation during submaximal exercise. In endurance performance as the distance increases then the intensity at which the event is performed decreases. Therefore the pilot study could be taken forward by firstly increasing subject numbers and defining a concentration of active ingredient that is optimum, or if the optimum conditions are subject dependant. Secondly determine if there is a difference between population for its affect i.e. between athletes, sedentary, and active individuals. This could also potentially develop the use of EGCG in cooperation with exercise programmes for prevention of cardiovascular events, and develop the role of EGCG during exercise.

If endurance athletes can benefit from the effects of EGCG then it would be interesting to understand if intensity of exercise has an impact. From this the concentration of GTE can be tailored towards the intensity at which it is most potent thus allowing the athlete to consume
Methods to optimise substrate utilisation during endurance performance. An amount on the basis of the intensity at which that rate will be performed at. This would be of benefit when an event is at an intensity that an athlete will oxidise greater amounts of fat, to know if consuming more of the more potent stimulator of fat oxidation have a greater effect.

Lastly I believe that it would be possible to target entrants to a mass participation endurance event (as was done for the Dublin marathon) i.e. ironman. To blindly provide a large group with GTE to consume in the run up to the event and again take a predicted performance from the groups. This would hopefully provide a simple idea as to the possibility of EGCG improving performance in an event were substrate utilisation is of great importance.
Methods to optimise substrate utilisation during endurance performance.

APPENDIX

APPENDIX I

Dublin marathon subject information sheet

TITLE OF INVESTIGATION: An investigation into the effect of different carbohydrate delivery strategies on marathon performance.

We invite you to participate in an investigation which we believe to be of potential importance. In order to help you to understand what the investigation is about, please read the following information carefully. Be sure you understand it before you formally agree to participate. If there are any points that need further explanation, please ask a member of the research team. It is important that you understand what you are volunteering to do and are completely happy with all the information before you sign this form.

What is the purpose of the study? There are many different compositions of sports supplements commercially available. This study aims to test the effectiveness of different methods of delivering carbohydrate via sports supplements, i.e., using a solid vs. a gel carbohydrate source. This study also aims to investigate the potential performance enhancing properties of caffeine when included in a gel based carbohydrate sports supplement.

Why have I been chosen? You have been selected as a possible participant in this investigation because you are participating in the Dublin marathon.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and you will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part? First you will be asked to complete a confidential questionnaire, which will allow us to obtain information related to your general health.

Your body weight will be measured before and after the race. You will be asked to provide a urine sample at the start and end of the race. At the end of the race we will take a small (<1ml) sample of blood from your finger tip, you will experience a mild pricking sensation when the skin is broken to obtain this sample. We will measure your core temperature before and after the race with a tympanic (ear) thermometer. You will be asked to recall what you ate and drank during the race.

You will be assigned to a particular sports supplement, manufactured by Highfive, and provided with as much of the product as you will require for the race.

You will be excluded from participating in this study if you take drugs (recreational or performance-enhancing drugs).

What are the side effects of taking part? There are none.

What are the possible disadvantages and risks of taking part? There are none.
Methods to optimise substrate utilisation during endurance performance.

Carbohydrate Supplementation and Marathon Performance: Effects of Altering Mode of Delivery

Glasgow

What are the possible benefits of taking part? The results will help you choose the most effective nutritional supplement for aiding your performance in future events.

What if something goes wrong? If you are harmed by taking part in this research project, there are no compensation arrangements. If you are harmed due to someone else's negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified, are fully trained in Basic Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff are on hand.

Will my taking part in this study be kept confidential? All information which is collected about you during the course of the research will be kept strictly confidential.

What will happen to the results of the research study? Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

If you are worried about any unwanted side effects from any of the above procedures, you should contact:

Dr Andy Cathcart
University of Glasgow,
Glasgow G12 8QQ
Phone: 0141 330 2585
E-mail: A.Cathcart@bio.gla.ac.uk
Methods to optimise substrate utilisation during endurance performance.

APPENDIX II

Example of Dublin marathon food recall sheet

### Dublin Marathon Food Diary

<table>
<thead>
<tr>
<th>Time/Distance</th>
<th>Description of Food or Drink</th>
<th>Portion Size</th>
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</thead>
<tbody>
<tr>
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</tbody>
</table>
Methods to optimise substrate utilisation during endurance performance.

APPENDIX III

GEL only was made to same specification as below with only the caffeine withdrawn.

Carbohydrate energy gel (including caffeine)

<table>
<thead>
<tr>
<th>Nutrition Information</th>
<th>Per Sachet (38g)</th>
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</thead>
<tbody>
<tr>
<td>Typical Values</td>
<td>ALL FLAVOURS - Orange Plus, Raspberry Plus</td>
</tr>
<tr>
<td>Energy (kJ/kcal)</td>
<td><strong>384/82</strong></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>23</td>
</tr>
<tr>
<td>of which sugars (g)</td>
<td>7</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0</td>
</tr>
<tr>
<td>of which saturates (g)</td>
<td>0</td>
</tr>
<tr>
<td>Fibre (Dietary) (g)</td>
<td>0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>35</td>
</tr>
</tbody>
</table>

GMO Free 🟢 Vegetarian 🟢 Nut Free 🟢

**Ingredients: Orange EnergyGel Plus**
- Maltodextrin, Water, Glucose, Fruit Juice Concentrate (Orange, Lemon), Natural Flavours, Sea Salt, Caffeine (30mg/pack), Taurine, Preservatives: Sodium Benzoate, Potassium Sorbate

**Ingredients: Raspberry EnergyGel Plus**
- Maltodextrin, Water, Glucose, Fruit Juice Concentrate (Raspberry, Cranberry), Natural Flavours, Sea Salt, Caffeine (30mg/pack), Taurine, Preservatives: Sodium Benzoate, Potassium Sorbate
Methods to optimise substrate utilisation during endurance performance.

Carbohydrate energy bar

<table>
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<th>Nutrition Information</th>
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<tr>
<td>SportsBar: 55g</td>
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<tr>
<td>Typical Values</td>
</tr>
<tr>
<td>Energy (kJ/kcal)</td>
</tr>
<tr>
<td>Protein (g)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
</tr>
<tr>
<td>of which sugars (g)</td>
</tr>
<tr>
<td>Fat (g)</td>
</tr>
<tr>
<td>of which saturates (g)</td>
</tr>
<tr>
<td>Fibre (Dietary) (g)</td>
</tr>
<tr>
<td>Sodium (mg)</td>
</tr>
</tbody>
</table>

GMO Free ✔ Vegetarian Or ✔ Nut Free ✔

SportsBar does not contain nuts, but other products containing nuts may have been manufactured on the same production line.

Ingredients: Banana: glucose, milk chocolate (20%)(cocoa butter, milk powder, cocoa mass, soy lecithin), oats (18%), rice crisps [rice flour, wheat gluten], raisins, maltodextrin, caramel paste (5%) [hydrogenated vegetable fat, caramelized syrup, emulsifier (E471)], banana (2%), honey, salt.

Values based on the standard 55g sports bar contents however our bars were specially made to the same specifications and scaled down to 20g portions.
### Example spreadsheet gas exchange calculator

<table>
<thead>
<tr>
<th>Pulmonary gas exchange calculator</th>
<th></th>
</tr>
</thead>
</table>

Input raw data into green cells (FiO2, body mass, PB and sampling time only need to be entered once), calculated:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FiO2 (%)</td>
<td>20.30</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>59.5</td>
</tr>
<tr>
<td>Barometric pressure (mmHg)</td>
<td>760</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gas Concentration sampling time (s)</th>
<th>120</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Collection time (s)</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>31.60</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
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</tr>
<tr>
<td>Volume in (L)</td>
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</tr>
<tr>
<td>FiCO2 (%)</td>
<td>18.03</td>
</tr>
<tr>
<td>PaCO2 (%)</td>
<td>3.11</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>145</td>
</tr>
<tr>
<td>treadmill speed (km/h)</td>
<td>rest</td>
</tr>
<tr>
<td>treadmill gradient (%)</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VO2 (L/min)</th>
<th>12.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCO2 (L/min)</td>
<td>3.65</td>
</tr>
<tr>
<td>VO2 (mL/kg/min)</td>
<td>2.02</td>
</tr>
<tr>
<td>VCO2 (mL/kg/min)</td>
<td>3.23</td>
</tr>
<tr>
<td>RER</td>
<td>1.00</td>
</tr>
<tr>
<td>VO2 (mL/min/mmHg)</td>
<td>34.33</td>
</tr>
<tr>
<td>VCO2 (mL/min/mmHg)</td>
<td>32.50</td>
</tr>
</tbody>
</table>

**APPENDIX IV**

Methods to optimise substrate utilisation during endurance performance.
Methods to optimise substrate utilisation during endurance performance.

APPENDIX V

University of Glasgow
Institute of Biomedical and Life Sciences
University of Glasgow

INFORMATION SHEET

TITLE OF INVESTIGATION: An investigation into the effect of green tea extract on the adaptation to endurance training.

We invite you to participate in an investigation which we believe to be of potential importance. In order to help you to understand what the investigation is about, please read the following information carefully. Be sure you understand it before you formally agree to participate. If there are any points that need further explanation, please ask a member of the research team. It is important that you understand what you are volunteering to do and are completely happy with all the information before you sign this form. This study is supported in part by Highfive, a supplier of sports nutrition products.

What is the purpose of the study? Long duration endurance exercise reduces the body’s carbohydrate stores thus limiting performance. An early switch to using fat for energy should effectively spare these carbohydrate stores and improve endurance performance. However the most effective supplement and method of supplementation to increase fat usage during endurance performance, is not known. This study aims to test the effectiveness of supplementing during training with green tea extract, a known promoter of fat oxidation.

Why have I been chosen? You have been selected as a possible participant in this investigation because you are an endurance trained, healthy male.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and you will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part? You will come to the laboratory on at least nine occasions before and after the training interventions.

During the first visit you will be asked to complete a confidential questionnaire, which will allow us to obtain information related to your general health. You will be given a chance to familiarise yourself with lab surroundings and equipment that will be used. You will then change into your exercise kit, then your height and weight will be measured and you will complete a 100 km course on a computer controlled cycle ergometer.

The following week you will return to the lab (2nd visit) and have resting breathing measurements taken, whilst lying down, for twenty minutes. Once we have made these measurement you will be asked to remain lying down and a needle will be used to insert a small plastic tube into a vein in your forearm. We will use this tube to draw blood samples from your vein. This method causes little discomfort as the needle is only inserted once and the whole procedure will last no longer than 5 minutes. Your blood samples will be placed into an anonymised collection tube and then processed and frozen to allow analysis of the levels of glucose, insulin and fats in your blood after the test has finished. Once the analysis has been satisfactorily performed your blood samples will not be retained for any reason. You will then sit in a closed chamber known as a ‘Bodpod’ in order to calculate your body composition. Once this is finished you will be asked to perform a one hour cycle at a fixed intensity to measure how your body uses its different fuel supplies during exercise.

During the next visit (3rd visit) in the same week you will be asked to repeat the 100 km time trial you performed during the first visit on a computer controlled cycle ergometer, which will take about 3 hours to complete. You will be given carbohydrate drinks at 15 minute intervals throughout the exercise period.

You will then follow your own training programme, based on two interval training sessions per week and three aerobic training sessions per week for 4 weeks. During this period you will take a small capsule three times a day, every day for the 4 week period. The capsule will either contain Green Tea Extract or a Placebo. We will provide you with a mobile power measurement device to monitor the intensity and duration of your training sessions.
Methods to optimise substrate utilisation during endurance performance.

You will also be required to keep a log of the training sessions you perform, including details of the sessions such as average speed, average heart-rate and average power output.

Within one week of finishing the 4 week training and supplementation period you will come to the lab for two testing visits. During these visits you will repeat, in the same order, the procedures of the second and third visits.

You will then be given a three week period without involvement in the study when you will be free to continue to train as normal. Following this you will, in what is known as a cross-over research design, repeat the procedures above from visit two onwards. The only difference between the first and second half of the study will be that you will receive the other supplement (Green Tea Extract or Placebo) to consume during the 4 week training period.

**What are the side effects of taking part?** No anticipated side effects, other than those normally associated with strenuous exercise.

**What are the possible disadvantages and risks of taking part?** Exercise has a negligible risk in healthy adults, although maximal exercise does carry a small risk of inducing myocardial ischaemia (“heart attack”). The primary symptom of myocardial ischaemia is chest pain on exertion. If you experience any unusual sensations in your chest during the experiment, you should cease exercising immediately. Your heart rate will be monitored via electrodes placed at points on the chest (an “electrocardiogram” or ECG). In the unlikely event you experience serious problems during the exercise, medically-qualified personnel are on call at all times during the test and approved emergency procedures are in place. At the end of the tests you will be very tired (exhausted), your legs will be very heavy and you will be out of breath. It is also not uncommon to feel a little light-headed and sometimes nauseous.

Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood.

**What are the possible benefits of taking part?** The information from this study will provide you with detailed feedback about your fitness levels.

**What if something goes wrong?** If you are harmed by taking part in this research project, there are no compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified, are fully trained in Basic Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff are on hand.

**Will my taking part in this study be kept confidential?** All information which is collected about you during the course of the research will be kept strictly confidential.

**What will happen to the results of the research study?** Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

If you are worried about any unwanted side effects from any of the above procedures, you should contact:

Dr Andy Cathcart  
University of Glasgow,  
Glasgow G12 8QQ  
Phone: 0141 330 2585  
E-mail: A.Cathcart@bio.gla.ac.uk
Methods to optimise substrate utilisation during endurance performance.
An investigation into the effect of green tea extract on the adaptation to endurance training.

Consent Form

I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, with no consequences.

I understand that there is a commercial sponsor with interest in the outcome of the study

I agree to take part in the above study.

Name  ....................................................
Signature ………………………………………
Date  ………………………………………
Please read.

It is important to take a record of your medical history. You may have, or may have once had a condition that would make this type of testing unsuitable for you. For this reason we ask you to be as truthful and detailed as possible. At no point will this information be made available to any one other than the principal investigators for this study. If you have any doubts or questions, please ask.
Methods to optimise substrate utilisation during endurance performance.

SUBJECT DETAILS:

NAME: 

AGE:  D.O.B: 

SEX (M/F): 

GP NAME & ADDRESS: 

SMOKING:  Never Smoked ...... 
Not for >6 months ...... 
Smoke <10 per day ...... 
Smoke >10 per day ...... 

ILLNESSES:

ALLERGIES: 

___________________________________________________ 

 HOSPITALISATIONS:

___________________________________________________ 

MUSCULO-SKELETAL DISORDER:
(Arthritis, Joint Pain, Fractures, Sports injury, Others) 

___________________________________________________ 

CARDIOVASCULAR DISORDER: (Fever, Heart Murmurs, Chest Pain, Palpitations, High Blood Pressure, Others) 

___________________________________________________ 

RESPIRATORY DISORDER: (Asthma, Shortness of Breath (SOB), Cough, Upper Respiratory Tract Infection (URTI), Others) 

___________________________________________________
Methods to optimise substrate utilisation during endurance performance.

GASTROINTESTINAL DISORDER: (Jaundice, Bleeding, Others)

 DIABETES:

 CNS DISORDER: (Fits, Blackouts, Tremor, Paralysis, Epilepsy, Other)

 PSYCHIATRIC TREATMENT:

 FAMILY HISTORY: (Sudden death in a first degree relative under the age of 35 years)

 ARE YOU CURRENTLY TAKING ANY MEDICATION?  No / Yes*  
 (*Please specify)

 ARE YOU CURRENTLY TAKING ANY SUBSTANCES TO HELP IMPROVE YOUR TRAINING OR CONTROL YOUR WEIGHT i.e. CREATINE, PROTEIN SUPPLEMENT?  No / Yes*  
 (*Please specify)

 ARE YOU CURRENTLY TAKING ANY OTHER SUPPLEMENTS i.e. FOOD SUPPLEMENTS, VITAMINS?  No / Yes*  
 (*Please specify)

 CAN YOU THINK OF ANY OTHER REASON WHY YOU SHOULD NOT TAKE PART IN ANY OF OUR TESTS?

 SYMPTOMS:
Methods to optimise substrate utilisation during endurance performance.

Do you experience any of the following, particularly on exercise?

- Breathlessness: No / Yes
- Chest Pain: No / Yes
- Dizzy Fits/Fainting: No / Yes
- Palpitations: No / Yes

Please note that if you feel unwell on the day of the proposed test, or have been feeling poorly over the preceding day or two, please inform the investigators and DO NOT TAKE PART in the exercise test.

DECLARATION:

I have completed this questionnaire fully and truthfully. I have not kept any information from the investigators that may put myself at risk during high-intensity exercise, or affect the results that they obtain. I understand that I may withdraw from any one test or the study as a whole if I feel unwell, or feel uncomfortable with any part of the testing procedure.

(Signature)………………………………

(Date)……………………..

PHYSICAL EXAM:

WEIGHT: ___________  HEIGHT: ___________

PULSE (Resting): ___________  BP (Resting): ___________

Screened by: ……………………………..

(Signature) ………………………………..  (Date) ………………………..
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REFERENCES

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