The effects of carbohydrate loading 48 hours prior to a simulated squash match.

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Abstract

Squash is a high-intensity, intermittent racket sport that is played by over 15 million people worldwide (Eime & Finch, 2002). Unlike other racquet sports such as tennis, squash has had limited attention in the area of sports nutrition and exercise performance. Furthermore, the effect of carbohydrate ingestion in the days (48 hours) leading up to a squash match has not been explored. Eight squash players who were graded at a Squash New Zealand grade of C1 or above were recruited. Following a familiarisation subjects completed two further trials which consisted of a bout of exhaustive exercise prior to completing a simulated squash match, following a controlled diet for 48 hours in between initial exhaustive exercise bout and match simulation. The match simulation was aimed to mimic the expected metabolic changes during a five-set match lasting about an hour to incorporate the endurance factor of exercise. Performance was measured by the time required to complete each set, which was designed to last ~12 minutes followed by a rest period of ~120 seconds in order to collect measurements. The interventions were assigned in a randomised, single-blind, cross-over design. The interventions consisted of a standardised diet with additional energy intake via the form of a 'sports drink'. The high-carbohydrate ($11.1 g \cdot kg^{-1}$) diet was primarily delivered in the form of a carbohydrate-containing solution containing maltodextrin; whilst the calorie-matched low-carbohydrate ($2.1 g \cdot kg^{-1}$) drink was made from a combination of milk powder, protein powder and oil. Oxygen uptake, respiratory exchange ratio (RER), fat and carbohydrate oxidation, and heart rate were continuously collected throughout the trial. Blood glucose and lactate samples were obtained before and after each squash set. Rating of Perceived Exertion (RPE) was also recorded after each set. There was an overall main effect of the intervention as seen from RER ($p = 0.016$). The difference in RER was further supported by a significant difference seen in fat ($p = 0.011$) and carbohydrate ($p = 0.013$) oxidation. Though an interaction of performance time and the intervention was progressing towards significance ($p = 0.076$), it narrowly missed the α-value of 0.05 to achieve significance. A significant main effect of the trial was not present in both blood glucose and lactate ($p > 0.05$). However, blood glucose and lactate had a significant effect of time of $p = 0.005$ and $p < 0.001$, respectively. These results point towards a beneficial effect of carbohydrate ingestion on squash performance. However, further research will be required to support the findings of this study.

Introduction

Squash is a popular racket sport that is played by over 15 million people worldwide (Eime & Finch, 2002). It is played by both males and females of all ages and can be taken on at any skill level. It is beneficial to health as it challenges several aspects of fitness including aerobic and anaerobic capacity, flexibility, and speed. A squash match is played over three to five sets and can last up to three hours during a five-set match (Steininger & Wodick, 1987).

Being the sport of interest in this study, squash has received limited attention from the sport science community and therefore the physiological stresses during match play are poorly understood. Hence, the rationale behind this study was to gain a better understanding of the metabolic, physiological, and nutritional aspect of squash. Previous squash research has primarily concentrated on game analysis (Girard *et al.*, 2007), with only a handful of studies investigating on-court performances via match simulations (Romer *et al.,* 2001,Kingsley *et al.*, 2006,Girard *et al.,* 2007).

This study aimed to simulate a five-set match, provoking the relevant physiological stresses required for success in competition. A nutritional intervention was introduced to this study which adds to the current literature, which had the aim of investigating the effect of carbohydrate loading on simulated squash performance. Unlike many other sports where there is a plethora of well-researched findings on the importance of carbohydrate on performance, this has not been researched in squash. Sensibly, we hypothesized that a high carbohydrate diet in the days prior to a simulated squash match would increase carbohydrate oxidation during the match, and thereby improve match performance.

The literature review covers a brief background of published knowledge regarding carbohydrate loading and feeding during exercise, the effect of carbohydrate stores in relation to performance, the processes of muscle glycogen depletion and synthesis, previous literature on high-intensity exercise, and a background of previous research and methodology used in squash-related research.

Literature Review

Exercise intensity and fuel selection

It is well understood that as exercise intensity increases, the proportion of energy produced from carbohydrate increases. This is due to two factors: 1) the increasing engagement of glycolytic fibres in the motor units sequentially recruited with increasing muscular tensions; and 2) the increase in activity of glycolysis promoting enzymes which occurs with cellular energetic stress. These effects are illustrated in the *Crossover Concept* (Figure 1). In a whole body context, fat is the main fuel source utilised at rest. When exercise is initiated, an increased proportion of energy will be derived from carbohydrate substrate whilst fat utilisation declines in relative terms. The crossover point occurs at around 70% of $\dot{V}O_2$max, but is dependent upon a number of factors including training status and nutritional status.

Further evidence of increased carbohydrate utilisation can be seen from the study of Saltin & Karlsson (1971) where the rate of glycogen depletion in the quadriceps muscle increased as exercise intensity increased. The optimal exercise intensity for the depletion of carbohydrate was found to be at 75% $\dot{V}O_2$max in which muscle glycogen values were approaching zero at the point of fatigue. Conversely, at maximal (100% $\dot{V}O_2$max) and supra-maximal (>100% $\dot{V}O_2$max) exercise intensities, glycogen depletion was not as severe because exhaustion likely occurred from non-fuel-related causes (probably cardiovascular in origin). Clearly, carbohydrate, particularly muscle glycogen, is the main source of energy for moderate- to high-intensity exercise.

There is a difference between trained and untrained participants in the utilization of carbohydrate during exercise. Well-trained participants possess a higher absolute oxygen uptake whilst maintaining a lower RER than an untrained participant at the same absolute intensity.

The higher circulating lactate levels seen in untrained individuals for the same relative workload can be, in part, explained by the fact that when trained individuals exercise at low-intensity, a greater proportion of the muscle fibres recruited are more oxidative and can utilize fat. Hence, in well-trained people, lower glycogen utilisation is observed and minimal lactate is produced. The seminal work by Henneman and colleagues (Mendell, 2005) who developed the *'Size Principle'* for motor unit recruitment, showed that as muscular tension

increases, larger motor units are recruited which are more glycolytic in their energetic requirement. This will, of course, result in increased lactate production in the exercising muscle. Training results in a shift to more oxidative fibres in those motor units utilized during training (Dudley *et al.*, 1982). An untrained individual recruits glycolysis-reliant fibres more quickly as muscular tension must be increased, so is more glycogen dependent as intensity increases (Saltin & Karlsson, 1971). In other words, a larger breakdown of muscle glycogen is experience in untrained subjects which at least partly explains the increased lactate concentrations at the same absolute workload for this group (Saltin & Karlsson, 1971).

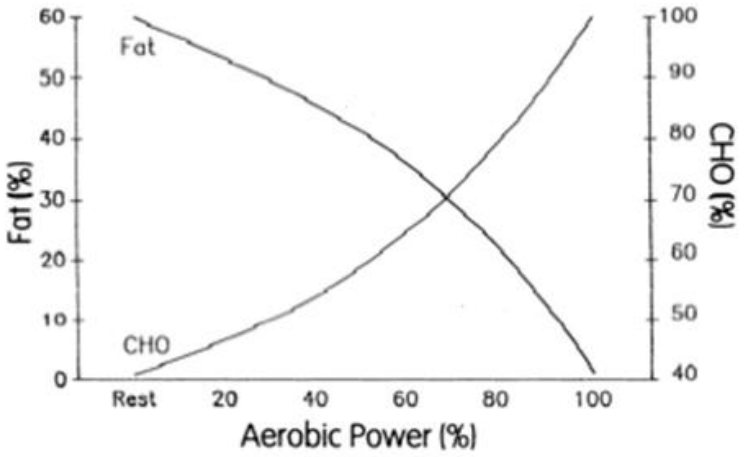

Figure 1. Crossover concept (Brooks & Mercier, 1994).

Background of carbohydrate feeding on performance

Pre-exercise nutritional interventions have been of interest to human physiologists since the early twentieth century. Nearly a century ago it was suggested by Christensen & Hansen (1939) that the ability to perform prolonged exercise is dependent on the type of diet before exercise, and that high carbohydrate diets provided an ergogenic advantage. Previous research has found that exercise capacity is related to the degree of glycogen stored in the muscle. Therefore as the duration of exercise progresses, the availability of glycogen as a fuel source becomes more limited from being utilised during exercise. The consequence of reduced muscle glycogen stores then impacts on exercise performance and/or capacity (Bergström *et al.,* 1967,Hermansen *et al.,* 1967).

Most research investigating fuel utilization during exercise, including that described above, has employed constant load, endurance exercise. Yet, most game sports are intermittent in nature, with varying degrees of intensity over 60-90 minutes. An early study by Hermansen *et al.* (1967) investigated the effects of intermittent exercise in muscle glycogen concentrations and found that the oxidation rate of carbohydrates was extremely high and constant throughout a 20-minute period of intermittent exercise at 75% of $\dot{V}O_2$max. These researchers also found that there was a close relationship between the rate of glycogen utilised along with the amount of carbohydrates combusted. As a result of this and subsequent studies, it is now well established that muscle glycogen content decreases during intermittent work and glycogen stores may be close to being completely emptied at the point of exhaustion.

Prior to the introduction of the of the biopsy needle (Bergström & Hultman, 1966), samples were taken by the open biopsy technique. In the late 1960s, Bergstrom and colleagues performed many studies investigating the effect of diet and exercise on muscle glycogen concentration. In one of these studies (Bergström & Hultman, 1966), the authors showed that when a muscle was previously exercised then provided with carbohydrate, its ability to accrue glycogen when carbohydrate was eaten was significantly greater than the other leg which did not take on the prior contractile activity. Further research by the same group (Bergström *et al.,* 1967) showed that when prior glycogen depleting exercise was followed by a high carbohydrate diet, the amount of glycogen stored within skeletal muscle was much greater than if a low carbohydrate diet was consumed. Thence developed a regime, variously termed ‘glycogen super-compensation’ or ‘carbohydrate loading’ whereby endurance athletes employed to maximise muscle glycogen levels prior to an important event. The protocol

consisted of three days of a high fat and protein diet, followed by another three days of a high carbohydrate diet with periods of exercise within a 7-day period. The parallel exercise protocol consisted of cycling to exhaustion at a work load corresponding to around 75% of the subjects $\dot{V}O_2$max on the first day, followed by a maintenance (low) training workload on the following days to keep muscle glycogen levels low.

Laboratory-based research on glycogen super-compensation showed that endurance cycling performance time was improved by at least 100% with the use of this traditional 'loading' regime. On the other hand, muscle glycogen concentrations after three days of a fat and protein diet following a bout of exhaustive work proved to be unsuccessful in resynthesising muscle glycogen to more than 50 percent of its initial value. The results of this study was further supported from the RER and lactate values taken during exercise which corresponded with the high-carbohydrate diet by demonstrating higher RER and lactate values when compared to the fat and protein (control) diet. This indicated greater utilisation of carbohydrate, greater use of larger motor units and thus generating greater force development within the muscle (Bergström *et al.*, 1967).

Carbohydrate ingestion three to four hours before exercise

Increasing carbohydrate intake three to four hours before exercise has been found to increase muscle glycogen concentrations. Coyle *et al.* (1985) investigated the effects of a high carbohydrate meal four hours before 105 minutes of exercise at 70% $\dot{V}O_2$max in endurance-trained cyclists. The trial was compared to a 16-hour dietary fast. As expected, muscle glycogen was significantly higher with pre-exercise feeding. However, the post exercise glycogen levels were not significantly different. This indicates that the total muscle glycogen utilised during the course of exercise was greater following pre-exercise feeding. This was supported by an increase in carbohydrate oxidation which would have been derived from the increased muscle glycogen stores.

A more recent study by Wright *et al.* (1991) investigated the effects of various combinations of carbohydrate feeding and timing before or during exercise. Subjects consumed $5g \cdot kg^{-1}$ body weight of a carbohydrate solution in a 25% carbohydrate solution which was made out of 21% glucose polymers and 4% sucrose. Following consumption of the carbohydrate solution, subjects rested for three hours before commencing exercise consisting of cycling at 70% of $\dot{V}O_2$max until exhaustion. When compared to a placebo, the RER was significantly

higher following pre-exercise carbohydrate feeding indicating a higher rate of carbohydrate oxidation. The result of this study found that a three-hour pre-exercise carbohydrate feeding resulted in a 19% improvement in total work output when compared to the trial without any carbohydrates. The improvement in performance suggests that the carbohydrate feeding delayed fatigue by maintaining carbohydrate availability. This would perhaps allow longer use of the larger motor units prior to fatigue (Wright *et al.*, 1991). This supports the results of the previously discussed study by Coyle *et al.* (1985) in which the increase in carbohydrate oxidation may have been due to an increase in muscle glycogenolysis or an increase in glucose uptake.

Even though carbohydrate feeding in the hours before exercise may result in a transient fall of glucose following the onset of exercise and a blunting of free fatty acid mobilisation, these metabolic perturbations are not detrimental to performance due to the increase in carbohydrate availability which compensates for greater carbohydrate oxidation and/or utilisation (Coyle *et al.*, 1985,Wright *et al.*, 1991).

Summary

Exercise intensity is closely related to fuel selection and utilization; vis-à-vis, the proportion of carbohydrates utilised for energy production increases with exercise intensity. It thus follows that endurance and intermittent exercise performance is also closely related to the amount of stored glycogen in the muscle at the onset of exercise. High carbohydrate diets, especially in the days (and even hours) following previous exercise, have the ability to maximize muscle glycogen concentrations. The majority of relevant research, however, has only studied endurance, constant-load exercise.

High intensity exercise

Carbohydrate utilization during intermittent exercise

As previously discussed, high-intensity, intermittent exercise is known to deplete muscle glycogen stores since it engages the larger (more glycolytic) motor units which rely heavily upon glycogen to support their energetic needs (Saltin & Essen, 1971). The end of a football match, for example, will result in low glycogen concentrations in the major locomotor muscles, and to facilitate recovery, carbohydrates must be quickly replenished (Bangsbo *et al.*, 2007).

Many sports such as soccer, tennis, and basketball involve bouts of all-out effort or maximal exercise during a prolonged period of continuous exercise of submaximal intensity (Smekal *et al.*, 2001,Bangsbo *et al.*, 2007). During a single bout of dynamic maximal exercise lasting less than 10 seconds, much energy is provided through anaerobic pathways; glycogenolysis leading to the formation of lactate and through the breakdown of phosphocreatine (Boobis *et al.*, 1982). Gaitanos *et al.* (1993) investigated the contribution of glycogenolysis and Creatine Phosphate (PCr) degradation to energy provision during ten six-second sprints on a cycle ergometer with 30 seconds of recovery between sprints. The magnitude of glycogen that was used anaerobically in the first 6-second bout was 44.1% while PCr degradation contributed to 49.6% of energy production. Following the tenth six-second sprint, the contribution of energy from anaerobic sources was reduced to 35.6%. Anaerobic glycolysis contributed to only 16.1% as the glycolytic rate had dropped 7.6 times from its original rate. Though the contribution of anaerobic glycolysis was reduced in the final sprint, it is likely that there was an increased reliance on aerobic metabolism of glycogen as a fall in muscle glycogen was observed. The reduction of power output and total work production is supported by Spriet *et al*. (1989) who also found a reduced average power output and total work production as the number of exercise bouts progressed. The authors found that reduced contribution from glycogenolysis was one explanation which may have affected the decline in performance. Down-regulation of glycogen phosphorylase activity was also affected by an increasing hydrogen ion, H^+ concentration $[H^+]$. The increased $[H^+]$ may have interfered with the calcium ion, Ca^{2+} concentration $[Ca^{2+}]$, activation thereby decreasing the amount of Ca^{2+} released from the sarcoplasmic reticulum resulting in a reduced force production and phosphorylase activation of the affected muscle fibres (Spriet *et al.*, 1989).

A later study by Hargreaves *et al.* (1998) investigated muscle metabolism in human muscle following repeated bouts of high-intensity exercise. Like the studies mentioned previously, a decline in muscle glycogen was observed as the number of sprints increased**.** The authors found that though peak power and work decreased during the initial three bouts, this was not evident in the fourth sprint. This may be explained through methodological differences in comparison to previous studies as the subjects cycled for 30 minutes between 30-35% $\dot{V}O_2$max between the third and fourth bout which would have facilitated the removal of lactate and H^+ ions from blood and muscle while minimising resynthesis of muscle glycogen. Though not significantly different, there was an increase in muscle glycogen following the 3rd exercise bout which may explain the increase in performance in the final sprint. Similarly to Spriet *et al.* (1989), Hargreaves *et al.* (1998) are in support of other factors such as PCr availability and the concentration of H^+ ion which may affect performance as similar changes were seen.

The studies discussed above found that the rate of glycogenolysis contributes to the reduction in performance seen with repeated bouts of high intensity effort. It also supports the contribution of anaerobic glycolysis during intermittent exercise. Since squash is an intermittent sport which can be completed over a prolonged period of time, the amount of glycogen stored in muscle would theoretically influence performance, even more so when exercise is prolonged from the aerobic component of glycogen utilisation.

It has been previously shown that nearly full replenishment of glycogen stores is possible in both type I and type II fibres within the first 24 hours following exercise-induced glycogen depletion (Piehl, 1974). A study by Nicholas and co-authors (Nicholas *et al.*, 1997) was designed to investigate the restoration of intermittent high-intensity running capacity following a diet containing additional energy in the form of carbohydrate or fat and protein. An additional amount of carbohydrates were calculated from the daily intake ($5.4 g \cdot kg^{-1}$) in order to increase each subject's daily intake to $10 g \cdot kg^{-1}$. Subjects were prescribed the diet with additional carbohydrates or an isocaloric diet in the form of fat and protein over a 22-hour recovery period following the first shuttle run test. Following the recovery period, subjects repeated the prolonged, intermittent, high-intensity shuttle run test. The results found that subjects were able to run for 5.8 ± 1.7 minutes longer in the carbohydrate trial when comparing the difference in performance time across experimental conditions. This improvement in endurance capacity allowed subjects to run 1.1 ± 0.3km further.

Carbohydrate feeding 2-3 days before intermittent exercise

Carbohydrate loading in the days leading up to intermittent exercise have been previously studied (Jenkins *et al.*, 1993,Casey *et al.*, 1996,Balsom *et al.*, 1999). The efficacy of a loading period between 24 and 72 hours has been investigated by Piehl *et al.* (1974) and Blom *et al.* (1987). A summary can be seen in Table 1 along with intermittent exercise completed after a short bout of carbohydrate feeding in the days leading to exercise. Jenkins and co-authors (1993) conducted a similar study to Snyder and colleagues (1993) looking at the effects of carbohydrate intake on performance on supramaximal intermittent exercise. Participants consumed a moderate CHO diet for 3 days before the first of two intermittent tests and were then randomly assigned to either a high, moderate or low carbohydrate diet for 3 days in between the first and second intermittent test. The intermittent tests consisted of five 60-second all-out periods of cycling with each period separated by five minutes of passive recovery. The authors found that participants who consumed a moderate or high CHO diet before the second maximal intermittent test significantly improved their average work output by 2.3% and 5.6%, respectively. In support of these improvements in performance, a significant relationship was found between the changes in work output and changes in carbohydrate consumption. Interestingly, the changes in work output between low carbohydrate and the moderate/high carbohydrate groups did not show a similar relationship with $\dot{V}O_2$ values during exercise.

Casey *et al.* (1996) found no improvement following 3 days of a high carbohydrate diet in work performed during four bouts of isokinetic cycling. Subjects went through a vigorous glycogen depletion protocol followed by 3 days of either a low (< 10%) or high (>80%) carbohydrate diet. The morning following the diet, subjects completed 30 minutes of cycling followed by a two hour recovery period before completing the four repeated bouts of 30-second maximal exercise. No significant differences were seen when comparing total work production during each bout of exercise before and after a high carbohydrate diet. However after a low carbohydrate diet, work decreased during the first three bouts of maximal exercise, but it had no effect thereafter.

Balsom and co-authors (1998) investigated the effects of a low- (4%) versus high-carbohydrate (67%) diet for 48 hours before completing an exercise regime. Two exercise protocols were completed 24 hours apart, on two occasions separated by at least one week. The first protocol consisted of 15 six-second bouts of high-intensity exercise with 30 seconds

of rest between periods. The second exercise protocol 24 hours later consisted of repeated six-second bouts of high-intensity exercise completed to the point of fatigue. Muscle glycogen was depleted 24 hours before completing the first protocol. The findings from the study found that following the high-carbohydrate diet, subjects were able to maintain a higher power output over the first protocol consisting of fifteen 6-secound bouts and completed an average of 183 more sprints in the second protocol where high-intensity exercise was repeated until fatigue. Since high intensity exercise is associated with a rapid utilisation of muscle glycogen, the maintenance of a higher average power output is in support of an increased availability of muscle glycogen (Gaitanos *et al.*, 1993). This is supported by glycogen concentrations which were significantly higher post-exercise following the high-carbohydrate trial. Since the second exercise protocol can last for at least 30 minutes, it was suggested that aerobic energy production plays a role in resynthesising ATP during this contractile activity. This is in support of Gaitanos *et al.* (1993) who suggested that there was a decrease in the contribution of anaerobic metabolism and a subsequent shift towards aerobic metabolism from repeated bouts of high-intensity exercise.

Therefore evidence of carbohydrate feedings before intermittent exercise still remains equivocal and needs to be investigated further. There is also further support for an increased aerobic contribution to energy production during exercise following repeated bouts of high-intensity exercise as ATP turnover declines (Spriet *et al.*, 1989,Bogdanis *et al.*, 1996). The reduced contribution of anaerobic glycolysis may also be supported by a reduced average power output during the last sprint. This can be explained by a higher mean power output in the first sprint where energy would have mainly been derived from PCr and anaerobic glycogenolysis. The reduced power output from an increased contribution of aerobic glycogenolysis may be explained by the muscle fibre type contribution as type II fibres are more glycolytic which support the higher mean average power output during the first sprint.

Summary

Sports such as squash, soccer and tennis consist of intermittent high-intensity bouts. However, since these sports can last for a prolonged period of time, the energy demands shift from an anaerobic source to greater use of the aerobic pathways. Carbohydrate feedings two to three days before short duration high-intensity intermittent tests have been found to result in an improvement in exercise performance of between 2.3% to 5.6%. Recent work investigating the effect of carbohydrate ingestion during prolonged intermittent exercise was found to be effective in studies simulating a soccer match and the duration of other sports such as hockey and basketball. However, available literature on carbohydrate ingestion on racket sports such as squash is very limited and has not received as much attention as it should have as the nature of the sport relies on a high proportion of carbohydrate utilisation.

Study	Participants	Depletion/Initial Protocol	CHO intake	Performance protocol	Muscle glycogen	Outcome of the study
Piehl *et al.* (1974)	4 active males	Glycogen resynthesis following glycogen depletion for 2 hours (1h of endurance exercise and repeated sprints)	60% CHO diet for 46 hours.		Glycogen = 23mmol ↑ to 124mmol glucose units/kg.	Glcyogen ↓ from 125 to 23mmol glucose units/kg. 46h recovery ↑ to 124mmol glucose units/kg.
Blom *et al.* (1987)	6 trained (T) and untrained (UT) runners	Glycogen resynthesis following exhaustive running @ 75% VO_{2max}	400g : 600g before : after exercise		↑ before exercise in (T) – 110 to 180 mmol/kg. ↑ glycogen in T and UT after 3 days.	Glycogen ↑ 26 to 87mmol/kg, and ↑ 57 to 127 mmol/kg in UT and T, respectively ($p < 0.001$).
Fallowfield & Williams (1993)	16 males	Ingested CHO after treadmill run @ 70% VO_{2max} for 90 min or until exhaustion	CHO = 5.8 (C) or 8.8g/kg (CHO) BW during a 22.5hr recovery	Repeated treadmill run.	None measured.	CHO trial ↑ time by 9.21 minutes. However, ↓ performance time by 15.57 minutes following control trial.
Jenkins *et al.* (1993)	14 moderately trained males	1st visit :VO_{2max} 2nd and 3rd : Maximal Interval Test (MIT).	3 diets lasting 3 days each: low (12%), moderate (55%) or a high (80%) CHO diet.	Repeat of interval test (MIT_2)	None measured.	Moderate and high-CHO ↑ work output by 2.3% and 5.6% respectively, when compared to low-CHO diet.
Casey *et al.* (1996)	11 healthy males	Glycogen depletion; Repeated cycling to exhaustion at 75% VO_{2max}.	2 CHO diets - three days each; low (<10%) and high (>80%)	30 min. cycling at 75% VO_{2max} with 2h recovery followed by four 30-s sprints	None measured.	No change in total work production before and after high CHO intake. Total work production ↓ in first 3 bouts following low-CHO intake. No change in 4th bout.
Balsom *et al.* (1999)	7 active males	Cycling for 90 min @ 70% VO_{2max} + supramaximal intermittent bouts.	2 diets: Low (4%) vs High (67%) CHO diet for 48h.	Short (IEx_{short}) and long (IEx_{long}) intermittent exercise during and at the end of feeding period, respectively.	Glycogen before IEx_{short} and IEx_{long} was sig. ↓ following L-CHO diet compared with H-CHO.	↓ work completed in L-CHO vs H-CHO. 265% ↑ in performance following H-CHO diet. Glycogen concentration significantly ↓ at point of fatigue in L-CHO vs H-CHO, 58 vs 181mmol/kg (dry weight).

Table 1. Summary of studies incorporating glycogen resynthesis between 22.5 - 72h with/without an intermittent exercise test.

Muscle glycogen metabolism

Glycogen utilisation

Gollnick *et al.* 1973a found that complete glycogen depletion occurred during prolonged exhaustive work at 65% $\dot{V}O_2$max. This suggests that all muscle fibres were used at some point in time during prolonged exercise. Five trained and untrained subjects were instructed to cycle at this workload for three hours or to the point of exhaustion. The trained subjects completed the three hours of exercise, whilst the remaining subjects lasted between 110 and 120 minutes.

The initial glycogen concentration for the untrained and trained groups was 96 & 182 mmol·kg^{-1}, respectively. These values declined to 11 and 34 mmol·kg^{-1}, respectively which identifies that the inability for untrained subjects to work beyond two hours was probably related to having lower initial muscle glycogen (Gollnick *et al.*, 1973a). The authors found that initial glycogen depletion occurred in the ST fibres. Additional ST motor units were recruited in order to maintain the ability to develop tension. When the ST fibres were glycogen depleted, FT fibres were recruited which led to their sequential glycogen loss.

During high-intensity exercise, with a calculated resistance of 150% of subjects' aerobic power, the low-oxidative, fast-glycolytic fast twitch fibres were the first to become glycogen depleted. Subjects performed six one-minute bouts at the calculated power output. The average pre-exercise glycogen was 132mM of glucose units·kg^{-1}. Following the first bout of exercise, this declined by 20%. There was a linear decline in glycogen following each successive sprint that was completed. The calculated average of final glycogen content was 49mM of glucose units·kg^{-1} (Gollnick *et al.*, 1973b).

The findings from this current study (Gollnick *et al.*, 1973b) found that FT fibres were recruited at the onset of heavy, high-intensity exercise. Though ST fibres were probably utilised during exercise, the glycogen stores would not have depleted as rapidly as FT fibres as it has a greater aerobic capacity than type II fibres. Their lower glycolytic potential may have also prevented the rapid depletion of glycogen.

A later study by Gollnick *et al.* (1974) examined the pattern of selective glycogen depletion in human muscle fibres following exercise of varying intensity; low, medium and high intensity which corresponded to ~31%, 64% and 84%, respectively of the subject's $\dot{V}O_2$max.

The results from this study support Gollnick's previous studies in which ST fibres are the first to lose glycogen during exercise at a workload lower than 100% of $\dot{V}O_2$max. It also confirms that FT fibres are only depleted of glycogen before ST fibres at supramaximal workloads.

During exercise at the lightest workload, total glycogen depletion was modest as expected. However, selective glycogen depletion did occur in ST fibres at an early stage following the onset of exercise which confirms the previous results (Gollnick *et al.*, 1973a). Exercising at the highest workload resulted in exhaustion in 60 minutes from the depletion of glycogen in ST fibres. However, an interesting point can be made in which there was still a substantial amount of glycogen available in FT fibres following termination of exercise.

A study by Vollestad *et al.* (1984) required subjects to exercise to exhaustion at 75% of $\dot{V}O_2$max. Subject's glycogen levels were similar in both fibre types at rest. After 40 minutes of exercise, a large proportion of type I and type IIa fibres were emptied of glycogen. The results from this study showed that all type I and type IIa fibres were recruited from the start of submaximal exercise at 75% $\dot{V}O_2$max. This result is in agreement with an earlier study conducted by Andersen & Sjogaard (1976) in which glycogen depletion occurred in type I, IIa and IIb fibres during 14 minutes of exercise at a higher exercise intensity.

In summary, high-intensity exercise results in the recruitment of type II muscle fibres which results in greater muscle glycogen depletion from those highly glycolytic fibres. On the other hand, lower intensity exercise that can be endured for prolonged periods recruit type I fibres (Gollnick *et al.*, 1973a,Gollnick *et al.*, 1973b,Piehl *et al.*, 1974,Greenhaff *et al.*, 1994). These two concepts need to be applied in the glycogen depletion bout for the proposed Masters study as the degree of depleted muscle glycogen is imperative before carbohydrate feeding. This is also important as participants in the study are not endurance trained athletes and therefore will not be able to follow traditional depletion methodologies such as cycling for two hours.

Glycogen breakdown

Glycogen stores in the liver and kidneys are broken down to release glucose into the blood by an enzyme called glycogen phosphorylase. The glycogen chain which is broken down is released as glucose-1-phosphate (G-1-P) and the associated glycogen chain that was attached to it. G-1-P is then converted to glucose-6-phosphate (G-6-P) by phosphoglucomutase. G-6-P

is then hydrolysed to release a glucose molecule into the blood. Glucose in the muscle cell is phosphorylated to glucose-6-phosphate before undergoing glycolysis in the muscle in order for a glucose molecule to be broken down (Elliott & Elliott, 2005). A summary of these processes can be seen in the figure 2 below.

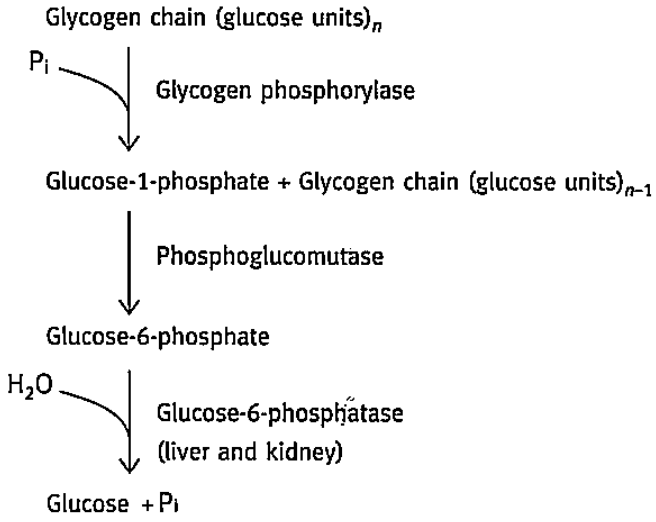

Figure 2. Breakdown of glycogen by glycogenolysis (Elliott & Elliott, 2005)**.**

The control of glycogen phosphorylase is regulated by reversible phosphorylation and allosteric effectors (Johnson, 1992). Glycogen phosphorylase is regulated by AMP and by Ca^{2+} release from the sarcoplasmic reticulum at the contractile level.

The breakdown of glycogen is an important process as it regenerates ATP, the energy source which allows for muscle contraction. ATP has to be regenerated as it is hydrolysed during a muscle contraction to ADP (adenosine diphosphate). In normal muscle contraction (non-panic), the chemical signal to activate glycogen phosphorylase activity is AMP which acts as an allosteric activator. An increase of AMP concentrations will increase ATP synthesis from the increase activity of glycogen phosphorylase. On the other hand, ATP and G-6-P will allosterically inhibit glycogen phosphorylase which reduces the breakdown of glycogen. The release of the calcium (Ca^{2+}) ion into the cytoplasm also has a partial contribution to the activity of glycogen breakdown which triggers the contraction following a motor nerve signal (Poortmans, 2004,Elliott & Elliott, 2005).

Glycogen phosphorylase in the muscle is bound within a glycogen-protein complex. This complex also includes other glycogenolytic enzymes such as phopshorylase kinase and

protein kinase (Entman *et al.*, 1980). The activity of glycogen phosphorylase a is closely related to the concentration of Ca^{2+} that is released from the sarcoplasmic reticulum. When muscle contraction occurs, the conversion of phosphorylase b to a is initiated as a result of the activation of phosphorylase kinase. Phosphorylase kinase is activated following Ca^{2+} binding to its calmodulin subunit, resulting from an increased concentration of Ca^{2+} which is released from the sarcoplasmic reticulum during the excitation-contraction coupling (Cohen *et al.*, 1980).

At the hormonal level, glycogen phosphorylase is activated by glucagon and epinephrine in the liver, and by epinephrine only in the muscle (Stallknecht *et al.*, 1998). In an emergency situation (flight-or-fight response), the conversion of phosphorylase b to a is most active during the absence of AMP. Following the binding of epinephrine to the hormone-receptor in the muscle, this activates adenylate cyclase to convert ATP to cAMP. cAMP activates protein kinase A (PKA). The activation of PKA activates phosphorylase b kinase via phosphorylation, which goes on to phosphorylate phosphorylase b to phosphorylase a. A summary of this conversion can be seen in the figure below (Elliott & Elliott, 2005).

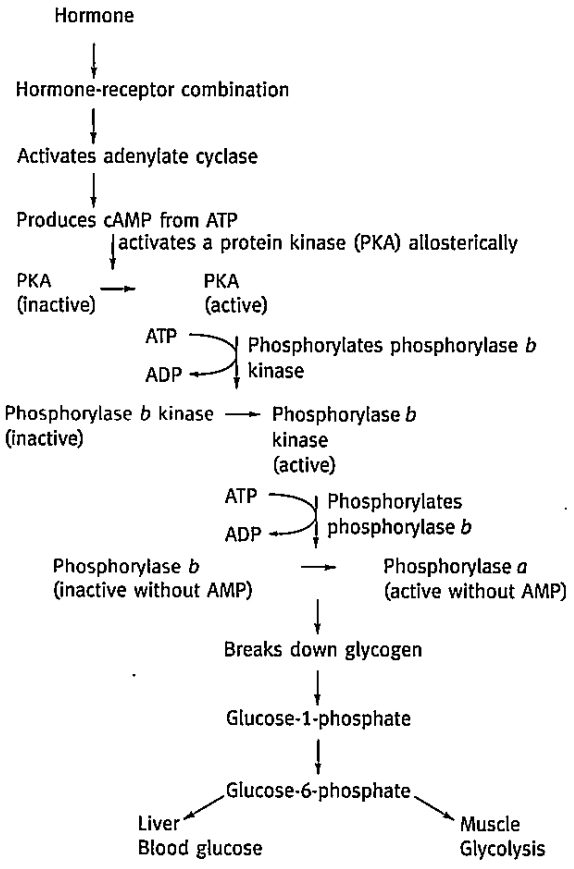

Figure 3. Mechanism to the activation of glycogen phosphorylase (Elliott & Elliott, 2005).

Muscle glycogen synthesis

Restoration of muscle glycogen following endurance exercise is very slow unless a carbohydrate-rich food is provided. However, when an adequate amount of carbohydrate is consumed by an athlete immediately after exercise and at two-hour intervals, the rate of muscle glycogen is rapidly increased and maintained for up to six hours following exercise (Ivy *et al.*, 1988,Reed *et al.*, 1989). It is well known that the rate of glycogen storage is consistent for the first couple hours following termination of prolonged exhaustive exercise. However, no added benefit has been found rate of glycogen storage by increasing the amount of carbohydrate consumption in the 6 hours following exercise (Ivy *et al.*, 1988). This highlights the timing importance of carbohydrate intake for glycogen resynthesis is at its highest and most effective rate following exhaustive exercise.

The rate of glycogen synthesis in human muscle fibres at a depleted state is well related to the activity of the enzyme, glycogen synthetase (Bergstrom *et al.*, 1972). From the results through periodic acid-Schiff (PAS) staining, the author found that resynthesis of glycogen began almost immediately after exercise-induced glycogen depletion. Furthermore, it was suggested that the entire initial feedings of various amount of carbohydrate was converted to muscle glycogen. This increase of initial glycogen immediately following exercise can be attributed to the activity of glycogen synthase (glycogen synthetase; interchangeably used) (Bergström *et al.*, 1967). In addition to the immediate resynthesis of glycogen following exercise-induced glycogen depletion, it has been reported that faster glycogen resynthesis occurs in type II fibres due to the greater glycogen synthase activity in type II fibres (Blanksby *et al.*, 1973,Piehl, 1974)

Glycogen synthase is the enzyme that catalyses the transfer of the glucose moiety of Uridine Diphosphate-glucose (UDPG) to the outer chains of glycogen, resulting in the enlargement of the glycogen molecule (Taylor *et al.*, 1972).

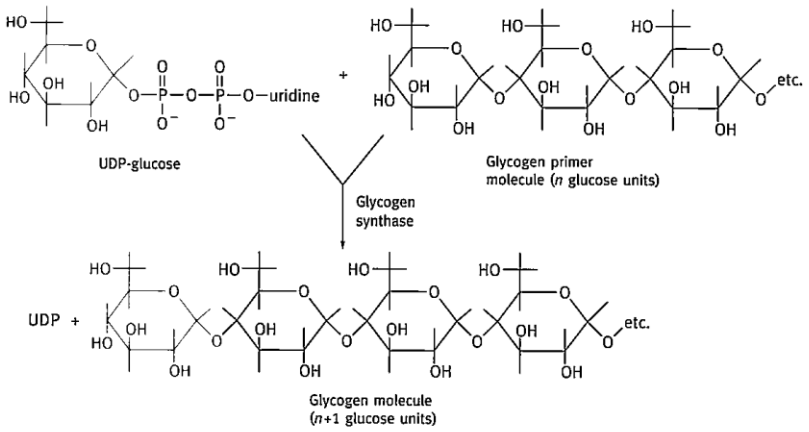

Figure 4. Glycogen synthesis via elongation of glycogen primer molecule using UDPG as a glucosyl donor (Elliott & Elliott, 2005).

Glycogen synthase exists in two interconvertible enzymatic forms; glycogen synthase I, is the non-phosphorylated form of the enzyme which is normoally active. I stands for independent, which is independent of G-6-P. The other form, glycogen synthase D is the phosphorylated form. It is opposite of glycogen synthase I whereby it is normally active, but can be activated in the presence of G-6-P, as it is dependent on G-6-P.

Glycogen synthase D is converted to glycogen synthase I when it is dephosphorylated by protein phosphatase. The conversion is reversed when glycogen synthase I is phosphorylated by either phosphorylase kinase or PKA. Since glycogen synthase D is dependent on G-6-P, an increased concentration of G-6-P will therefore induce glycogen synthesis. Furthermore, an increase concentration of insulin will further activate protein phosphatase resulting in activating of glycogen synthase (Houston, 1995,Elliott & Elliott, 2005).

The activity of this enzyme is inhibited when glycogen levels are high. However, when the glycogen level is reduced as a result of exercise, the inhibitory effect of the enzyme declines resulting in an increase in the activity of the I form of glycogen synthase (Bergstrom *et al.*, 1972,Taylor *et al.*, 1972,Piehl, 1974). Piehl, 1974 also suggested from the study that glycogen resynthesis was equally as a fast, if not faster in FT fibres, which were not as glycogen depleted after exercise as ST fibres were. Further investigation is needed to confirm the higher glycogen synthase activity in FT fibres as compared to ST fibres. As mentioned briefly earlier, a relationship between glycogen content and the percentage of the activity of

the I form exists. This is an inverse relationship between glycogen content and synthase I activity at a low glycogen level. Furthermore, there was no increase in synthetase I activity when the glycogen levels were above the normal mean value at rest (Bergstrom *et al.*, 1972).

Following exhaustive exercise, it has been shown that consuming between 525 to 648g of carbohydrate during the first 24 hours after running will result in the increase of muscle glycogen and return muscle glycogen values to its pre-exercise values (Costill *et al.*, 1981). Burke *et al.* 1995 suggested that the dietary guideline for optimal daily glycogen synthesis to be between 8 to 10g of absolute carbohydrate intake per kg per day. However in more recent literature, Burke and co-authors have suggested that carbohydrate intake for fuel needs for athlete for moderate exercise should be 5 to 7$g \cdot kg^{-1} \cdot day^{-1}$ (Burke, 2010).

In terms of percentage of total daily energy intake that should be derived from carbohydrate, this is calculated to be around 65 to 70% of energy intake. The study by Burke *et al.* (1995) found that there was no further increase in muscle glycogen levels when compared between a control diet (7 $g \cdot kg^{-1} \cdot day^{-1}$ of carbohydrate) and a matched energy diet (11.8 $g \cdot kg^{-1} \cdot day^{-1}$of carbohydrate). Therefore this result supports the previous statement for optimal carbohydrate intake exists between 8 to 10 $g \cdot kg^{-1} \cdot day^{-1}$. However, this is in disagreement with Acheson et al. (1988) who suggested that the glycogen storage capacity in man is around 15 $g \cdot kg^{-1} \cdot day^{-1}$. However, methodological differences may attribute to the disagreement between these recommendations.

Summary

Muscle glycogen can be depleted following a previous bout of exhaustive exercise. The exercise protocol completed plays a role in the proportion of type I or II fibres that are depleted. Prolonged exercise at 65% $\dot{V}O_2$max have been found to be effective in depleting muscle glycogen. Initial muscle glycogen depletion occurred in type I fibres, followed by the eventual recruitment and sequential glycogen depletion in type II fibres in order to maintain the ability to develop tension. However, it was later discovered that exercise intensity is a determinant of the muscle fibres that are recruited. High intensity exercise results in the recruitment of type II fibres which are more glycolytic leading to glycogen depletion of those fibres. Two enzymes in the breakdown and synthesis of glycogen are glycen phosphorylase and glycogen synthetase. Glycogen phosphorylase is activated by AMP, and inhibited by ATP and G-6-P. On the other hand, the active I form of glycogen synthetase is present in the

absence of G-6-P. An inverse relationship between glycogen content and the activity of the I form exists, whereby the lower the glycogen concentration, the higher the activity of the I form which will stimulate glycogen synthesis.

Squash background

Squash metabolism

Since squash is a high-intensity, intermittent sport which can last up to several hours (Sharp *et al.*, 1998), both (aerobic) endurance and anaerobic capacity are important to be able to perform well at a competitive level (Girard *et al.*, 2005).

Match analyses, though rare, have found that players covered a mean distance of only 12m during rallies lasting between 16 to 21 seconds and have reported post-match blood lactate concentration of 8 mmol·L^{-1} (Mercier *et al.*, 1987).

Chin *et al.* (1995) has previously compared the intensity of squash through mean heart rates between squash players from different parts of the world. The author found that the mean heart rate of 168 beats per minute (bpm) or 89.8 % heart rate max (HR_{max}) of the participants was similar to physically fit players and players from South Africa (Van Rensburg *et al.*, 1982) and Australia (Pyke *et al.*, 1974). The maximum lactate value observed during the treadmill and squash-specific test were 11.0 mmol·L^{-1} and 10.8 mmol·L^{-1}, respectively. This shows that the maximal intensity from the squash-test closely replicates the intensity of maximal steady state rhythmic exercise such as treadmill running (Candau *et al.*, 1998).

The use of a portable gas analyser is currently the most effective solution for assessing metabolic changes during game sports such as squash and has previously been used by Todd *et al.* (1998) and Girard *et al.* (2007) to observe the metabolic demands of the game. Nevertheless, there has been limited previous research on the physiological requirements or changes that occur in a squash match. Available information indicates that HR_{max} in squash competitions was similar to the age-related HR_{max}. A recent review of racket sports by Lees, (2003) found that the mean heart rate often exceeded 75% of the HR_{max}. On-court oxygen uptake has also been estimated from the heart rate recorded during matches from players of similar standards to each other (Van Rensburg *et al.*, 1982). However, this method of

estimating oxygen uptake is inaccurate because on-court heart rate can be influenced by factors such as ambient temperature, humidity, stress of competition or use of the arms (Girard *et al.*, 2007). For these reasons, this method has also been known to overestimate oxygen uptake during high-intensity intermittent exercise (Ballor & Volovsek, 1992).

Exercise protocols

Squash is characterized by movements incorporating frequent bursts of rapid acceleration and deceleration over short distances in a range of directions; involving frequent turning, lunging and side-stepping with regular short recovery periods (Wilkinson *et al.*, 2009b).

It has been previously suggested that competitive squash consists of high-intensity, intermittent activity lasting between 10 to 20 second with a work to rest ratio of 1:1. Previous match-play analysis has observed a mean rally time of 13.6 seconds with 49% of rallies lasting 10 seconds or less and 80% lasting less than 20 seconds (Montpetit, 1990). Meanwhile other authors have suggested that the mean duration of a rally is between 5 and 20 seconds with rest periods of between 7 and 8 seconds (Sharp *et al.*, 1998). This can be confirmed from the results of Girard *et al.* (2007) who found that rally durations lasted less than 21 seconds and greater than 21 seconds were 67% and 33%, respectively. This was also in support of previous findings indicating that 80% of rallies lasted less than 20 seconds and 49% of rallies lasted for 10 seconds or less in professional and A-grade players (Montpetit, 1990).

It is, of course, very difficult to make measurements, particularly of physical performance, during competition, so a simulated match model is necessary. One of the difficulties in collating the small amount of scientific information regarding squash relates to the different simulated model used in different studies. A standard exercise protocol has not been agreed upon as the nature of the sport results in the difficulty in developing a protocol that can account for reaction time, skill and/or match fitness.

The use of on-court sprints such as ‘court-ghosting’ is commonly used during training as it involves multiple direction changes, reflecting the high frequency of change-of-direction that occurs during a match (Sherman *et al.*, 2004).

A variety of squash-specific exercise protocols have been used in the last couple of decades for research purposes. These range from an incremental treadmill test, to an on-court ghosting

protocol to the more specific squash-simulation matches (Steininger & Wodick, 1987,Romer *et al.*, 2001,Girard *et al.*, 2005,Bottoms *et al.*, 2006,Girard *et al.*, 2007).

One of the first on-court squash protocol was developed by Steininger & Wodick in 1987. This procedure was devised to mimic the physiological demands and techniques of squash movement. The authors arranged 3 lamps on each side wall of the squash court; front corners, middle of the side-walls and back corners. These lamps were connected to a computer which was located outside the court. Each lamp had a squash ball mounted under each lamp. Different sequences of light flashes were selected according to the exercise intensity. Players were required to react and run from a central point of the squash court (the 'T') toward each of the balls as soon as the corresponding bulb was lit and to strike it in an appropriate manner. This was performed in 3-minute periods of exercise. There were 12 light pulses per minute initially with a total of 36 dashes at the first stage. The intensity was increased by 6 pulses (or dashes) per level until exhaustion was reached. The results from this study found that the test of validity of the protocol was sufficient to estimate the level of fitness from measurements during exercise of a protocol closely replicating squash.

However, the limitation of this protocol was that the intensity and duration are unlike that of squash match-play. This is due to the incremental maximal test which does not occur during a squash match. However, the outcome of an incremental squash-test as such may be useful to players and coaches as a quantification of squash-specific endurance.

Examples of on-court ghosting being used as an exercise protocol have been previously incorporated by Romer *et al.*(2001) and Girard *et al.* (2005). Romer and co-authors' ghosting routine consisted of 10 sets of 2 repetitions of ghosting. Each repetition required participants to simulate positional shots at 6 positions during a game of squash; front left and right corner, back left and right corner, right-forehand volley and a left-forehand volley. The end of each set was met with 30 seconds of passive recovery.

The protocol used by Girard *et al.* 2005 consisted of two incremental protocols; a treadmill test and a squash graded test. The treadmill test initiated at 10 $km \cdot h^{-1}$ followed by increases of 1 $km \cdot h^{-1}$ every two minutes until voluntary exhaustion. The squash test consisted of two bouts of nine shuttle runs (a stage). This was used as it simulated the repeated nature of multi-directional displacement as seen in a game of squash. The nine displacements included two forward, three lateral, and four backward courses, performed randomly. A 10-second rest

period was taken between each stage and a 30-second rest between the end of stage three and the beginning of stage four (end of warm up period).

Another study from which an on-court squash protocol was used was completed by Bottoms *et al.* (2006). The authors investigated the effects of carbohydrate ingestion on skill maintenance during a squash-specific test. The exercise protocol consisted of on-court shuttle running for 20 minutes, running between the front and back wall of the squash court along with the tempo of a metronome. Following the shuttle run, subjects performed three, three-minute intervals of maximal effort court-ghosting to all corners of the court with a one-minute rest interval.

A recent investigation by Kingsley *et al.* (2006) developed and used two on-court squash simulation protocols that closely replicated the physiological demands of elite junior squash. The multistage squash test required players to complete simulated rallies that lasted for one minute. During each subsequent stage, the time allowed to reach each court position was reduced progressively. Stages were separated by seven seconds of recovery. The participants completed the test until voluntary exhaustion. The second squash test; the squash simulation protocol (SSP) consisted of four simulated rallies of varying length (between six to eleven shots performed by the participant) and movement patterns. These factors were used to replicate typical rallies as obtained from analysis of elite male squash. This simulation protocol also had the same seven-second recovery period. The squash simulation protocol lasted 12.2 minutes in which 24 rallies were simulated; resulting in a total of 186 simulated shots.

The results from the protocol by Kingsley *et al.* (2006) found no differences between the length of play, physiological responses or RPE during intense match-play and the SSP which highlights the similarities and replicability of the SSP to a normal squash match. This supports the reason why many other researches have used squash simulation as an exercise protocol. These recent studies have shown that on-court squash specific tests have become more popular in squash-related research and that the use of on-court ghosting is repeatedly used as it closely simulates the multi-directional movement patterns as seen in squash.

A more recent paper by Girard *et al.* (2007) took squash-testing to a new level by requesting subjects to play three squash games in order to simulate the physiological responses from an actual match. Here, participants carried a portable gas analyser whilst playing 3 competitive squash games against a player of similar standard. During each game $\dot{V}O_2$, heart rate, RER,

breathing frequency and expired volume were measure in five-second averages. There was a two-minute rest period in between each game. These games were performed according to the normal 'point-per-rally' scoring method. Since this protocol could not be controlled and was mainly descriptive, the focus of the protocol was on collecting physiological and metabolic data using the portable gas analyser during the three-game match simulation.

Creating a squash-specific exercise protocol provides a unique challenge to exercise physiologists as the test would have to simulate a squash match as accurately as possible in order to examine squash performance. This difficulty arises as the game intensity and the patterns of activity are difficult to quantify and replicate precisely. As a result, the protocol employed in this thesis had to be developed in order to evaluate the effect of constantly changing physiological demands along with the need to replicate the stochastic nature of squash movements. The neuromuscular ability to rapidly accelerate and decelerate is a crucial performance marker in squash but it is likely to go undetected by a test that uses predictable movement sequences; such as a fixed pattern to simulate a rally. Hence a protocol was developed with a randomly sequenced pattern of shots to simulate a rally in each set.

Squash and nutrition

Only a handful of studies have looked at a nutritional component on its effect on squash performance (Noakes *et al.*, 1982,Graydon *et al.*, 1998,Romer *et al.*, 2001,Bottoms *et al.*, 2006), a summary can be seen in Table 2 below. A study conducted by Noakes *et al.* (1982) investigated the metabolic response in squash following pre-exercise carbohydrate ingestion. Subjects were given either 250ml of tap water or the same volume of water with 67g of additional carbohydrates dissolved in the solvent. These drinks were consumed 25 minutes before a 90-minute squash match. Blood samples were collected following the warm-up period, at 15 minutes, 45 minutes and 90 minutes. The results found that the metabolic effect of 67g of carbohydrate ingestion 25 minutes before exercise had little effect. As expected, glucose, insulin and growth hormone levels were elevated for at most, the first 15 minutes of the game. This result is likely to be explained by the rise in insulin levels leading to hypoglycaemia. This is in line with previous work by Costill *et al.* (1977) who found a fall in blood glucose levels following ingestion of 75g of carbohydrates 45 minutes before a treadmill run.

One of the first studies looking at carbohydrate ingestion on the skill aspect of squash was conducted in 1988 by Graydon and co-authors. Eight subjects were given a glucose polymer at a dose of 8g per 100ml per kg of body weight diluted in a solution. The protocol of the trial was to complete three simulated games of squash whilst consuming half of the solution before the first game and a quarter after each of game 1 and 2. The accuracy test was conducted with a feeder and required participants to move from the 'T' (centre of the court) to hit a straight forehand drive into the back corner of the court. The scores given followed a marking scheme in which higher points will be awarded if the ball landed close to the back corner of the court. The results of the study found that there was a trial and time interaction effect ($p < 0.01$) and also a significant main effect of the trial ($p < 0.001$). This indicated that the participants were able to maintain accuracy of performance in the carbohydrate feeding trial but not in the placebo trial.

Another nutritional study was conducted by Romer *et al.* (2001) who looked at the effects of creatine supplementation on simulated positional play. Participants were required to perform an on-court ghosting routine which was intended to simulate shots played during a match. Participants were supplemented four times a day over five days. The ghosting routine has been previously explained in the previous section summarising the various squash protocols that have been previously used. The authors found that there was an improvement in the mean sprint time in the experimental group of 3.2%. The results also showed that there was a significant improvement in time between sets 2 and 10 following creatine supplementation compared to the placebo condition. This is the only study investigating the effects of creatine supplementation on squash which has found a beneficial effect on squash performance.

A more recent study by Bottoms *et al.* (2006) have repeated the original work from Graydon *et al.* (1988), with the exception of using a valid skill test which has been assessed in previous pilot work. The study used a double-blind cross-over design. Participants were provided with two beverages (total volume of 1 litre) which were consumed over the duration of the protocol. The carbohydrate-containing drink was made up to a 6.4% carbohydrate-containing beverage with a suitable placebo drink. Participants completed several tests which included a visual and auditory reaction test, maximal voluntary contraction fatigue test, shuttle running, on-court ghosting and a squash skill test. The results from the study found that skill declines following exercise in an absence of carbohydrate supplementation, while some evidence of skill maintenance is present following carbohydrate ingestion.

It is clear that apart from investigating the effect of carbohydrate and fluid supplementation during exercise, there is a dearth of good scientific research concerning the importance of diet on squash performance. Given the reliance upon carbohydrate, and in particular muscle glycogen as fuel for high intensity intermittent exercise, there is scope to investigate how dietary carbohydrate content prior to a match may be manipulated to optimize performance.

Unfortunately, available literature on nutritional studies on squash is not as widely available as tennis. A recent review (Hornery *et al.*, 2007) has identified several tennis studies that have included a carbohydrate intervention and performance measures on various tests (ground stroke accuracy, the Sargent vertical jump test, sprint test etc.) looking into ways to mitigate a performance decrement in tennis. Some of the studies mentioned have found a performance benefit following the consumption of carbohydrates which increases the plausibility of researching a carbohydrate intervention in squash. Though previous carbohydrate-related studies used in squash have only investigated changes in skill and sprint outcomes, a match simulation following a nutritional strategy in the days leading to an event is yet to be investigated. Therefore the intended study in this thesis will be one of few, if not the only 'carbohydrate-loading' study available in squash literature.

Summary

Although receiving some recent scientific attention, the science of squash is limited compared to other sports such as cycling, soccer or tennis. Due to the difficulty in investigating exercise performance in squash, earlier studies consisted of match analysis and match observation only. However, advances in technology have allowed for more targeted research to be conducted in order to assess the physiological and metabolic demands experienced in simulated competition. This was particularly aided by the introduction of portable gas analysis and timing devices which can be worn and store data. The use of more specific exercise protocols has also led to higher quality research which increases face-validity of the sport; from an incremental speed test to the more realistic squash match simulations. Nevertheless, there is a gap in the literature concerning what is optimal nutritional practice for squash competition, especially in relation to pre-competition diet.

Study	Participants	Protocol	Intervention	Measurements	Outcome of the study
Noakes *et al.* (1982)	6 experienced squash players	Drinks consumed 25 min before the start of a 90-min match, on two separate occasions.	250mls water vs 250mls solution containing 67g of CHO.	3 x 10ml venous blood samples: before, 15 and 45 min into the match.	Result: mild hyperglycaemia, ↑ GH and blood lactate concentrations which plateaued after 45 minutes. Progressive ↑ free fatty acid concentrations.
Graydon *et al.* (1988)	8 club standard male players	Drinks consumed 30 min prior to simulated match before game 1, and after games 1 and 2.	8g/100ml/kg of CHO vs placebo sugar-free drink	Rating of perceived exertion (RPE), heart rate (HR) and accuracy test were measured.	19% ↓ in performance accuracy in placebo. No change in performance accuracy in CHO trial. Non-significant ↑ in HR was seen as the number of games ↑. CHO may have attenuated ↑ in RPE.
Romer *et al*. (2001)	9 competitive squash players	Subjects performed on-court ghosting routine involving 10 sets of 2 repetitions of simulated positional play.	Creatine vs control group supplemented four times daily for five days with 0.075g/kg of creatine monohydrate and maltodextrin, respectively.	HR, lactate and performance sprint times were measured.	The experimental (creatine) group improved mean set sprint time by 3.2% (0.8) when compared to the control group, whilst an improvement of 4.7% (0.3) was evident for all 10 sprints in the creatine group in comparison to control group
Bottoms *et al.* (2006)	16 squash players	A 2-hour fast completed before attending test. 1L beverage consumed over the duration of the protocol.	2 beverages; orange-flavoured CHO-containing (6.4%) beverage and placebo.	2 trials measuring pre- and post-exercise performance. Completed reaction time (RT) test, wrist flexion maximal voluntary contractions, squash skills test and on-court ghosting.	CHO ingestion no main effect on total skill scores. ↓ 9% ↓ total score pre- to post-exercise in placebo, and 1% ↑ following CHO. Improved in RT in CHO trial. A significant difference in blood glucose was also seen between trials, but blood lactate response during trials was not significant.

Table 2. Studies that have included nutritional interventions in relation to squash performance.

Summary and Rationale

Carbohydrate supplementation and its effect on endurance performance has been a well-researched topic. Recent studies have started to further investigate the influence of carbohydrate supplementation in high-intensity intermittent exercise (Bangsbo *et al.*, 1992,Bussau *et al.*, 2002,Fairchild *et al.*, 2002). This has taken step forward as a couple of studies have now looked at carbohydrate supplementation on a specific sport such as seen in a soccer simulation test (Bangsbo *et al.*, 1992,Ali & Williams, 2009).

Literature on squash is limited and is not as widely available as other mainstream sports such as soccer and tennis. Previous research has looked at physiological profiles of squash players from different countries and age groups (Van Rensburg *et al.*, 1982,Chin *et al.*, 1995,Kingsley *et al.*, 2006). The metabolic and physiological responses in squash is also another area which has had similar attention to the physiological profiles of squash players (Blanksby *et al.*, 1973,Garden *et al.*, 1986,Mercier *et al.*, 1987,Montpetit, 1990,Sharp *et al.*, 1998). In recent years, research output on squash has increased and more studies simulating squash match-play (Steininger & Wodick, 1987,Romer *et al.*, 2001,Girard *et al.*, 2005,Kingsley *et al.*, 2006,Girard *et al.*, 2007) have emerged which have led to the validation of these tests (Wilkinson *et al.*, 2009a,Wilkinson *et al.*, 2009b,Wilkinson *et al.*, 2009c).

Though there have been a couple of studies which have looked at the metabolic and physiological responses during a squash match simulation with the use of a portable gas-analyser (Todd *et al.*, 1998,Girard *et al.*, 2007), a nutritional strategy has not been investigated using this method. Previous studies testing ergogenic aids such as creatine and carbohydrate supplementation on skill and accuracy have been looked at, but the influence of carbohydrate feedings on a prolonged squash-match simulation have not been tested. Nevertheless, this presents a challenge for researchers such as myself to take and to investigate a 'field-test' which is never as straight-forward and cannot be as strictly controlled as a treadmill run simulating a marathon or a 40-km bicycle ride on a cycle ergometer.

The aim for the following Masters research project is to look at the effects of carbohydrate feeding following a bout of exercise-induced glycogen depletion during a 48-hour recovery period. The rationale of the study follows:

1. Develop a match simulation based on match data analysis that can closely replicate a real-life squash match to last for a prolonged period of time.
2. Test the efficacy and influence of a carbohydrate feeding strategy on squash performance.
3. To add to the current library of scientific research papers available on squash as a sport.
4. Have a better understanding of the metabolic and physiological responses that occur during a squash match since previous studies have only tested for short periods.

Hypotheses

1. The nutritional intervention will successfully show a difference in RER from an additional carbohydrate intake of 9 $g \cdot kg^{-1}$ body weight.
2. The results of the study will show a similar pattern in fuel utilisation following prolonged exercise, changing from high carbohydrate oxidation to an increase in fat oxidation.
3. A difference in performance time is expected to be seen, as previous research on carbohydrate feedings show a general improvement in performance.

Methods

Experimental Overview

Nine healthy and recreationally active male squash players at a Squash New Zealand grade of C1 or above (played at least three times a week and had been competing for at least five years) were recruited and completed a simulated squash match which lasted for ~60 minutes following 48 hours of a dietary intervention. Data from eight subjects is displayed as the ninth subject was not able to complete the second trial. The simulated squash match consisted of five sets lasting for ~12 minutes each. Participants were instructed to complete each set as quickly as possible and to treat each set as they would in a competitive match. The protocol developed was aimed to simulate the physiological responses during a squash match. Each subject completed a single-blind, cross-over design of two experimental trials that took place at the same time of day and were separated by at least 8 days. Performance and/or trial bias was avoided by use of a counter-balanced study design and by enforcing the same level of encouragement to subjects in order to perform with maximal effort.

Subjects

Nine healthy and recreationally active males agreed to participate in this study. The subjects' characteristics were (mean ± SD): age 24 ± 8 years, height 1.8 ± 0.1 m, weight 76.9 ± 6.8 kg. Each subject was fully informed of the experimental procedures and possible risks before giving informed, written, consent. The protocol was approved by the Institutional Human Ethics committee and performed according to the Declaration of Helsinki.

Preliminary Session

At least a week before the first experiment trial, subjects reported to the squash courts for a familiarisation session to the protocol of the experimental trial. The familiarisation session required subjects to complete a short run through of the simulated squash match. They ran through five lighted timing gates in a random order at different intensities to firstly, get used to the idea of the protocol and secondly, to familiarise themselves to the intensity of the protocol when exercising maximally. Subjects completed several rounds of the simulation until they were comfortable with how the protocol worked along with the lighting and audio cues that was incorporated with the timing gates. Subjects also completed the familiarisation

session with the portable gas system attached; which allowed subjects to get used to the extra weight and also to the feeling of running with the mask attached from the portable gas analyser.

Experimental Protocol

Glycogen depletion and dietary manipulation

Before each experimental trial, subjects completed a bout of strenuous exercise in order to reduce muscle glycogen. The glycogen depletion bout consisted of running the 'Yo-Yo Intermittent Recovery Level 1' (IR1) test to exhaustion to focus on depleting type II muscle fibres followed by a 30-minute treadmill run at 85% of heart rate max (HRmax; as calculated from the Yo-Yo IR1 test) to focus on depleting type I muscle fibres. Subjects also ran with a 2% incline on the treadmill. Upon completion of the depletion bout, subjects were assigned to either a high-carbohydrate (H-CHO; $11.1 g \cdot kg^{-1}$) or low-carbohydrate (L-CHO; $2.1 g \cdot kg^{-1}$) treatment. Subjects were provided with a standardised low-carbohydrate ($1.3 g \cdot kg^{-1}$) diet to follow over 48h. The energy intake for the standardised diet provided was identical for all subjects. More importantly, the high- or low-carbohydrate treatment was mixed in a 2.1L solution that was isocaloric within subjects. The high-carbohydrate drink was made up of maltodextrin with a dextrose equivalent of 10 units. The low-carbohydrate drink consisted of 200g milk powder (Pams whole milk powder, New Zealand**)** and 120g of protein powder (DNA Nutrition, New Zealand) in order to create a standard base of energy content, flavour and palatability. The difference in energy content between the high- and low-carbohydrate drink was calculated and then balanced with olive oil (Pams olive oil, New Zealand) for the low-carbohydrate drink. Since individual variability in dietary proportions exists, subjects were allowed to eat additional foods which only contained protein and/or fat in order to minimise dietary carbohydrate intake. This was repeated for the subsequent trial.

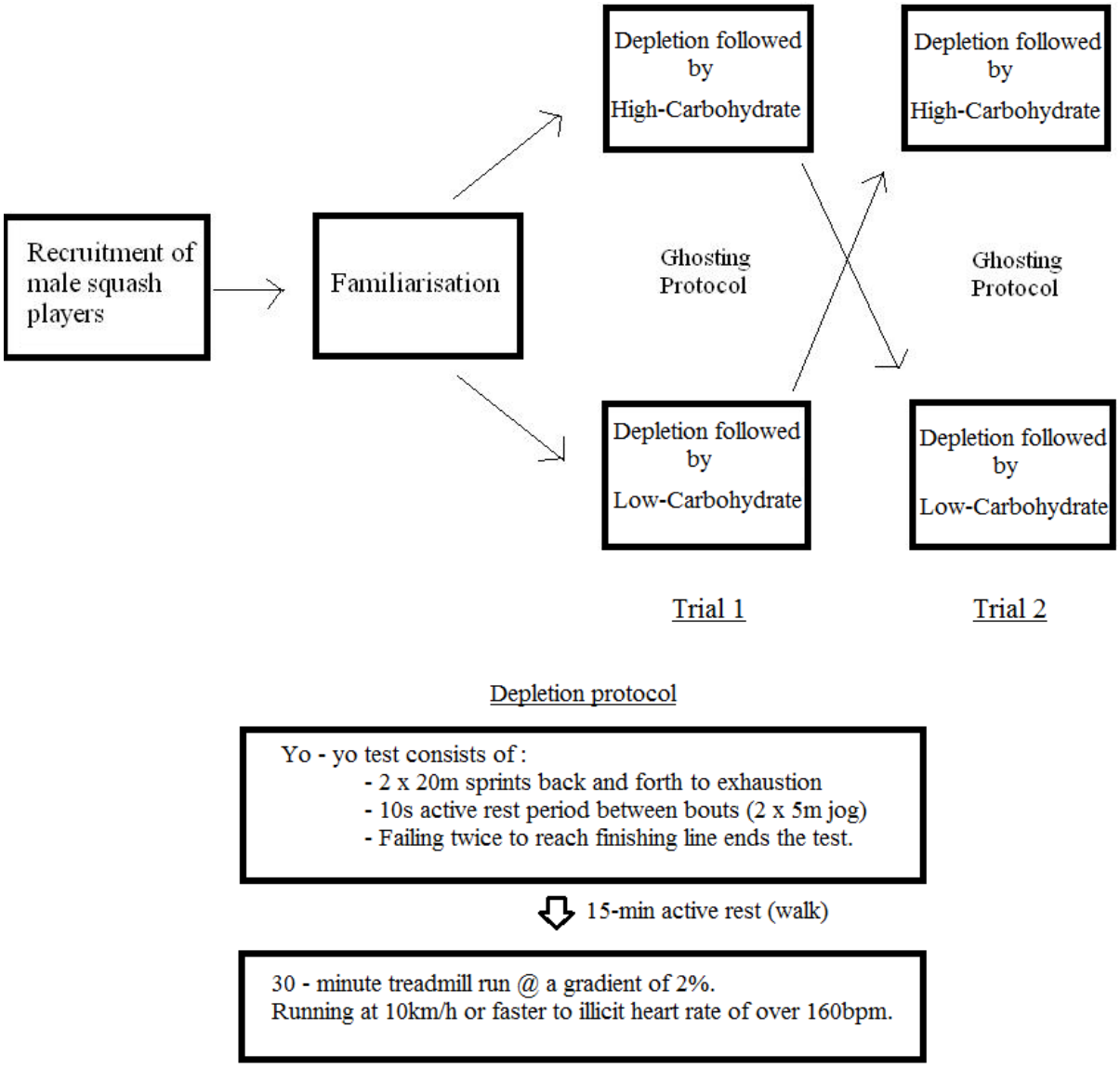

Figure 5. Overview of experimental procedures.

Simulated squash match

The protocol for the squash match was created to simulate a five-set match to last ~60 minutes. Five timing gates (Fusion Smartspeed, Australia) were used to simulate a home base (the 'T') and four shots at each corner of the court. Each set was designed to last for ~12 minutes each. The match was simulated by subjects starting from the centre of the squash court, also known as the 'T'. The first of five timing gates was located at the 'T' and served as the home (start and finish) gate to one of four other timing gates located in each corner of the squash court. Each 'rally' required subjects to run from the 'T' to and from the four gates

in a random pattern as fast as they could. This order of movement in the set had been randomly generated during the development of the protocol. Subjects used a squash racket to break the laser on the timing gate; in order to simulate a shot. Subjects completed a total of 30 rallies per set. The general outline of the set-up can be seen in the diagram below.

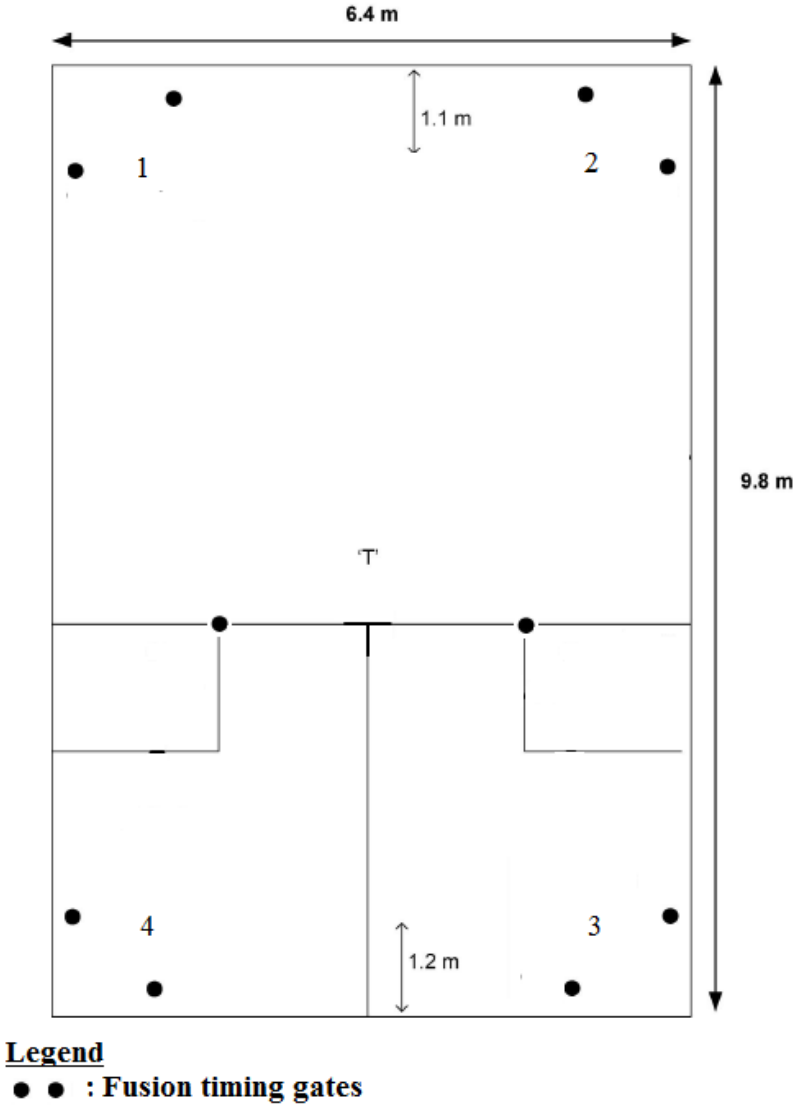

Figure 6. On-court schematic and position of Fusion timing gates.

It may be worth pointing out that the squash protocol used in this study was developed from new based on previous squash research in order to produce a modern protocol that can accurately measure performance and reaction time. This meant that protocol had to be created by creating a run sheet with the proportion of shots played in the front and back court considered along with the random distribution to the four gates (See Appendices). This was done so that the software engineers of the timing gates can then create a software code for the gate system to work in order to generate a simulated squash protocol. Since all subjects completed the same protocol, this meant that the total distance covered during the squash simulation protocol is identical.

The distribution of shots played in the protocol was derived from real match analysis where 74% of the match was found to be played from back corner movements and 26% from front corner movements. This allowed the match simulation to be as realistic as possible since top-level players tend to rally more at the back of court. Four gates were used as shot positions based from a previously validated squash-specific test (Wilkinson *et al.,* 2009a).

Several studies that have used squash simulation as an exercise protocol. Steininger & Wodick, (1987) conducted a simple but effective squash simulation protocol using audio and visual cues to simulate players running toward a 'shot' and striking the balloon with the corresponding bulb that was lit.

Kingsley *et al.* (2006) have recently found that there were no differences between the length of game play, physiological responses (heart rate and blood lactate concentrations) or perceived exertion recorded during intense match-play and a squash simulation protocol. Consistent patterns of players' movement during squash match-play has encouraged players to incorporate 'court-ghosting' as part of their training in order to improve in their squash-specific movements without hitting a ball. Since these movements are similar to that seen during match-play, simulating this movement over a period of ~60 minutes was used in order to create a controlled and consistent squash simulation protocol.

Furthermore, this type of protocol is more suitable as the incremental squash-specific test (Wilkinson *et al.,* 2009a) does not adequately simulate squash match-play as it is preferred as a protocol to quantify squash-specific endurance (Kingsley *et al.,* 2006). The rest period was chosen to be 10 seconds long as various studies (Girard *et al.,* 2005,Kingsley *et al.,* 2006,Wilkinson *et al.,* 2009a) have incorporated a rest period of between 7 to 16 seconds. Previous studies by Girard *et al.* (2007) and Montpetit, (1990) have reported that the mean

duration of a squash rally lasted between 16 and 21 seconds. Since the protocol of the current study looked at a prolonged (~60 minutes) period of squash simulation, the five-set protocol had to be designed to last around 12 minutes each. Therefore each 'rally' of four shots, should last around 15 seconds each with 10 seconds of rest in between each rally.

Experimental Procedures

Subjects were requested to refrain from exercise, caffeine and alcohol for at least 24 hours before the glycogen depletion bout. Subjects were also asked to keep a food diary from the day before the depletion bout until the simulated squash match (four days in total). After 48 hours of the dietary intervention subjects arrived at the squash courts to complete the simulated squash match.

The portable gas analyser (Cortex Metamax 3B, Germany) was turned on and calibrated at least 30 minutes before the arrival of subjects by calibrating for digital barometric pressure reader (Cortex, Germany), volume (Hans Rudolph 3L, USA), oxygen and carbon dioxide concentrations. Upon the arrival of subjects at the squash courts following the 48h recovery diet, subjects changed into the appropriate sporting attire following a nude body weight measurement. Subjects were then seated for five minutes before resting blood lactate (Lactate Pro, Arkray Inc, Kyoto, Japan) and glucose (Accu-Chek Advantage, USA) were collected via finger prick blood sampling method (Accu-Chek Safe-T-Pro Plus, Germany). Once collected, the portable gas analyser was attached to the subject and subjects then completed a five minute warm-up.

Upon commencement of the trial, continuous breath-by-breath recording with the portable gas analyser was active until completion of the trial. Time-point markers were entered to identify the beginning and ending of each set. At the end of each set, a rating of perceived exertion (RPE) was immediately recorded after subjects exited the court, followed by the collection of blood lactate and glucose. RPE was measured on the 15-grade Borg rating of perceived exertion scale; from 6 to 20 (Borg, 1970). Subjects were also given a standardised volume ($2ml \cdot kg^{-1}$) of water to drink between sets to maintain hydration status. At the end of the trial, final blood glucose and lactate samples were collected. Subjects then toweled off and measured their final nude body weight.

Measurements

The subjects' height and weight were measured using a stadiometer (Seca, Bonn, Germany; accurate to 0.1cm and scale (Jadever, Taiwan; accurate to 0.01kg). Heart rate was monitored using a Polar heart rate monitor (Polar T11, Polar Electro, Finland) which was wirelessly transmitted and recorded into the software included with the gas analyser (Metamax, Cortex, Germany). Expired gases were continuously collected for the duration of each experimental trial via the portable gas analyser. The expired gases were analysed for CO_2 and O_2 concentrations and volume, and values converted to STPD. Rates of O_2 uptake ($\dot{V}O_2$), CO_2 elimination ($\dot{V}CO_2$) and the respiratory exchange ratio (RER) were calculated.

Carbohydrate and fat oxidation was calculated using the algorithm developed by Frayn which ignores the concentration of nitrogen and the utilisation of protein (Frayn, 1983).

A summary of the protocol can be seen in Figure 7 below.

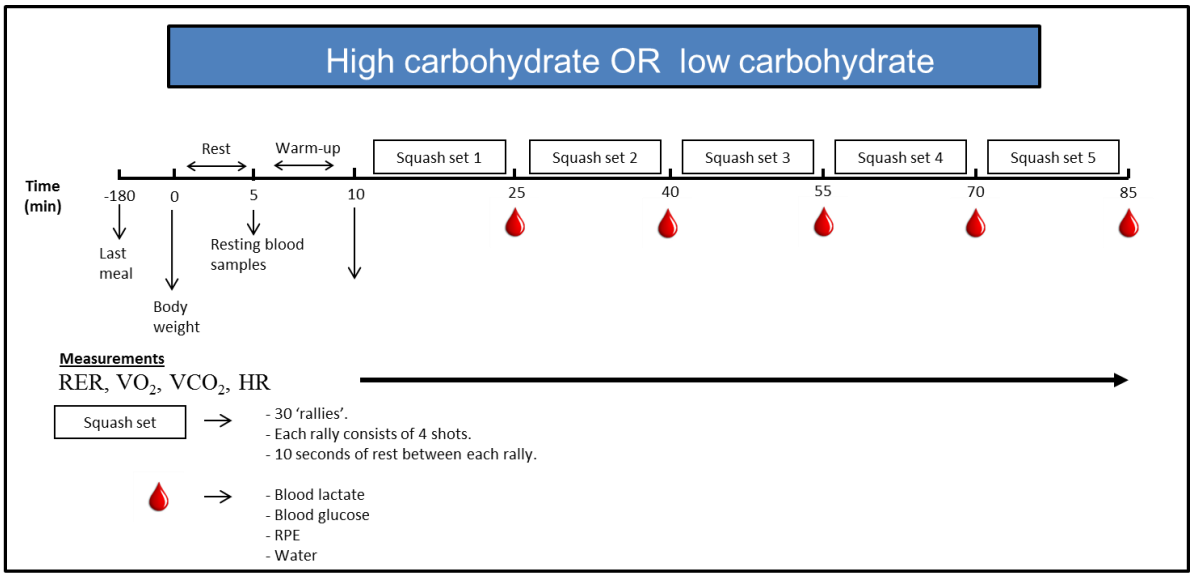

Figure 7. Time-line of events during each squash trial.

Statistical analysis

Dependent variables (RER, $\dot{V}$O2, $\dot{V}$CO2, RPE, carbohydrate and fat oxidation, heart rate, glucose, lactate and performance time) were analysed using a two-way (trial x time) repeated measures analysis of variance (ANOVA). Where a significant time effect was present, post-hoc analysis to determine specific differences was used using the Bonferroni correction.

A one-way ANOVA was completed for RER, carbohydrate oxidation and fat oxidation as they were found to have a significant trial effect. All data were assessed for sphericity and the Huynh-Feldt correction was used if necessary. All data were analysed using SPSS statistical software (V. 18, Chicago, IL, USA) with a priori statistical significance set at $p < 0.05$. All data are reported as mean ± standard error of mean (SEM).

Results

Figure 8 shows the respiratory exchange ratio (RER) over a period of five sets. There was a significant effect of time ($p < 0.001$), and trial ($p < 0.05$) such that RER decreased in both trials from 0.86 ± 0.02 to 0.76 ± 0.02 and 0.8 ± 0.01 to 0.75 ± 0.01 in the H-CHO and L-CHO trial, respectively. However there was no significant time by trial interaction seen ($p > 0.05$).

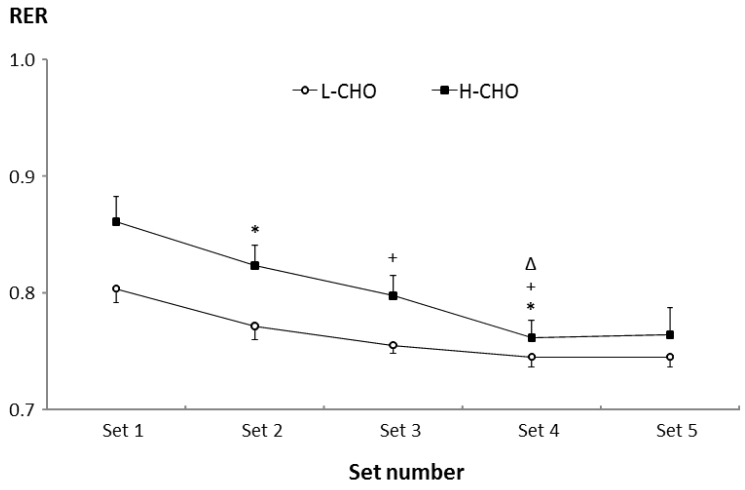

Figure 8. RER (mean ± SEM) during each trial. * denotes significantly different at the particular time point ($p < 0.05$), + denotes significantly different than set 1 ($p < 0.01$), Δ denotes significantly different than set 2 ($p < 0.05$).

Figure 9 shows the rate of carbohydrate oxidation over a period of five sets. There was a significant effect of trial ($p < 0.05$), and time ($p < 0.01$) such that oxidation rates decreased from 1.38 ± 0.21 to 0.40 ± 0.16 $g \cdot min^{-1}$ (L-CHO) and 2.32 ± 0.45 to 0.64 ± 0.39 $g \cdot min^{-1}$ (H-CHO). However there was no significant time by trial interaction seen ($p > 0.05$).

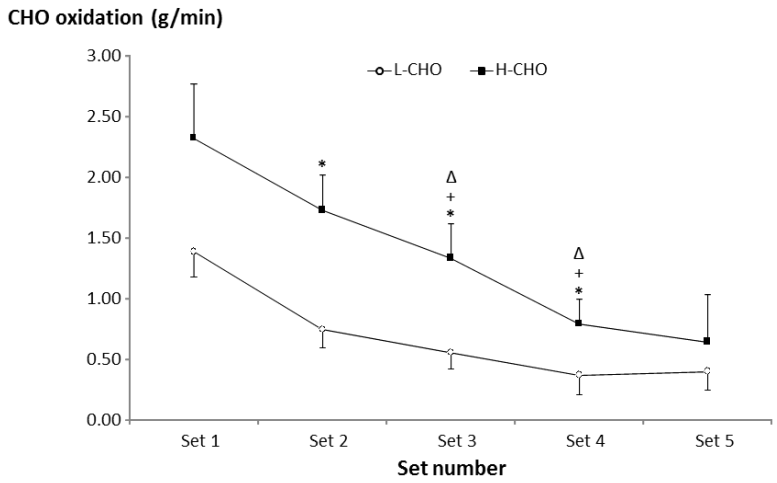

Figure 9. Rate of carbohydrate oxidation (mean ± SEM) during each trial. There was a significant main effect between trials ($p < 0.05$). * denotes significantly different at the particular time point ($p < 0.05$), + denotes significantly different than set 1 ($p < 0.05$), Δ denotes significantly different than set 2 ($p < 0.05$).

Figure 10 shows the rate of fat oxidation over a period of five sets. There was a significant effect of trial ($p < 0.05$) such that the rate of fat oxidation started at 1.05 ± 0.19 $g \cdot min^{-1}$ and increased to 1.68 ± 0.23 $g \cdot min^{-1}$ in the H-CHO trial, unlike the L-CHO trial which had a small increase in fat oxidation starting from 1.44 ± 0.15 $g \cdot min^{-1}$to 1.61± 0.13 $g \cdot min^{-1}$. There was neither a significant time effect nor a significant time x trial interaction seen ($p > 0.05$).

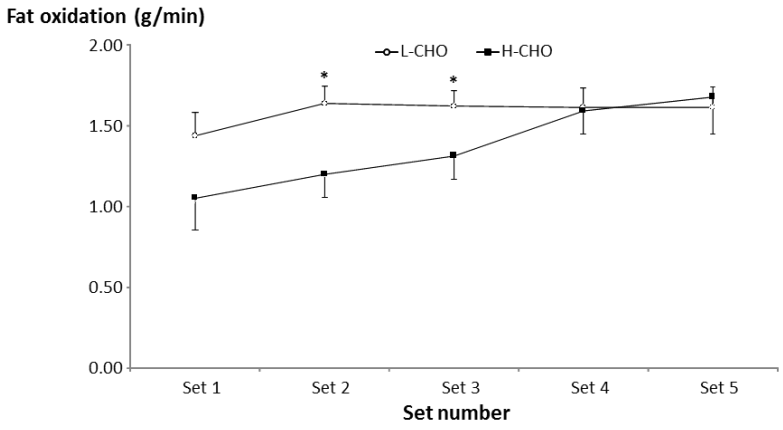

Figure 10. Rate of fat oxidation (mean ± SEM) during each trial. * denotes significantly different at the particular time point ($p < 0.05$).

Figure 11 shows the rate of oxygen uptake over a period of five sets. There was a significant effect of time ($p < 0.05$) such that oxygen uptake increased (H-CHO) and decreased (L-CHO) after the first 3 sets. However, there was neither a significant trial effect ($p > 0.05$) nor a time by trial interaction effect ($p > 0.05$). No significant differences in post-hoc between time points were found.

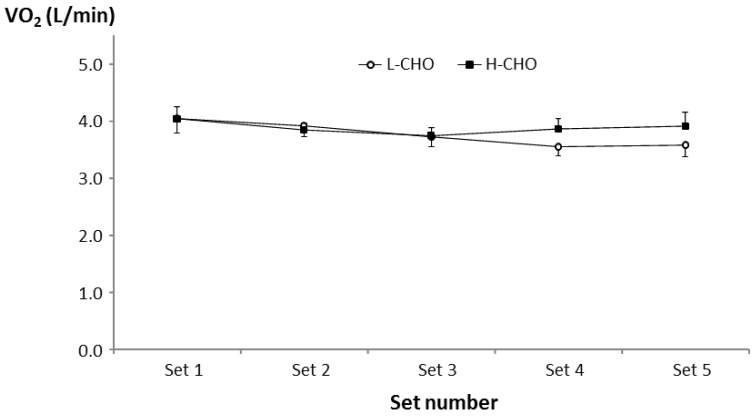

Figure 11. $\dot{V}O_2$ (mean ± SEM) during each trial.

Figure 12 shows the rate of Carbon Dioxide production over a period of five sets. There was a significant effect of time ($p < 0.001$) such that set 4 ($p < 0.05$) and set 5 ($p < 0.01$) were lower than set 1, and set 4 was similarly lower than set 2 ($p < 0.05$). This is displayed by the similar descending trend in both trials as time progressed. There was neither a significant trial effect ($p > 0.05$) nor a time by trial interaction effect ($p > 0.05$).

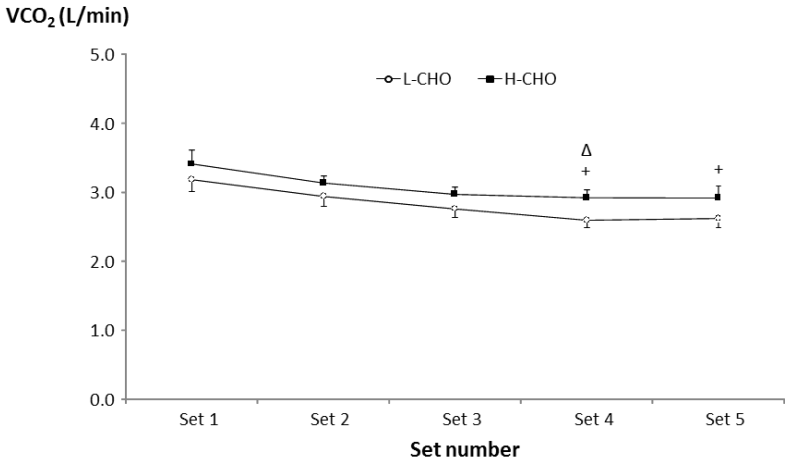

Figure 12. $\dot{V}CO_2$ (mean ± SEM) during each trial. + denotes significantly different than set 1 ($p < 0.05$), Δ denotes significantly different than set 2 ($p < 0.05$).

Figure 13 shows the changes in heart rate over a period of five sets. There were neither a significant effect of time ($p > 0.05$) nor trial ($p > 0.05$), and no significant effect of a time by trial interaction ($p > 0.05$).

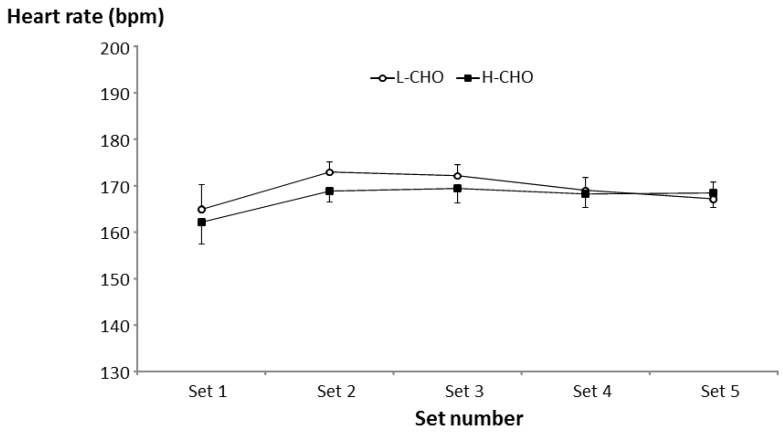

Figure 13. Heart rate (mean ± SEM) during each trial.

Figure 14 shows the changes in the concentration of glucose over a period of five sets. Following the end of the first set, there was a similar increase in glucose concentration following the rest period (5 $mmol \cdot L^{-1}$ ± 0.21 L-CHO; 5 $mmol \cdot L^{-1}$ ± 0.33 H-CHO) in both trials (7.3 $mmol \cdot L^{-1}$ ± 0.54 L-CHO; 7.6 $mmol \cdot L^{-1}$ ± 0.71 H-CHO). There was a significant effect of time ($p < 0.01$). However, there were no significant differences in the post-hoc analysis. There was neither a significant effect of trial ($p > 0.05$) nor a time by trial interaction ($p > 0.05$).

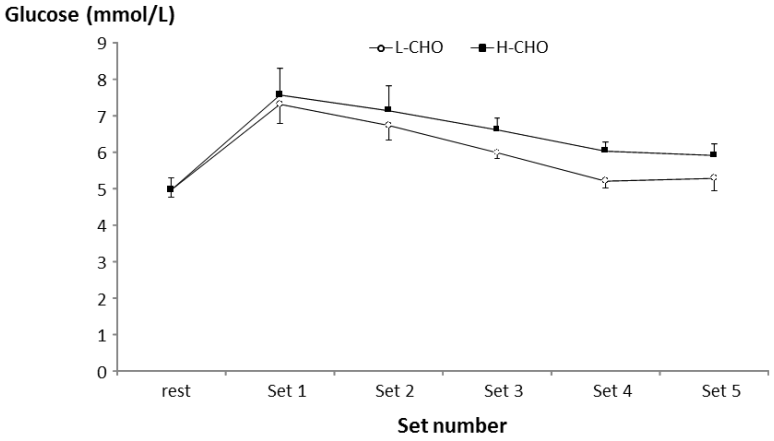

Figure 14. Glucose concentration (mean ± SEM) during each trial.

Figure 15 shows the changes in lactate concentration over a period of five sets. There was a significant effect of time ($p < 0.001$) such that lactate increased at rest from 1.9 ± 0.49 to 5.5 ± 0.92 mmol·L^{-1} and 2.0 ± 0.50 to 6.1 ± 0.63 mmol·L^{-1} at the end of the first set in L-CHO and H-CHO trial, respectively. Both trials followed a similar trend from rest where there was an initial increase in lactate concentration from rest. Lactate values then continued to drop until the 3rd set which was when a different pattern was seen. There was neither a significant effect of trial ($p > 0.05$) nor a time by trial interaction ($p > 0.05$).

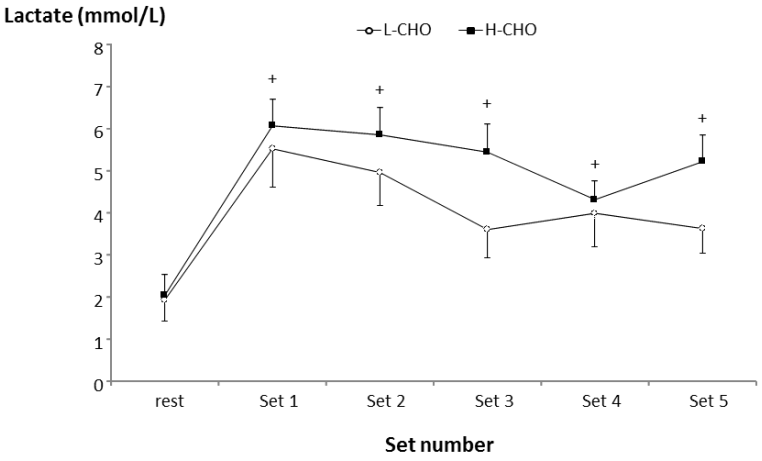

Figure 15. Lactate concentration (mean ± SEM) during each trial. + denotes significantly different than rest ($p < 0.05$).

Figure 16 shows the changes of performance time over a period of five sets. There was no significant effect of time ($p > 0.05$), trial ($p > 0.05$) or a time by trial interaction ($p > 0.05$).

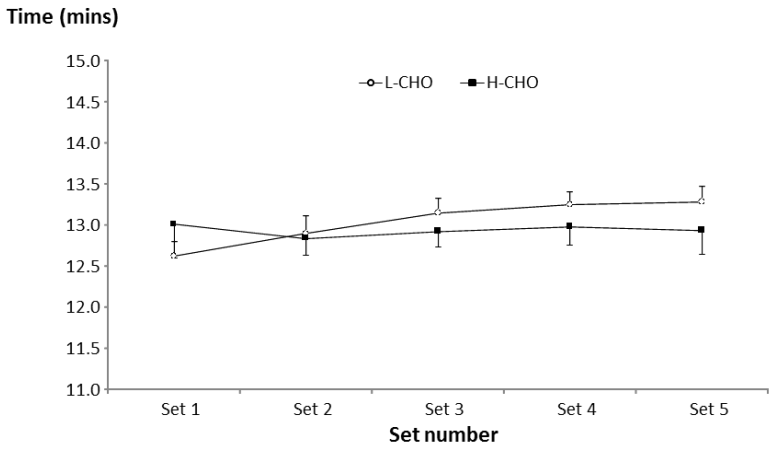

Figure 16. Performance time (mean ± SEM) during each trial.

Figure 17 shows the changes in RPE over a period of five sets. There was a significant effect of time ($p < 0.01$) such that RPE started and ended at the same point in both trials; 17 to 20.

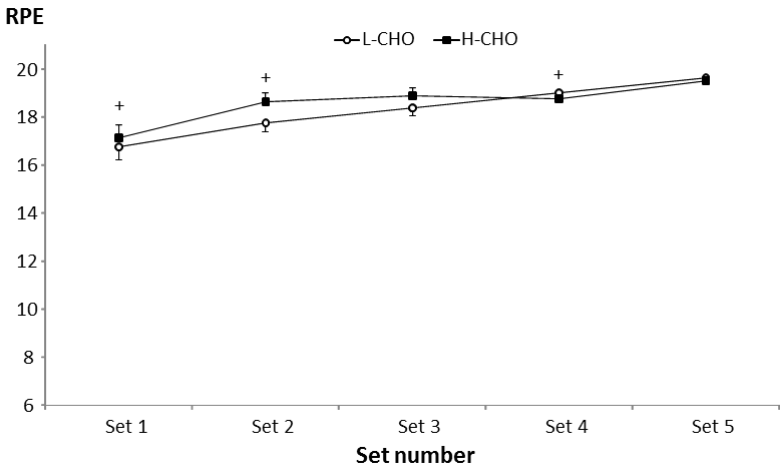

Figure 17. RPE (mean ± SEM) during each trial. + denotes significantly different than set 5 ($p < 0.05$).

Discussion

Interpretation of results

This study was undertaken primarily to test the hypotheses that ingestion of a high carbohydrate diet in the days leading to simulated squash performance will: 1) reveal an increase in carbohydrate utilization during exercise; 2) that the simulated squash performance, like other high intensity game sports, will show an increase in fat oxidation as the game progresses; and 3) that this will be associated with improved simulated performance.

In confirmation of the primary hypothesis, RER measurements indicate a significantly greater rate of carbohydrate utlilisation during the high carbohydrate trial. The data clearly show a difference in RER indicating that the preferred fuel utilised during the duration of the match-simulation was different between trials. This also confirms the success of the intervention. However, despite the success of the intervention, a significant improvement in performance time was not detected, though a strong trend towards improved performance in the carbohydrate trial can be seen in Figure 16.

Since both heart rate and RPE were not significantly different between trials, this indicated that subjects were exercising at the same intensity as required at all points in time. Heart rate results (average of 167 and 169 bpm in the low- and high-carbohydrate trial, respectively) are also comparable to previous work which expected heart rate to reach a minimum of 150 beats per minute during a squash match (Montpetit, 1990). RPE followed a similar pattern such that the same values were seen in the first and last set, and were different from each other.

Metabolic

The average RER values of the low-carbohydrate and high-carbohydrate trials were 0.76 and 0.80, respectively. The difference in fuel utilisation is most likely explained by the increased amount of muscle glycogen that was stored in the high carbohydrate condition (Bergström *et al.*, 1967). However, as muscle glycogen was not measured this cannot be confirmed. Nevertheless, a bout of exhaustive exercise before a period of a high-carbohydrate diet increases the activity of glycogen synthase and allows for a greater rate of glycogen synthesis and storage when glucose becomes available to the muscle (Bergström *et al.*, 1967). Since the consequence of carbohydrate loading is now a well-established physiological effect, it was

assumed that the capacity of muscle glycogen storage from a dose of 9 $g \cdot kg^{-1}$ of additional carbohydrate intake would synthesise muscle glycogen to an equivalent magnitude to other studies (Hawley *et al.*, 1997,Bussau *et al.*, 2002).

Previous literature has shown that exercise intensity is related to the fuel utilised (Brooks & Mercier, 1994), though cause and effect is hard to establish. That is, it is difficult to determine whether the increase in intensity mediated the increased RER, or that the increased RER due to fuel availability allowed a greater intensity. In the current study, the higher RER in the high-carbohydrate trial may indicate higher exercise intensity compared to the low-carbohydrate trial, though increased performance (and thus intensity) did not quite reach significance. That intensity was not significantly different only permits the explanation that increased glycogen concentrations in the high carbohydrate condition encouraged greater glycogen utilisation.

In terms of the secondary hypothesis (i.e. that carbohydrate utilization would decrease as the trial increased in duration), the expected outcome was observed. Taken together (main effect), the difference in RER through time indicated a reduction in carbohydrate utilization towards the end of the simulated performance trials. This was also reflected in the significant (main effect of time) decrease in carbohydrate oxidation. The rate of carbohydrate oxidation was close to 1g/min higher following the first set in the high-carbohydrate trial than the low-carbohydrate trial. The rate of fat oxidation mirrors this trend but the main effect of time on calculated rate of fat oxidation did not reach significance.

It is interesting to note that the rate of fat oxidation is greater in the high-carbohydrate than the low-carbohydrate trial in the final set. This observation is difficult to explain with the data available. Reduced efficiency in fuel utilisation may be another explanation to the difference in fat oxidation.

The application of this study is to provide knowledge regarding dietary carbohydrate over two days prior to competition and its effect on squash performance. Unfortunately an effect for time, trial and an interaction was not seen for performance time. Hence we cannot definitively state that a high carbohydrate diet is beneficial to squash performance. However, a p-value of 0.076 for the time by trial interaction points towards an ergogenic effect of the high carbohydrate diet. This is likely the result of a type 2 error as what seems to be a large difference in performance time between trials was not significant (Figure 16). Therefore if an extra one or two subject were completed, this would potentially reveal a significant effect.

During the high carbohydrate trial, the rate of oxygen uptake is maintained at a higher level compared with the low carbohydrate trial. This may be reflective of the improved performance time for the fourth and fifth set. Additionally, the performance times were seen to be quicker during the high carbohydrate trial from the second set onwards. However, this observation cannot be confirmed since there were no significant effects of time, trial or an interaction despite a time by trial interaction which was approaching significance. A likely explanation to the improved times in the high-carbohydrate trial may be attributed to greater power or speed being produced as a greater proportion of carbohydrates were available to the contracting muscle.

Physiological

The lactate concentrations for both trials at rest averaged at 2.0 mmol·L^{-1}. The average lactate concentration of all squash sets were 4.3 and 5.4 mmol·L^{-1} in the low- and high-carbohydrate trials, respectively. Interestingly, individual values here are comparable (9.1 mmol·L^{-1}) to that of Mercier *et al.* (1987) who reported lactate values as high as 8 mmol·L^{-1} for highly skilled players against an opponent of equal ability. The mean increase in blood lactate in the present study is also comparable to that of Noakes *et al.* (1982) who found a mean lactate increase of 2.5 mmol·L^{-1}. Mean lactate increase of the present study were 2.4 and 3.3 mmol·L^{-1} in the low- and high-carbohydrate trials, respectively. The lower increase in lactate in the low-carbohydrate trial is likely to be due to the reduced exercise intensity and the fact that glycogen is not as readily available as a substrate for glycolysis. The higher lactate concentration thus indicates a higher contribution of energy from glycogenolysis since plasma glucose concentrations were not different between conditions.

Subjective rating

RPE in both trials were of the same value following the end of the first and last set. This supports the level of effort participants are committing to each trial. However, it was interesting to note that RPE was not lower in the high-carbohydrate trial. This is inconsistent with previous literature (Davis *et al.*, 1997) where, when carbohydrate was made available during the trial in the form of a beverage ,increased carbohydrate intake was associated with a lower RPE. However, when any well-trained persons undertake self-paced exercise performance it is expected that, regardless of conditions, they will choose an intensity which relates to their highest sustainable pace, and this is likely to be related to perceived exertion (Ulmer, 1996).

Design of match simulation

There are a number of reasons to why a good scientific description of racket sports such as squash is not as widely available as tennis or other high-intensity intermittent sports such as football. These include the level of professionalism of the game – more money means a greater scientific effort in investigating what can produce optimal performance. Another issue is the practicality of research in a small environment and a general lack of research interest in the sport. However, scientific research on squash is progressing with several relevant studies being undertaken in more recent times (Montpetit, 1990,Chin *et al.*, 1995,Sharp *et al.*, 1998,Todd *et al.*, 1998,Romer *et al.*, 2001,Girard *et al.*, 2005,Bottoms *et al.*, 2006,Kingsley *et al.*, 2006,Girard *et al.*, 2007,Wilkinson *et al.*, 2009a,Wilkinson *et al.*, 2009b,Wilkinson *et al.*, 2009c).

The design of the current study had been adapted from several studies to accommodate and develop a modern face-valid squash simulation to mimic a practical five-game match lasting approximately an hour. Thus, each set had to be designed to last around 12 minutes. In the case of individuals completing the set between 11 and 12 minutes, this would have been counteracted from fatigue as performance time was generally slower as the duration of exercise progressed. Several studies have shown that a large proportion of squash rallies last for a period of less than 20 seconds (Montpetit, 1990,Sharp *et al.*, 1998,Girard *et al.*, 2007). The rest periods that have been incorporated for these squash trials were between 7 to 10 seconds (Sharp *et al.*, 1998,Girard *et al.*, 2005,Kingsley *et al.*, 2006). With the latter of these parameters in mind, the exercise protocol was developed to include 4 shots per rally. Each rally lasted around 15 seconds, with a 10 second rest period. There were a total of 30 rallies that were completed in each set. The duration of each set was calculated to last for 12.5 minutes using the above example. This duration is likely to change as exercise progresses as participants will either improve or decline in performance time. In addition to the design of the protocol, the logistics of how the match-simulation was completed was also important to make the simulation as realistic as possible to a real encounter. Previous match data analysis have found that shot distribution in a match were 74% in the back corners and 26% in the front corners. This distribution of shots adds support to the design of the study and adds to its validity as top level players tend to rally more at the back of the court (Wilkinson *et al.*, 2009a).

The current protocol was similar to that of Kingsley *et al.* (2006) who designed a squash simulation protocol that lasted for 12.2 minutes and consisted of 24 rallies which included between 6 to 11 shots in each rally. This resulted in a total of 186 simulated shots. On the other hand, the current study completed 120 simulated shots from 30 rallies. More importantly, it should be kept in mind that the purpose of this simulation was to simulate prolonged exercise (at least 60 minutes) and that the design of the simulation had to be reliable to allow for a treatment effect to be resolved. This goal was achieved as most subjects were able to complete both trials with maximal effort, which really simulates the intensity of a real squash match (one subject was not able to complete both trials due to an unrelated injury). Furthermore, feedback from the participants indicated that the design of the protocol was successful in replicating a squash match and that it was as hard, if not harder than a squash match, especially during the last two sets.

Design of study

A 48-hour feeding period before an exercise event has previously been shown to be long enough to refill glycogen following exercise-induced glycogen depletion (Piehl, 1974). An additional carbohydrate intake of $9g \cdot kg^{-1}$ of body weight was supplemented to a standardised diet provided to participants. The diet was provided in order to control the macronutrient composition of participants' diet as well as maintaining a controlled and consistent energy intake across all participants. As mentioned in the Methods section, participants were allowed to add additional foods to their diet since variation in dietary proportions of subjects cannot be excluded. In this case, subjects were encouraged to eat only protein and/or fat rich foods in order to minimise carbohydrate intake. Only two subjects found that the provided diet was not sufficient to maintain daily satiety. The additional foods consumed by participants were reported to be in the form of grilled chicken breast and/or canned tuna.

The exercise-induced glycogen depletion bout consisted of two types of running exercise; Yo-Yo intermittent recovery test and a treadmill run. These two exercises were necessary as the proven glycogen depletion bouts of endurance running or cycling described in the literature would not have been able to be completed by squash players. Upon completion of the depletion bout, subjects were immediately advised to start consuming their 'recovery' drinks which were made up of either low- or high-carbohydrate drinks. Though it would have been ideal to have three interventions in this study; low, "normal" and a high carbohydrate diet, the normal carbohydrate diet was not included in this study as it would have been

extremely difficult to gain particpants' full commitment since each trial required commitment for a period of four days in order to control for diet, exercise and the completion of each trial. The logistics of running three interventions for a relatively new area of research in a sport such as squash may have also been unnecessary at the present time since it was still to be established if a diet high in carbohydrates would be beneficial in improving squash performance. The main purpose of this study was to identify whether or not a high-carbohydrate diet would improve squash performance. Therefore the low-carbohydrate diet was the most appropriate diet to determine the performance effect of additional carbohydrates on squash performance.

Practical Applications

The use of carbohydrate supplementation in squash has had very little attention. Previous work by Noakes and co-authors, (1982) found that pre-ingestion of carbohydrates 25 minutes before a squash match may impair the normal metabolic response to squash and may adversely affect performance. This is due to the lowered glucose and free fatty acid concentrations. A hypoglycemic effect has also been concluded in another related study looking at pre-exercise carbohydrate ingestion (Costill *et al.*, 1977).

On the other hand, skill maintenance has been found to be better when a 6.4% carbohydrate beverage was consumed during the duration of exercise (Bottoms *et al.*, 2006). In comparison to a placebo beverage, carbohydrate ingestion was found to significantly improve shot accuracy and visual reaction time. Auditory reaction time was also improved, but this was evident in both the placebo and carbohydrate trial. The use of this ergogenic aid to maintain the skill aspect of squash is practical as many carbohydrate-electrolyte containing drinks are readily available and vary between 6-8% in carbohydrates. This makes the practice of this method likely, and anecdotally, is actually widely practiced in squash tournaments. However, carbohydrate feeding during a game and dietary carbohydrate content in the days prior to a game are two different occasions in which the timing of carbohydrate intake will produce various results between the events.

The results from the present study certainly suggest that the practice of maximizing carbohydrate intake in the days prior to competition should be considered. Certainly ingesting additional carbohydrates on top of a normal diet would not cause any harm nor

would it be detrimental to performance and if two matches were schedule for the same day the likelihood of a better performance with two days of prior carbohydrate supplementation is high.

Limitations

The obvious limitation of the present study was the absence of muscle glycogen from participants to provide a mechanistic explanation for the observations. However, since the topic of carbohydrate loading is well researched, and a clear difference in RER was seen, we are confident in our explanations (above).

An expected result from the failure to measure muscle glycogen is the lack of ability to measure the difference in glycogen concentrations pre- and post-exercise. A previous study by Rauch *et al.* (2003) suggested a theory in which the presence of a pacing strategy may influence the performance outcome between a carbohydrate-loaded and a non-loaded individual. This can be explained from the authors' finding from which the non-loaded subjects resulted at the same endpoint of muscle glycogen concentration regardless of initial muscle glycogen concentration. This suggested that the non-carbohydrate loaded subjects paced themselves at a lower workload throughout the trial in order to reach a similar endpoint. The explanation to this theory was the possibility of a chemoreceptor in the muscle which "is used by the central nervous system to calculate optimum pacing strategy". This suggestion was introduced as subjects decreased their power output during a 2-hour submaximal ride when they had an abundance of glycogen reserves. The lowest value was seen between the 30 and 32 minute mark, but reached peak values during the final 1-2 minutes of a 1-hour time trial which followed from the 2-hour submaximal ride. Therefore, this certainly suggests that power output was constrained for the first 58 minutes of the time trial, with the use of a 'pacing strategy' instead of a limitation of muscle glycogen.

In relation to the current study, this should have been ameliorated as subjects were instructed to exercise maximally in both trials in order to maintain the consistency of the high-intensity nature of the sport allowing the expected metabolic perturbation to take place when playing a competitive match. Nevertheless, a pacing strategy cannot be disregarded as subjects knew the endpoint of the trial, as opposed to a real match where it can only be decided on the day. Therefore the suggestion of a pacing strategy may be possible and if present, is only natural. The instruction of exercising maximally and treating the match simulation as a competitive

match to simulate the high-intensity nature of the match at all times highlights the thought of minimising any external factors which may influence the result of the study.

Though data for nine subjects were collected initially, the incomplete trial from one of the subjects resulted in the use of data from only eight subjects. This resulted in the outcome of the study to be slightly underpowered. This was evident in the performance time where a time by trial interaction was approaching significance. Part of the problem with the pool of these subjects was the fact that the pool of subjects who fitted the inclusion criteria was limited. Having better access to more players from across the country would have ameliorated this issue. Due to the small sample size and available participants, the grade level of players ranged between A grade and C1. Having a larger pool to recruit subjects from would also minimise this issue, allowing more A grade players to be recruited which will give an improved consistency across results as all participants would be of an equal skill and/or fitness level. Nevertheless, recruitment of good players for this study means that application of the results to this level and probably also the elite, is warranted.

A feeding period of two days was used as other studies have used a feeding period of between two to three days, thus providing support to the chosen methodology. However using a 24-hour feeding period may be more realistic, especially when since the recovery period faced between games in tournaments may be less than 24 hours. Future research could investigate carbohydrate ingestion on squash performance by ingesting a carbohydrate beverage in between each game which is commonly seen in competitive matches, yet no research has ever been carried out on this. Another possible study could look at carbohydrate feeding four hours before exercise which has been shown to be beneficial to endurance exercise performance.

Conclusion

The practice of carbohydrate loading is common in endurance events such as a marathon or a cycle race which covers a long distance over each race. Additional carbohydrate intake loading is practiced to super-compensate the muscle with glycogen following previous exhaustive exercise in order to initially deplete muscle glycogen stores which in turn increases the activity of glycogen synthase to maximise glycogen storage capacity (Bergström *et al.*, 1967,Karlsson & Saltin, 1971,Taylor *et al.*, 1972,Piehl, 1974,Ivy *et al.*, 1988). Research on a nutritional intervention during prolonged, high-intensity intermittent exercise such as squash is scarce. This study is only one of a handful of other studies that have implemented a nutritional intervention to improve an aspect of squash performance in either the form of skill and accuracy (Graydon *et al.*, 1998,Bottoms *et al.*, 2006) or an improvement in exercise performance (Romer *et al.*, 2001).

This study has investigated the effect of carbohydrate ingestion on squash performance, 48 hours following a strenuous bout of exercise. The exercise bout consisted of an aerobic and anaerobic exercise bout. The two exercises were completed as a treadmill run and an intermittent sprint test were aimed to deplete glycogen in type I and type II muscle fibres, respectively.

Subjects were provided with an additional $9g \cdot kg^{-1}$ of carbohydrates on top of a standardised low-carbohydrate diet. The experimental trial consisted of a squash-simulation match which was designed to last for around 60 minutes, by simulating a 5-set match in order to provoke the physiological changes as expected during prolonged exercise, but more importantly to examine the ergogenic effect of carbohydrate ingestion 48 hours before prolonged exercise. The intervention of the experiment was successful, as supported from a difference in RER and from a greater utilisation of carbohydrate. However, this did not result in a significant effect of performance time. A possible explanation to this could be as a result of the study being slightly underpowered. However, the p-value of an interaction between the intervention and performance time was very close to achieving significance. Therefore, this effect cannot be discounted and more data will be collected in the near future in order to get more power for publication. A significant interaction for this parameter would indicate that an increase in carbohydrate intake would support previous studies on carbohydrate feeding, resulting in an improvement in exercise performance. In this case, it would be an ergogenic effect by delaying fatigue in the form of maintaining speed or power, not endurance capacity.

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Appendices

Participant information sheet

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PARTICIPANT INFORMATION SHEET

The effects of carbohydrate loading 48 hours prior to a simulated squash match.

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You are invited to participate in a study evaluating the effect of pre-exercise carbohydrate loading on simulated squash performance. Participation in this study is on a voluntary basis and you have the right to pull out, or ask questions at any time. This project forms part of my MSc.

Why are we doing this study?

Squash is an ever growing sport, currently with more than 15 million players worldwide. The impact of nutrition on squash performance is an area which has had very limited attention with only a few studies having previously

looked at the influence of ergogenic aids such as carbohydrate or creatine ingestion on exercise performance and squash skill and/or accuracy. Carbohydrate loading is a nutritional strategy commonly practised by athletes several days leading to an endurance event in order to maximise glycogen stores in muscle. It is well understood that a high carbohydrate diet results in an improvement of performance in a long distance running or cycling event. However, evidence on carbohydrate loading during repeated bouts of short-duration high-intensity exercise performed over a prolonged period of time is limited with only a couple of studies showing an improvement in performance following 48 hours of a pre-exercise carbohydrate loading regimen. Available literature of pre-exercise carbohydrate loading is further limited when focused at racket sports such as squash which uses a large proportion of both anaerobic and aerobic energy systems. Carbohydrate loading may be beneficial for racket sports due to a large demand of energy from anaerobic glycolysis which rapidly breakdown muscle glycogen.

What is the aim of this study?

The aim of this project is to investigate if carbohydrate loading before a simulated squash performance would lead to delayed fatigue and/or improve in performance.

If I agree to take part, what will I be asked to do?

You will be asked to come to the courts on three occasions and twice at Massey's recreation centre to complete the depletion bout with all experimental trials separated by at least 7 days. All experimental trials will be taken at the same time each day (+/- 1 hour). Each experimental trial will last ~2 hours.

Session 1: Familiarisation

This session will consist of giving consent to participate and will also involve a familiarisation of the exercise protocol to be used.

Session 2-3: Performance Trials

In all experimental trials, you will consume either a placebo or carbohydrate-containing beverage and perform an on court ghosting protocol using programmable timing gates for approximately an hour. During this time, heart rate, lactate, glucose and rating of perceived exhaustion (RPE) will be measured, along with the total number of times the timing gates are passed through during the protocol. These trials will be conducted in a single-blinded, cross-over design and will be preceded by 48 hours of dietary and physical activity control including abstinence from any caffeine or alcohol containing products and minimising a high carbohydrate diet for the purpose of the study. You will also be provided with meals 48 hours prior to and on the day of the experimental trial.

Experimental Protocol

Before each experimental trial, subjects will undertake a bout of strenuous exercise to reduce muscle glycogen stores. After this strenuous exercise bout, you will randomly ingest either a low- or high-CHO diet consisting of 0.3 $g.kg^{-1}day^{-1}$ or 9 $g.kg^{-1}day^{-1}$, respectively, which will be mixed into a 7.5L drink **for consumption during the 48 hours prior to** the experimental trial. Subjects will be instructed to avoid all forms of exercise in the 48 hours prior to the experimental trial. On the day of the trial, you will warm up for five minutes using a "boast and drive drill" with the researcher and then undergo the ghosting protocol. You will also be provided with meals 48 hours prior to and on the day of the experimental trial.

Blood Collection

Capillary blood will also be collected from a finger throughout each trial (before, during and after) to analyse for blood lactate and glucose. **There will be a total of 6 blood samples collected in each trial.**

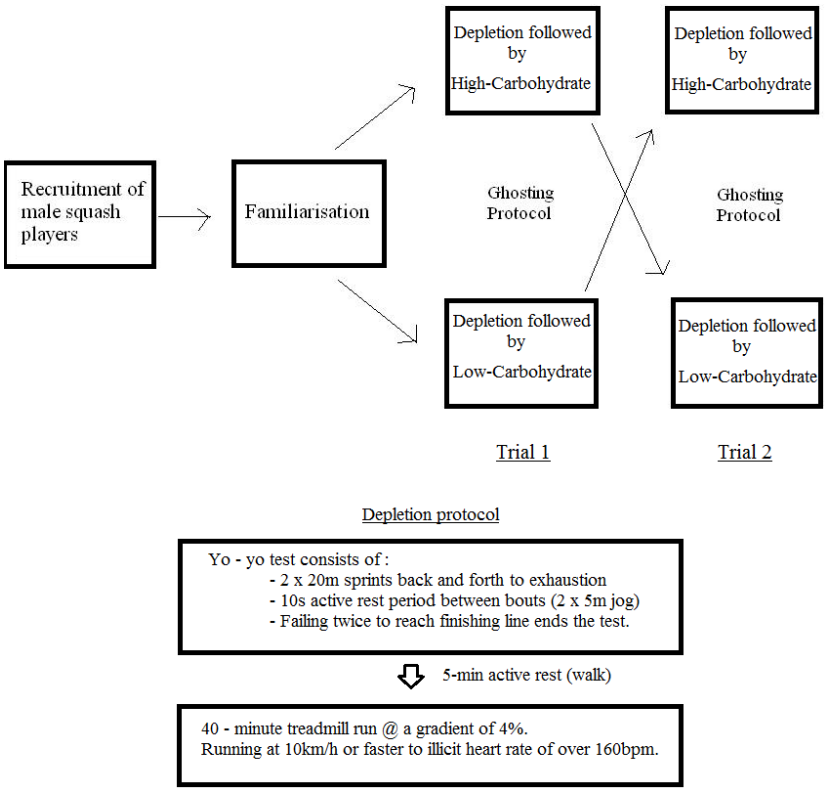
Exercise Protocol

The protocol for the glycogen-depleting exercise will consist of a 'Yo-Yo intermittent test' which consists of 2 x 20m sprints which increases in speed over time interspersed with 10 seconds of recovery time. You will run till exhaustion in order to deplete muscle glycogen to its greatest extent. During the experimental trial, you will be required to perform an on-court ghosting protocol using programmable timing gates that is a modification of the protocol based from Wilkinson *et al*. (2009). The protocol involves you to start on the "T" position and running to each timing gate as it lights up and emits a noise – a gate is placed in each of the four corners of the court. The player then places his racket in the middle of the gate and returns to the T as quickly as he can, and gets ready for the next timing gate to activate – there is slight delay between passing through a timing gate and activation of the next gate in order to let the player return to the T. The order and sequence of the gate activations are programmed into the timing gates by the researcher prior to the experiment and are based on actual squash match analysis data. The timing gates are programmed so that the protocol is split into rallies of differing length with a rest of eight seconds between rallies. Furthermore, based on match analysis data, the protocol is divided into five 'games' each of twelve minutes duration. Between each game, a 90 second break will be given to you where you will go off court and drink 150ml of water. You are asked to perform at your best ability for the duration of the trial.

Experimental Measures

In all experimental trials, heart rate will be measured prior to, during every 90-second break of the protocol and upon completion of the exercise task. Furthermore, ratings of perceived exertion (RPE) will be taken during every 90-second break and upon completion of the ghosting protocol. Finger prick samples of blood lactate and glucose (via capillary blood) will also be measured before, during the 90-second intervals and upon completion of each trial. The time taken to trigger each gate will be taken as the measure of performance.

Summary of Protocol



Wilkinson M, Leedale-Brown D & Winter EM (2009). Validity of a squash specific fitness test. *International Journal of Sports Physiology and Performance* **4**, 29-40.

What are the risks?

Squash places large amounts of stress on the body in terms of joints and muscles and the cardiovascular system. For this reason, you must pass selection criteria (see the health screening questionnaire) before participating.

Since blood glucose will be measured, if any abnormalities are seen during the trial, participants will be notified. You will be advised to see your GP if an abnormal blood glucose level is observed.

Additionally, a high carbohydrate diet might provide you with a bloating feeling or extra wind.

What are the benefits?

You will receive reimbursement for your time with a voucher and will also be provided with food for ~48 hours.

What are my rights?

- You can ask questions on any aspect of the project at any time, and we will do our best to answer them to your satisfaction.
- As a participant in the study you will provide information on the understanding that your name will not be used unless you give permission to the researcher.
- You have the right to view your own data at any stage and have it explained to you.
- You will also be given access to a summary of the project findings when it is concluded.
- You can withdraw from the project at any time, without giving any reason and without penalty.

What about compensation for injury?

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

Am I eligible?

Although voluntary, your participation will also be confirmed on criteria relating to health and safety; namely, in this study we are seeking healthy, moderately active squash playing (play squash a minimum of 3 times per week) males aged between 18-40 and currently holding a New Zealand Squash grade of C2 or above. For health/safety reasons, you should **not** participate if any of the following apply to you:

- You have any known heart or cardiovascular condition or if a member of your family died below the age of fifty (50) as a result of a heart condition.
- You have (a) condition(s) that could be made worse by exercise.
- You have ever had an injury or any medical condition that you think may affect your ability to sense pain or discomfort.
- You are taking prescribed medication.
- You have any other reason to consider that you are not in good health and of average, or better than average, fitness.

Anything else I need to know?

You will be asked to wear your normal squash shorts, shirt and footwear that you feel comfortable exercising in. Water will be provided throughout the testing procedure. We would also like you to fill in a food and physical activity diary and you will need to be well hydrated ***(on arrival you will be provided with 500mL water to optimise hydration status).***

All data obtained from this study will be kept strictly confidential. Data will be identified as a code only. Results will be made available to you at the completion of the study.

If you are interested in taking part:

Contact:

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Massey University, Palmerston North Campus

Palmerston North, New Zealand

Phone: (021) 2122 897

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"This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 11/20. If you have any concerns about the conduct of this research, please contact Professor Julie Boddy, Chair, Massey University Human Ethics Committee: Southern A telephone 06 350 5799 x 2541, email humanethicsoutha@massey.ac.nz."

Health screening questionnaire

Pre-Exercise Health Screening Questionnaire

Name: _____

Address: _____

Phone: _____

Age: _____

This questionnaire has been designed to identify the small number of persons (15-69 years of age) for whom physical activity might be inappropriate. The questions are based upon the Physical Activity Readiness Questionnaire, originally devised by the British Columbia Dept of Health (Canada), as revised by Thomas *et al.* (1992) and Cardinal *et al.* (1996), with the added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

You should be aware that even amongst healthy persons who undertake regular physical activity there is a risk of sudden death during exercise. Though extremely rare, such cases can occur in people with an undiagnosed heart condition. If you have any reason to suspect that you may have a heart condition that will put you at risk during exercise, you should seek advice from a medical practitioner before undertaking an exercise test.

Please read the following questions carefully. If you have any difficulty, please advise the exercise specialist who is conducting the exercise test. Please answer all of the following questions by ticking only <u>one</u> box for each question:

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

Yes ☐ No ☐

2. Do you feel a pain in your chest when you do physical activity?

Yes ☐ No ☐

3. In the past month have you had chest pain when you were not doing physical activity?

Yes ☐ No ☐

4. Do you lose your balance because of dizziness or do you ever lose consciousness?

Yes ☐ No ☐

5. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

Yes ☐ No ☐

6. Do you have a bone or joint problem that could be made worse by vigorous exercise?

Yes ☐ No ☐

7. Do you know of any other reason why you should not do physical activity?

Yes ☐ No ☐

8. Have any immediate family had heart problems prior to the age of 60?

Yes ☐ No ☐

9. Have you been hospitalized recently?

Yes ☐ No ☐

10. Do you play squash 3 or more times per week?

Yes ☐ No ☐

11. Do you have diabetes or any other medical conditions which may put you at risk from a high carbohydrate intake?

Yes ☐ **No** ☐

12. Are you lactose intolerant?

Yes ☐ **No** ☐

13. Are you allergic to any artificial sweeteners?

Yes ☐ **No** ☐ **If yes, please explain :**

14. *Are you taking any of the following pharmaceutical preparations?*

Yes ☐ No ☐

- Antihypertensive (high blood pressure) medications of any sort
- Antiarrhythmic drugs
- Allergy, cold and flu, or cough preparations containing pseudoephedrine
- Bronchodilators containing: theophylline, albuterol, epinephrine (adrenaline)
- Pseudoephedrine

I have read, understood and completed this questionnaire.

Signature: _____ Date: _____

References

Thomas S, Reading J and Shephard RJ. (1992) Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 17(4): 338-345.

Cardinal BJ, Esters J and Cardinal MK. (1996) Evaluation of the revised physical activity readiness questionnaire in older adults. *Med Sci Sports Exerc* 28(4): 468-472

Consent form

Institute of Food, Nutrition and Human Health

Private Bag 11 222

Palmerston North

New Zealand

CONSENT FORM

Project Title: The effects of carbohydrate loading 48 hours prior to a simulated squash match.

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: ..... **Date:** .....

Full Name - printed .....

Ethical letter of approval

MASSEY UNIVERSITY

5 May 2011

Aaron Raman
School of Sport & Exercise
PN452

Dear Aaron

Re: HEC: Southern A Application – 11/20
The effects of carbohydrate loading 48 hours prior to a simulated squash match

Thank you for your letter dated 4 May 2011.

On behalf of the Massey University Human Ethics Committee: Southern A I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

JMBoddy

Prof Julie Boddy, Chair
Massey University Human Ethics Committee: Southern A

cc A/Prof Steve Stannard
School of Sport and Exercise
PN452

Dr Toby Mundel
School of Sport and Exercise
PN452

Set list of timing gates

Range: From 1 to 4 - Unsorted

Set 1	Set 2	Set 3	Set 4	Set 5
1	3	1	3	1
2	4	3	4	4
3	2	2	3	3
4	4	3	2	1
3	1	3	3	4
2	3	3	2	4
4	3	2	3	1
4	3	4	3	3
1	2	3	4	1
3	2	4	2	3
3	4	1	3	2
2	1	4	3	3
3	3	4	3	3
4	3	3	4	4
2	2	4	1	4
2	4	3	4	2
1	4	3	3	1
4	1	4	4	3
3	3	4	1	1
3	3	3	2	3
3	4	2	3	3
4	4	3	4	1
1	1	4	3	4
4	4	1	3	3
1	3	4	4	4
4	2	3	3	3
2	3	3	2	4
3	4	1	3	1
4	1	3	3	1
3	4	2	1	4
4	3	4	3	3
3	2	2	4	4
4	4	4	4	3
4	3	4	3	3
3	4	3	1	4
2	1	3	3	2
3	2	4	3	3
1	4	1	3	1
3	4	3	1	4
3	3	3	3	4
1	4	2	2	3
4	3	3	4	4
4	2	4	3	1
3	3	3	4	3
3	3	3	4	2
4	2	4	2	4

1 & 2 are front gates
3 & 4 are back gates

74% back = 89 gates
26% front = 31 gates

1	2	1	2	4
2	3	3	4	4
3	2	4	4	3
2	4	4	3	4
4	3	2	2	1
4	4	1	1	4
4	3	3	4	2
3	4	3	4	4
2	4	4	3	3
3	3	2	4	4
3	3	1	3	4
2	3	4	3	1
3	3	3	3	4
4	4	3	2	4
3	3	4	4	1
2	2	2	3	2
3	3	3	4	3
3	4	4	4	4
4	2	1	2	4
1	3	3	3	3
4	3	4	3	3
3	2	2	3	3
4	4	3	1	3
4	1	3	3	2
4	4	2	4	3
3	4	3	3	4
4	3	3	1	3
2	3	3	1	4
3	4	4	3	1
3	4	3	3	3
4	4	3	3	3
2	1	4	1	4
3	3	4	3	3
4	2	3	4	4
4	4	4	3	4
3	4	4	4	1
4	3	3	4	3
3	4	4	1	3
4	4	4	1	3
4	3	4	3	1
4	3	3	1	4
1	4	1	4	4
3	1	1	3	4
3	3	4	3	4
4	4	1	2	1
3	3	4	4	2
4	2	1	3	4
1	3	3	4	3
4	4	2	3	3
1	4	4	3	4

3	3	4	4	4
3	4	2	2	3
4	3	4	3	2
3	3	1	3	3
3	2	3	1	1
3	3	1	3	4
2	4	3	4	3
3	3	4	4	3
4	2	3	3	4
1	3	4	3	4
3	4	3	2	3
4	1	3	4	2
3	4	4	1	3
3	3	3	4	4
4	3	2	3	3
1	4	1	4	3
4	1	4	3	1
2	4	1	3	4
3	3	4	3	4
3	3	3	1	3
4	4	4	2	4
1	4	4	3	2
3	1	3	3	4