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Author name : Clarke, N.D. , Drust, B. , MacLaren, D.P.M. and Reilly, T. Title: Carbohydrate ingestion and pre-cooling improves exercise capacity following soccer-specific intermittent exercise performed in the heat. Article & version: Final pre-print Original citation: Clarke, N.D. , Drust, B. , MacLaren, D.P.M. and Reilly, T. (2011) Carbohydrate ingestion and pre-cooling improves exercise capacity following soccer-specific intermittent exercise performed in the heat. . *European journal of applied physiology*, volume 111 (7): 1447-1455 Publisher: Springer Publication website: http://dx.doi.org/10.1007/s00421-010-1771-5 DOI: 10.1007/s00421-010-1771-5

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All alterations are highlighted in red.

## Introduction

Page 3, line 34 – The references have now been separated into protocols that simulate the work-load that corresponds to soccer and actual match play.

Page 3, lines 44-45 have been removed so that there is not a repetition of lines 38-39.

Page 3, line 47 – 'of' added after 'impact'.

Page 3, paragraph 4 - The introduction to the paragraph now states that soccer is regularly played in hot conditions which can increase the thermal stress.

## Method

Page 5, lines 7-19 – This paragraph already stated that fluid was ingested at 15 min intervals and the volume equated to 223 ml at each time point. This is now followed by a statement that this was calculated on the basis of 3 ml/kg body mass. Furthermore, the drink formula has been added along with information regarding the amount of CHO ingested.

Information now included about what the participants did when not undergoing pre-cooling i.e. just sitting in the lab.

Method relating to the two performance tests – These tests were separate and the published version of the Cunningham and Faulkner test was employed. In addition, information has been added providing a rationale for the choice of the self-selected speed test, in that it was a psychological measure designed to monitor the subjective feeling of the pace that the participants could sustain. This is due to the impact a high core temperature has on exercise performance which may attenuate the starting pace during a subsequent self-paced bout of exercise. This is as a consequence of self-chosen exercise intensity potentially being centrally regulated in an anticipatory feed-forward manner necessary to control the rate of heat storage and avoid the development of hyperthermia which may be attenuated by reducing core temperature as a result of pre-cooling.

## Results

Page 7, line 5 – 'been' has been replaced with 'being'.

Pre data for all conditions now included in figures 3 and 6 and table 1. These were originally omitted in an attempt to make the figures clearer.

P-values now included for all of the core temperature time points as well as selected data for the other variables, especially the performance measures.

## Discussion

Page 9, line 17 - 'effects' has been replaced with 'affects'.

Page 9, lines 21-22 have been rewritten as the sentence was incomplete.

Page 9, lines 26-37 – This is a hypothetical suggestion however after reading the 2010/11 laws of the game it does not appear that there are any specific rules relating to what a player must ware during the warm up.

# Carbohydrate Ingestion and Pre-Cooling Improves Exercise Capacity Following Soccer-Specific Intermittent Exercise Performed in the Heat

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#### Abstract

Ingestion of carbohydrate and reducing core body temperature pre-exercise, either separately or combined, may have ergogenic effects during prolonged intermittent exercise in hot conditions. The aim of this investigation was to examine the effect of carbohydrate ingestion and pre-cooling on the physiological responses to soccerspecific intermittent exercise and the impact on subsequent high-intensity exercise performance in the heat. Twelve male soccer players performed a soccer-specific intermittent protocol for 90 min in the heat (30.5°C and 42.2% r.h.) on four occasions. On two occasions, the participants underwent a pre-cooling manoeuvre. During these sessions either a carbohydrate-electrolyte solution (CHOc) or a placebo was consumed at (PLAc). During the remaining sessions either the carbohydrate-electrolyte solution (CHO) or placebo (PLA) were consumed. At 15-min intervals throughout the protocol participants performed a mental concentration test. Following the soccer-specific protocol participants performed a self-chosen pace test and a test of high-intensity exercise capacity. The period of pre-cooling significantly reduced core temperature, muscle temperature and thermal sensation (P < 0.05). Self-chosen pace was greater with CHOc ( $12.5 \pm 0.5 \text{ km} \cdot h^{-1}$ ) compared with CHO ( $11.3 \pm 0.4$ km·h<sup>-1</sup>), PLA (11.3±0.4 km·h<sup>-1</sup>) and PLAc (11.6±0.5 km·h<sup>-1</sup>) (P<0.05). High-intensity exercise capacity was improved with CHOc and CHO when compared with PLA (CHOc; 79.8±7 s, CHO; 72.1±5 s, PLAc; 70.1±8 s, PLA; 57.1±5 s; P<0.05). Mental concentration during the protocol was also enhanced during CHOc compared with PLA (P < 0.05). These results suggest pre-cooling in conjunction with the ingestion of carbohydrate during exercise enhances exercise capacity and helps maintain mental performance during intermittent exercise in hot conditions.

Key words: Body temperature, carbohydrate, intermittent exercise, mental performance, exercise capacity, selfchosen pace.

#### Introduction

Soccer match-play requires players to cover 10-12 km (Stolen et al. 2005) and perform a wide variety of activities ranging from walking to sprinting, with frequent and irregular changes in intensity. Soccer also requires mental concentration and situational and cognitive awareness in order to make the right tactical and technical decisions. As such, successful soccer performance can be said to incorporate both physical and mental dimensions (Stolen et al. 2005).

Low substrate availability is a potential cause of fatigue in soccer (Reilly et al. 2008). A significant reduction in the glycogen content of the thigh muscles of players has been observed at the completion of a soccer match (Jacobs et al. 1982). The decline in glycogen stores is reflected in lower running speeds and shorter distances covered during the second half compared to the first half of a match (Saltin 1973) as well as reduced sprint performance (Krustrup et al. 2006). Lowered blood glucose levels are associated with impaired cognitive performance (Donohoe and Benton 1999), and ingestion of a carbohydrate-electrolyte solution during exercise minimises the negative effects of fatigue on mental performance during prolonged exercise (Collardeau et al. 2001; Reilly and Lewis 1985) and intermittent high-intensity exercise with physical demands similar to those associated with team sports (Winnick et al. 2005). These findings indicate that low carbohydrate availability has the potential to limit both physical and mental performance during soccer, and therefore impact both the physical performance of players and their technical/ tactical skills.

Many authors have investigated the impact of carbohydrate ingestion on the performance of exercise protocols that simulate the work-rate of soccer (Nicholas et al. 1999; Nicholas et al. 1995; Walton and Rhodes 1997) and during actual match-play (Kirkendall et al. 1988; Leatt and Jacobs 1989; Zeederberg et al. 1996) in moderate temperatures. However, the effect of ingesting fluid containing carbohydrate during intermittent exercise performed in the heat has not been extensively investigated (Morris et al. 2003). Some authors have reported performance benefits such as improved cycling time trials (Davis et al. 1988; Below et al. 1995), whilst others have suggested that carbohydrate availability may not be a limiting factor when exercise is performed in hot environments (Morris et al. 2003). This would suggest that there is a requirement for carefully controlled investigations on the impact of carbohydrate ingestion on simulated soccer performance in the heat. Such investigations may provide more specific fluid replacement strategies for players during tournaments under conditions of climatic stress.

Soccer matches at major tournaments, such as the FIFA World Cup finals between 1994 and 2006, are regularly played in temperatures exceeding 30°C and playing in such conditions results in an increase in core temperature. As a consequence another factor inducing fatigue during exercise in the heat is hyperthermia (Nielsen et al. 1993). A high core temperature may reduce physical performance due to increased cardiovascular strain or perhaps more importantly, reduction in the central drive from the nervous system to the active musculature

(Reilly et al. 2008). Pre-cooling limits the increase in core temperature caused by a fixed exercise task or increases the margin to the maximal core temperature that can be tolerated by reducing the temperature of the core prior to exercise (Quod et al. 2006). This procedure increases the storage of metabolic heat and the time required to reach a critical limiting temperature when a given exercise intensity may no longer be maintained (Reilly et al. 2006). Previous research has indicated that pre-cooling can improve the performance of endurance exercise (Lee and Haymes 1995; Booth et al. 1997; Cotter et al. 2001) and high-intensity cycling (Marsh and Sleivert 1999), although the available data on soccer-specific exercise is equivocal (Drust et al. 2000a). This may be a consequence of the limited thermal challenges that were provided by the environmental conditions included in the investigation. Pre-cooling may therefore have the potential to be of benefit during 90 min of soccer-specific exercise when conditions are hot and where there is a likelihood that core temperature increases to levels that may limit exercise performance.

The available literature on the metabolic responses to exercise in the heat indicates that there may be some additional benefit to performance by combining carbohydrate ingestion with pre-cooling. It appears that during sub-maximal exercise in the heat, a marked increase in core body temperature (>0.5°C) is associated with an augmented intramuscular carbohydrate utilization (Febbraio 2001). This suggests that a combination of reducing core temperature through pre-cooling prior to exercise and then consuming carbohydrate during the activity may spare muscle glycogen and lead to greater improvements in exercise capacity than those associated with using each individual approach in isolation. The aim of this investigation was therefore to examine the combined effects of carbohydrate supplementation and pre-cooling on the metabolic and thermoregulatory responses during, and performance measures following, soccer-specific exercise. We hypothesise that a combination of pre-cooling and carbohydrate ingestion enhances both physical and mental performance.

#### Methods

Twelve male university soccer players (mean age:  $25\pm1$  years; body mass:  $73.8\pm2.6$  kg; height:  $1.80\pm0.02$  m;  $\dot{V}O_{2max}$ :  $61.3\pm1.4$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) participated in the study. All participants provided written informed consent to participate, in accordance with the ethical procedures of Liverpool John Moores University. Participants were tested in a post-absorptive state (3-4 hours), having performed no vigorous exercise i.e. competitive match or intense training for 48 hours prior to testing. Test sessions were performed at the same time of day to minimise the circadian variation of the measured variables.

Participants undertook two familiarisation sessions, consisting of two blocks (Figure 1) of the soccer-specific protocol (i.e. 30 minutes). The protocol was a modified version of that designed by Drust *et al.* (2000b) and reported in Clarke *et al.* (2008). The soccer-specific protocol consisted of various exercise intensities that are regularly observed during competitive soccer matches (i.e. walking, jogging, cruising and sprinting) over a 90

min period divided into 2 x 45-min identical periods, separated by a period of 15 min, representing half-time. Each 45-min period consisted of three 15-min blocks. The full soccer-specific protocol (Figure 2) was completed on a motorised treadmill on four occasions in the environmental chamber  $(30.5\pm0.1^{\circ}C)$  and  $42.2\pm0.2\%$  relative humidity).

On two occasions, the participants underwent pre-cooling. The pre-cooling strategy involved wearing a cooling vest (Cool Vest, Jackson Technical Solutions Ltd, Kent, UK) for 60 min prior to exercise. At half-time, the cooling vest was again worn for the entire 15 min. During these sessions either a carbohydrate-electrolyte solution [Still Lucozade Sport, (6.6 g·100 ml<sup>-1</sup> CHO, 49 mg·100 ml<sup>-1</sup> Na, 296±1 mOsm·kg<sup>-1</sup>), GlaxoSmithKline, Gloucestershire, UK] (CHOc) or a placebo (a similarly coloured, flavoured and textured electrolyte solution) (GlaxoSmithKline, Gloucestershire, UK) was consumed at 0, 15, 30, 45, 60 and 75 min of exercise (PLAc). During the remaining trials either the carbohydrate-electrolyte solution (CHO) or the placebo (PLA) were consumed at the same time points but without being preceded by pre-cooling (i.e. participants remained seated in the laboratory for 60 min without donning the cooling vest). The total volume of fluid ingested during each trial was  $1338\pm41$  ml, which equated to  $223\pm7$  ml (3 ml·kg<sup>-1</sup> body mass) at each time point. During the rank trials the total amount of carbohydrate ingested was  $88\pm9$  g at a rate of  $59\pm6$  g·h<sup>-1</sup>. All of the trials were performed in a randomised counter-balanced fashion.

Prior to undergoing pre-cooling, muscle temperature was recorded and a venous blood sample was drawn. Throughout pre-cooling, and the exercise protocol, core temperature was monitored. At the completion of the pre-cooling procedure and prior to the commencement of the soccer-specific protocol, muscle temperature was measured again and a further blood sample collected. Muscle temperature assessment and blood sample collection were also performed at half-time and on completion of the exercise protocol. During the soccer-specific protocol mental concentration and thermal sensation were measured at 15 min intervals and after the soccer-specific protocol participants performed a self-chosen work-rate test and a measure of exercise capacity.

Core body temperature was monitored continuously by means of an ingestible temperature sensor pill and external data logger (HQ inc., Florida, USA). Data were presented as the mean value for each 15-min block of exercise. Muscle temperature was determined by means of a needle thermistor (Model MKA-A, Ellab, Denmark) inserted into the quadriceps muscle to a depth of 30 mm. Participants also rated their thermal sensation during exercise according to a 17-point thermal sensation scale (Toner et al. 1986) before and after the cooling procedure, and at the completion of each block of activity.

After 4 min of every 15-min block of the protocol (Figure 2) the participants performed a mental concentration test (Hardy and Fazey 1990). A test grid was projected onto the wall in front of the treadmill. The task required participants to scan 16 lines of figures from left to right and identify each pair of figures that added up to ten.

The participant had 90 seconds to complete as many lines as possible whilst performing the soccer-specific protocol. When the 90-s period was completed, the number of "tens" correctly identified was recorded as a percentage of those attempted.

After performing the soccer-specific protocol participants completed a self-chosen work-rate test (Atkinson et al. 1993) which involved running for 3 min at a speed that the subjects felt could be sustained for 30 min. This test was designed to identify the perceived ability of the participants to complete a sustained period of activity following the soccer-specific simulation. The rationale for this test was partly to investigate the potential impact of exercise induced hyperthermia on the pacing strategies that players may use in exercise that has some relevance to extra time periods in competition football. Such ideas are in-line with the concept of self-chosen exercise work-rate being centrally regulated in an anticipatory feed-forward manner in response to changes in the rate of heat storage (Tucker et al. 2004). The self-chosen work-rate test was immediately followed by Cunningham and Faulkner's (1969) test of fatigue resistance to high-intensity exercise. This test was chosen as it is a recognised exercise protocol that assesses anaerobic work capacity (Green, 1995), as required during soccer match-play.

Blood samples were centrifuged and the plasma frozen at -80°C for later analysis. Samples were analysed for glucose (Glucose oxidase, Instrumentation Laboratory, Monza, Italy), and glycerol and non-esterified fatty acids (NEFA) (Randox Laboratories ltd, Co. Antrim, UK).

All variables were analysed using two-way ANOVA with repeated measures except for the performance variables. The performance variables were analysed using a one-way ANOVA with repeated measures. Results are reported as the mean  $\pm$  the standard deviation (SD) and a level of *P*<0.05 was considered statistically significant.

#### Results

Pre-cooling significantly reduced core temperature (PLAc,  $37.2\pm0.1^{\circ}$ C to  $36.6\pm0.1^{\circ}$ C; CHOc,  $37.3\pm0.1^{\circ}$ C to  $36.6\pm0.1^{\circ}$ C; *P*=0.000, Figure 3a). As a consequence, core temperature during CHOc and PLAc was significantly lower prior to the start of exercise compared with PLA and CHO. This trend remained throughout the first 30 min (i.e. blocks 1 and 2). During exercise core temperature increased significantly during all trials throughout the exercise period with the exception of the half-time recovery period where it decreased significantly (*P*=0.000). Core temperature between 30 min and half-time was significantly lower in CHOc and PLAc compared with CHO. During block 4 (i.e. after 60 min) core temperature during CHO was also significantly higher than CHOc (*P*=0.014). During the final 30 min of the protocol (i.e. blocks 5 and 6) core temperature was significantly higher during PLA, PLAc and CHO compared with CHOc (*P*=0.000).

Pre-cooling significantly reduced muscle temperature (Figure 3b; PLAc,  $36.1\pm0.2^{\circ}$ C to  $35.5\pm0.2^{\circ}$ C and CHOc,  $36.2\pm0.1^{\circ}$ C to  $35.5\pm0.2^{\circ}$ C; *P*=0.000). Muscle temperature subsequently increased significantly between each time point during exercise in all trials (*P*=0.000) although at half-time and at the end of exercise there was no difference between trials. Pre-cooling also significantly reduced thermal sensation (Table 1) prior to performing the soccer-specific protocol (*P*=0.000) and at half-time compared with CHO and PLA (*P*=0.013). This would seem to indicate improved thermal comfort before the start of exercise and at half-time. There was, however, no significant treatment effect (*P*=0.101) for thermal sensation during the exercise period though thermal sensation ratings did increase significantly during each half of the soccer-specific protocol (*P*=0.000).

The speed during the self-chosen work-rate test (Figure 4a) was significantly higher (P=0.006) during CHOc (12.5±0.5 km·h<sup>-1</sup>) compared with the other trials (CHO; 11.3±0.4 km·h<sup>-1</sup>, PLAc; 11.6±0.5 km·h<sup>-1</sup>, PLA; 11.3±0.4 km·h<sup>-1</sup>). There was also a significant treatment effect (P=0.000) on exercise capacity during the Cunningham and Faulkner test (Figure 4b) with performance times in the CHOc (79.8±7 s) and CHO (72.1±5 s) being significantly higher compared to PLA (57.1±5 s). The trend for performance to be improved with pre-cooling during the placebo trial, approached significance (P=0.072). Performance in the mental concentration test was also significantly influenced by the trial (P=0.025; Figure 5). The percentage of correct responses after 4, 34 and 49 min of exercise was significantly higher during CHOc compared with PLA. The percentage of correct answers was relatively constant throughout the protocol for all trials (P=0.264).

The plasma glucose concentration was significantly higher at half-time and the end of the soccer-specific protocol during CHO and CHOc compared with PLA and PLAc (P=0.001; Figure 6a). These values were significantly above resting levels (P=0.000). The concentration of NEFA (Figure 6b) was significantly higher during PLA and PLAc compared with CHO (P=0.017). These values increased significantly between each time point as exercise progressed during all trials (P=0.000) with NEFA concentrations increasing more after half time during PLA and PLAc compared with CHO and CHOc (P=0.019). The plasma concentration of glycerol was significantly higher during the PLA trial compared with CHO and CHOc throughout the protocol (P=0.003) with the Glycerol concentration remaining significantly higher in PLAc than CHO and CHOc at 90 min (Figure 6c). Plasma glycerol concentration increased significantly between each time point in each trial (P=0.000) although these increases were markedly more during PLA and PLAc compared with CHO and CHOc (P=0.000).

#### Discussion

The major findings of this study were that the combined effect of carbohydrate ingestion and pre-cooling significantly enhances exercise capacity, improves mental performance but does not affect metabolism during

exercise in the heat despite significant reductions in core temperature. Our data would suggest that carbohydrate ingestion when combined with pre-cooling may improve performance to a greater extent than when these two strategies are employed in isolation. The observed performance improvements when pre-cooling and carbohydrate ingestion were combined were significantly greater for self-chosen pace and exercise capacity when compared with PLA and PLAc. Self-chosen pace was also significantly increased when CHOc was compared with CHO. There was also a trend for self-chosen running speed (3%) and high-intensity exercise capacity (23%) to be improved with placebo and pre-cooling compared with placebo ingestion alone although this did not reach the required level of significance. This would suggest that the combination of pre-cooling and carbohydrate ingestion appears to be a potential strategy to improve performance in soccer players when playing in the heat. The strategy may also be applicable to other intermittent field-based endurance sports.

A possible explanation for such performance improvements observed in CHOc following exercise is the lower core temperature at the completion of the soccer-specific protocol compared with the other trials. The 'artificially' enhanced capacity for heat storage following pre-cooling has become one of the most prominent explanations for improvements in performance under such conditions (Quod et al. 2006). Such improvements are thought to be partly related to a reduced likelihood of attaining the high core body temperatures that are associated with fatigue under similar exercise conditions. This temperature related fatigue mechanism may also have an anticipatory component that may adjust the work-rate at any given time in the exercise bout to ensure that the task is completed within homeostatic limits (Lambert et al. 2005; Marino et al. 2004). Feedback from the thermal inputs of core and muscle temperature will therefore allow the body to adopt a pacing strategy for the workload to ensure that a critical core temperature is not exceeded (Gonzalez-Alonso et al. 1999). The higher core temperatures observed at the completion of the soccer-specific protocol during CHO, PLA and PLAc when compared with CHOc may therefore have resulted in a selection of a slower speed in the selfchosen pace test thereby reducing the capacity for performance. The increased availability of carbohydrate in the CHOc condition may also have influenced the participants' willingness to exercise. Kringelbach (2004) reported that carbohydrate may activate the brain regions that mediate emotional and behavioural responses such as the dopaminergic system of the ventral striatum. This brain region has been implicated in arousal, motivation and the control of motor behaviour (Berridge and Robinson 1998). As a consequence, Chambers et al. (2009) suggested that oral carbohydrate may increase the activity of the dopaminergic pathways and counteract the effects of fatigue. Higher dopaminergic activity as a result of the lower core temperature and higher availability of carbohydrate in CHOc may have resulted in the perception that the participants could exercise at a higher intensity thereby explaining the faster self-chosen speed. The higher carbohydrate availability and increased activation of the dopaminergic system may also explain the longer time to exhaustion in the Cunningham and Faulkner test observed during CHOc and CHO. These factors may have delayed the need to reduce power output during the Cunningham and Faulkner test thereby enabling a longer run time to exhaustion.

Better maintenance of cerebral blood flow and elevated metabolism of glucose as a consequence of carbohydrate ingestion and lower core temperature associated with pre-cooling may explain the improvements in mental performance during CHOc compared with PLA. It has been reported that cerebral blood flow is reduced in exercising hyperthermic participants compared with normothermic participants (Nybo et al. 2002). Such reductions in blood flow may reflect the availability of substrate, especially blood glucose. This may lead to a depletion of the available glycogen stores in the brain. Carbohydrate ingestion during prolonged exercise can increase cerebral glucose uptake and oxygen consumption thereby delaying the impaired central nervous system function that may occur late in exercise (Nybo et al. 2003). Consequently, the increased availability of carbohydrate and lower core temperature that may be observed in the CHOc condition could potentially prevent a decrease in cognitive functioning. A number of research papers support these ideas by demonstrating that glucose administration is capable of enhancing cognitive performance in healthy young adults at rest (Kennedy and Scholey 2000; Scholey et al. 2001) and after exercise (Collardeau et al. 2001; Reilly and Lewis 1985). Therefore, maintaining blood glucose, and reducing changes in cerebral blood flow as a consequence of lowering core temperature during exercise could have beneficial effects in sports such as soccer, which require tactical decision-making where carbohydrate availability may become limited.

Thus far the metabolic responses to exercise after pre-cooling have not been extensively investigated. In the present study glucose, NEFA and glycerol concentrations were not significantly different between CHO and CHOc or PLA and PLAc. These observations are similar to those of previous studies (Booth et al. 2001; Castle et al. 2006; Drust et al. 2000a). Booth *et al.* (2001) reported a reduced body temperature and heart rate after whole-body pre-cooling, but found no differences in muscle concentrations of glycogen, triglyceride, adenosine triphosphate, creatine phosphate, creatine and lactate. As a consequence Booth *et al.* (2001) proposed that core and muscle temperatures have to exceed 39°C before muscle metabolism is significantly altered. In addition, Febbraio *et al.* (1998) established that the level of circulating adrenaline affects muscle metabolism. Although not presented in this paper, catecholamines levels were measured in the present study and the response was found to be similar in all conditions. This suggests that when muscle temperature and adrenaline concentration are not significantly different during exercise and when core temperature is not manipulated to sufficient levels metabolism is not significantly altered.

On a practical level, whilst the combination of pre-cooling and carbohydrate ingestion appears to be a strategy that could be used by soccer players to improve performance when playing in the heat, it seems unlikely that this particular pre-cooling manoeuvre could be employed prior to competitive matches. The pre-cooling regime in this study required the participant to wear the cooling vest for 60 min and remain in a seated position to facilitate the reductions in core temperature. This strategy is not practical for professional players as they are required to move around the changing room and to complete a warm-up prior to the match. One potential compromise may be the wearing of a cooling vest during the warm-up period as has been employed to improve 5-km run performance in the heat (Arngrimsson et al. 2004).

In conclusion, pre-cooling in conjunction with the ingestion of carbohydrate during exercise can enhance exercise capacity and maintain mental performance. Whilst this combination may be a suitable strategy for soccer players when performing in the heat, the exact mechanisms for this improvement remain to be identified. In addition, carbohydrate ingestion alone can also significantly improve exercise capacity, although to a lesser extent, following soccer-specific exercise performed in the heat.

#### Acknowledgements

This study was supported by GlaxoSmithKline.

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	Time point									
	Pre-	Post-	1	2	3	HT	4	5	6	
PLA	5±0	5±0	7±0	7±0	7±0	6±0	7±0	7±0	8±0	
PLAc	5±0	3±0*	6±0	7±0	7±0	5±0*	7±0	7±0	8±0	
СНО	5±0	5±0	7±0	7±0	7±0	6±0	7±0	7±0	8±0	
CHOc	5±0	3±0*	6±0	7±0	7±0	5±0*	7±0	7±0	8±0	

**Table 1:** Thermal sensation ratings during the soccer-specific protocol.

Note: Pre – before pre-cooling; Post – after pre-cooling; 1-6 – Blocks; 1-6, HT – Half-time.

\* PLAc and CHOc significantly lower than PLA and CHO



Figure 1: Activity profile of a single 15-min block of the soccer-specific protocol.



Figure 2: Schematic illustration of the experimental protocol.



Pre – before pre-cooling; Post – after pre-cooling; 1-6 – Blocks 1-6, HT – Half-time.

\* PLA and CHO significantly greater than PLAc and CHOc. † PLA, PLAc and CHO significantly greater than CHOc. ‡ CHO significantly greater than PLAc and CHOc.

# CHO significantly greater than CHOc.



† CHOc significantly higher than PLA, PLAc and CHO. \* CHOc and CHO significantly higher than PLA.



Figure 5: Mental concentration during soccer-specific protocol. \* CHOc significantly greater than PLA.



‡ CHO and CHOc significantly greater than PLA and PLAc. \* PLA and PLAc significantly greater than CHO and CHOc.