Title of article: Addition of Caffeine to a Carbohydrate Feeding Strategy Prior to Intermittent Exercise.

Preferred running head: CHO and Caffeine feeding strategy for soccer.

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Abstract

The ergogenic effect of caffeine is well established, although no investigations providing a high carbohydrate feeding strategy (pre-exercise meal = 2g/kg BM) co-ingested with caffeine exist for soccer. This investigation examines the effect of caffeine in addition to a pre-exercise carbohydrate meal and drink mid-way through a soccer simulation. Eight recreational soccer players completed an 85-minute soccer simulation followed by an exercise capacity test (Yo-yo Intermittent Endurance test level 2) on two occasions. Prior to exercise participants consumed a high carbohydrate meal, with placebo or 5mg/kg BM^{-1} caffeine. No significant performance effect was identified ($p=0.099$) despite a 12.8% (109m) improvement in exercise capacity following caffeine. Rates of carbohydrate and fat oxidation did not differ between conditions and nor were differences apparent for plasma glucose, fatty acids, glycerol, β-hydroxybutyrate ($p>0.05$). However, an increase in lactate was observed for caffeine ($p=0.039$). A significant condition effect on rating of perceived exertion was identified ($p<0.001$), with the overall mean for the protocol lowered to 11.7±0.9au for caffeine compared to 12.8±1.3au. Caffeine supplementation with a carbohydrate feeding strategy failed to affect metabolic and metabolite responses, although reductions in perception of exercise were observed. While a 12.8% increase in exercise capacity was noted the findings were not significant, possibly due to the small sample size.

Introduction

The requirement for carbohydrate (CHO) to fuel high intensity intermittent team sport activity is a key necessity [1], especially if the duration is longer than 60 minutes. Studies have illustrated a significant reduction in muscle glycogen stores during soccer match play that may be linked to a decreased distance covered at high intensities, and a limit in sprint performance [2, 3]. Similar findings of positive performance outcomes in favour of CHO feeding prior to laboratory controlled high intensity intermittent exercise simulations concur with those findings during match play [4, 5]

A significant number of athletes, including soccer players, also co-ingest caffeine or caffeine-containing products prior to matches to gain an ergogenic response [6, 7]. The performance benefits of caffeine have been long established and early investigations observed increases in exercise capacity when cycling at 80% $\dot{V}O_{2max}$, [8], similar to the overall intensity during soccer that is suggested to be approximately 75% [9], and enhanced fat metabolism during 120 minutes of cycling [10, 9] leading to an original concept that the ergogenic effects of caffeine are mediated by stimulation of circulating adrenaline resulting in elevated plasma fatty acid and a decreased use of the limited muscle glycogen stores [8]. More recently several studies have demonstrated performance gains during intermittent activity after caffeine ingestion when fat oxidation and glycogen stores have not been a limiting factor [12, 13, 14, 15]. These findings cast doubt on the Costill model concerning the likely effects of caffeine mobilising fats and sparing glycogen.

The most likely mechanism for caffeine action concerns the fact that caffeine acts as an adenosine antagonist in the central nervous system (CNS) and so stimulates this system [16]. Significant improvements in concentration, response speed and detection, and an improved complex of rapid visual information processing following the consumption of a caffeine-containing performance bar have been reported [17]. These findings illustrate that caffeine may be key to success when concentration and reactions are a crucial element. In addition, further mechanisms purported to promote the ergogenic effect of caffeine are an increase in Na+/K+ ATPase activity [18] and the mobilisation of intracellular calcium [19], both of which may improve muscular function during the high intensity components of soccer.

In addition to caffeine ingestion alone and the ergogenic properties this may induce, studies have suggested that the co-ingestion of caffeine with CHO can also increase glucose absorption and CHO oxidation [20, 21, 22, 23]. The co-ingestion of caffeine and CHO have been reported to improved cycling time trial performance by 9% compared to water and 4.6% compared to CHO alone [24], although with regards to intermittent exercise the literature is limited. Significant improvements

during intermittent sprint cycling have been observed [25], but this investigation lacked relevance to team sports. However, researchers [26] have investigated the effect of the co-ingestion of caffeine and CHO on rugby simulation by providing a beverage 60 mins prior to the start of the protocol and then three further boluses throughout the protocol. They found improvements in sprint performance, motor skills tests and a reduction in subjective ratings of exertion. In regard to soccer it has been demonstrated that improvements in a soccer-specific passing test can be achieved with caffeine [27], whilst further improvements in running, power-based activities and sprint performance have also been observed [28, 15]. However, these studies did not specify the pre-exercise meals consumed and are unlikely to be specific to match day nutritional intake [29], with regards to the timing and macronutrient content. The current investigation compares a high CHO pre-match meal fed 3.5 h prior to 85 min of intermittent high intensity exercise when caffeine or placebo were ingested 45 minutes prior to the exercise bout. The novelty of this investigation is that caffeine is administered following typical pre-match nutritional preparation with a high CHO meal and with the protocol commencing at an appropriate start time in line with the start of a match in professional sport (15:00). It is hypothesised that the caffeine would provide enhanced endurance performance following the high intensity intermittent exercise, but without significant changes in metabolism and metabolites.

Methodology

Participants

Eight male recreational soccer players (Age: 20±1 yrs., body mass [BM]: 78.36±11.67 kg, body height 1.81±0.05 m), all of whom trained twice a week and played a weekly competitive match were recruited for this investigation. Participants were informed of the potential risks and signed written informed consent. The investigation was approved by the institution's Research Ethics Committee and was conducted in accordance with the Ethical Standards in Sport and Exercise Science Research: 2020 Update [30].

Experimental design

The investigation was completed with a repeated measure, counterbalanced, double blind study design. Participants were required to attend the laboratory on four separate occasions. The first two were for familiarisation of the intermittent soccer protocol, in which participants completed 15 min of the high intensity intermittent protocol, followed by the Yo-yo Intermittent Endurance test level 2 (YIE2) [31], which has been found to be reproducible, sensitive and valid [32]. If the difference between the participants YIE2 score was greater than 2 shuttles, which equated to 80 meters,

additional laboratory visits were replicated in the same fashion as described above until test-retest distances were similar. Three participants had to attend the laboratory for an extra familiarisation session to account for this. The final two visits were to complete the two experimental conditions (Caf or Pl), separated by 7 days. Participants abstained from alcohol and physical exercise for 48 hours prior to testing and their diet 24 hours prior to the test was also kept constant for all trials. The same standardised breakfast of cereal, toast and fruit juice was consumed on all test days at 08:00 a.m. (total energy = 377 kcal; CHO = 79 g; Fat = 3 g; Protein = 13 g). Trials were conducted following the participants verbal confirmation that the previous diets had been followed.

Pre-exercise meal, CHO solution and Caffeine.

The CHO meal (Table 1) contained 984 kcal, with an estimation of Glycaemic Index calculated at 41 [33]. The macronutrient content was CHO: 61%, protein: 17%, fat: 22%. Prior to exercise and at half time the participants consumed 5 mL/kg^{-1} BM of a 5% glucose (dextrose powder) solution (Thornton & Ross Ltd, Huddersfield, England). In addition to the meals and drinks, participants were given either a 5 mg/kg^{-1} BM of caffeine, 98.5%, USP/BP (Acros Organics, NJ, USA), or a placebo 45 minutes prior to exercise.

Enter Table 1 here

Protocol

Following collection of the first venous blood sample (20 mL) at approximately 11:10, participants consumed the test meal at 11:30. During the postprandial period, participants drank 1 L of water (between 11:30 – 14:15) and rested until they returned to the laboratory at 14:15 for the caffeine or placebo supplement and pre-exercise blood sample.

Prior to the high intensity intermittent protocol, participants completed a standardised warm up consisting of light jogging and static stretching at 14:55. The protocol was an amended version of a soccer specific protocol [34] and completed on a motorised treadmill. This protocol has been used previously [35, 36]. The protocol imitates the activity patterns that occur in soccer matches and include bouts of walking (31 – 39 s), jogging (41 – 46 s), cruising (41 – 45 s), sprinting (16 – 21 s) and standing. The proportion of each individual activity is similar to those obtained from early motion analysis studies [37] and comprises of two 22 min 30 s blocks for each half of 45 min; although participants completed 85 min of the high intensity intermittent protocol before undertaking the YIE2. A 15 min half time break was provided during which participants drank 5mL.kg^{-1} of CHO

solution and a further venous blood sample was collected. The second half of the protocol was then completed (40 min). A final venous blood sample was collected immediately post-exercise before completion of the YIE2.

Venous blood samples were collected from the antecubital vein pre-meal, pre-exercise, at half-time, and post exercise, and analysed for glucose, fatty acid (FA), glycerol, β-hydroxybutyrate, and lactate. These metabolites were measured on a RX Daytona clinical chemistry analyser (Randox, Co. Antrim, UK). Expired gases were measured at four time points during the protocol (10, 35, 55 and 78 min). Furthermore, the first sample was not collected until after 10 min to ensure CO_2 metabolism was in a steady state. Expired air was collected using Douglas Bags (Cranlea, UK) for 90 seconds and analysed on a Servomex 1440 Gas Analyser (Servomex, UK). Substrate oxidation calculations for fat and CHO oxidation were made using the non-protein stoichiometric equation previously validated [38]. Subjective measurements of rating of perceived exertion [39] were recorded every 5 minutes. Heart rate was continually measured and averaged over 5 minutes periods using a Polar S610 (Polar, Kempele, Finland). Schematic of protocol can be seen below in Figure 1.

Insert Figure 1

Statistics

SPSS software (version 25 SPSS, Chicago, IL) was used for data entry and analysis for all studies. Analysis of variance for repeated measures on 2 factors (experimental condition and time) was used to analyse differences in the physiologic and metabolic responses in all trials, followed by Bonferroni post hoc test for determination of differences between specific groups. A paired t-test was used to analyse the YIE2 results between conditions. Effect size was calculated using Cohen's d effect size value calculation [40]. Differences were considered significant at $p<0.05$, and all data are presented as the mean $\pm$ SD.

Results

Performance (Figure 2): Only 7 out of the 8 participants performed the performance test due to a muscular injury sustained to one participant during the intermittent high intensity treadmill run, resulting in a low post hoc power (1-β err prob) = 0.12. No significant difference was found between conditions for the YIE2 between Caf (960 $\pm$ 282 m) and Pl (851 + 328 m), p = 0.099, CI_{95} -248.10-465.24. However, following the Caffeine trial, participants completed an additional 109 m, which equates to a 12.8% improvement. In fact, 5 out of the 7 participants produced a greater result in

YYIE2 after the Caf trial compared to the Pl. Further, Cohen's effect size value (*d* = 0.35) suggested low to medium practical significance.

Insert Figure 2

CHO oxidation and fat oxidation (Table 2): No significant differences were observed between conditions for CHO oxidation or fat oxidation during either half of the high intensity intermittent protocol.

Insert Table 2

Plasma metabolites: Table 3 represents measures of plasma glucose, FA, glycerol, β-hydroxybutyrate and lactate. No significant differences were observed between conditions or interactions for all metabolite apart from lactate, which produced a significant difference between conditions ($p=0.039$), time ($p=0.009$) and an interaction effect ($p=0.013$). However, no post hoc difference was reported. Furthermore, all plasma metabolite concentrations increased from the start of exercise and thereby produced significant time effects (plasma glucose $p<0.001$; FA $p<0.001$; glycerol $p<0.001$; β-hydroxybutyrate $p=0.003$; lactate $p<0.001$).

Insert Table 3

Heart rate and rating of perceived exertion (RPE): Significant increases were seen over time for both HR and RPE ($p<0.001$). The overall mean HR for each condition was 158 $\pm$ 7.8 bpm and 160 $\pm$ 7.1 bpm for the Pl and Caf trial respectively, which did not signify differences between conditions or interactions. However, a significant effect of condition on RPE was identified ($p<0.001$, CI_{95} -0.10-2.30), with the overall mean for the duration of the exercise reported as 12.8 $\pm$ 1.3 and 11.7 $\pm$ 0.9 for the Pl and Caf trial respectively (Figure 3). This result highlights a large practical significance with an effect size *d* = 0.95, and a post hoc medium power (1-β err prob) = 0.71 achieved.

Insert Figure 3

Discussion

The aim of the present study was to investigate the effects of caffeine prior to a high intensity intermittent protocol following an ecologically valid nutritional strategy. The main finding was that

performance was not statistically improved following additional caffeine supplementation, although an increase in performance of 109 m that equates to a 12.8% improvement was observed. Of further interest was that RPE was significantly reduced throughout the soccer simulation, most notably in the latter stages of the exercise bout, which is in agreement with previous studies [26, 41, 42]. This may offer some practical relevance for the use of caffeine within a nutritional strategy during competition, although whether this translates to a performance enhancement remains unclear. In addition, the low number of participants recruited for this study is a major limitation and therefore, caution should be exercised when interpreting these findings.

The efficacy of caffeine on exercise capacity tests have provided conflicting results. A previous report [43] failed to show any significance of caffeine ingestion versus a placebo during a run to exhaustion at 80% $\dot{V}O_{2max}$, despite slight increases in time to fatigue (Caffeine = 12.6 + 3.8 min; Placebo = 11.8 + 3.4 min), the authors suggested that the lack of CHO may have been the reason for the failure to produce favourable results. On the other hand, caffeine significantly enhanced progressive high-intensity intermittent exercise by 16%, employing the yo-yo intermittent recovery test level 2 even though CHO was not provided [14]. It may be plausible that the caffeine dose between studies is an important factor to consider, since the higher dose of 6 mg/kg BM^{-1} [14], compared to an acute does of 80 mg, that equated to approximately 1 mg/kg BM^{-1} [43], showed performance improvements.

When performance measures have been recorded during soccer simulations, positive results have been observed following caffeine ingestion as part of a strategy for performance enhancement. Sprint performance has been shown to improve during simulated soccer performance as well as repeated sprint ability, power and high intensity distance covered [15, 28]. These studies compared the effects of energy drinks with a moderate dose of caffeine (3-4 mg/kg BM^{-1}) against a placebo condition. In addition to the physical improvements highlighted the subjective experiences of players were also enhanced, leading to a conclusion that caffeine has the ability to offset many fatigue-inducing declines in self-selected components of performance specifically linked to soccer. Within a rugby union simulation, the authors [26] investigated the effects of the co-ingestion of caffeine and CHO and identified significant improvements in sprint performance. Although no pre-exercise meal was provided, an ecological approach was followed by providing a caffeine and CHO drink prior to exercise and throughout the rugby protocol. The authors observed a 3.6% improvement in 15 m sprint performance following the addition of caffeine to the CHO drink, with an improved mean sprint time of 2.71 $\pm$ 0.17s compared to the CHO drink and placebo solution resulting in similar times of 2.81 $\pm$ 15s and 2.81 $\pm$16s respectively. However, observations from

previous investigations [44] also utilising a rugby union protocol, noted that sprinting over 20 m produced unclear practical assessment whether the results had a substantial effect with improvements of 0.5-2.9%. Furthermore, in a soccer simulation in which caffeine was provided prior to the Loughborough Intermittent Shuttle Test, no improvements in mean 20 m sprint performance throughout the simulation were found [27].

Although it is not entirely clear what mechanisms can be attributed to any likely performance enhancement, the current understanding is that the primary mechanism of action is adenosine receptor antagonism [45]. Due to the similarity in structure between caffeine and adenosine, caffeine can block adenosine`s inhibitory neurophysiological action, as it binds to its cell membrane receptors providing a stimulatory action on the CNS. Caffeine increases the release of excitatory neurotransmitters that, among other factors, contributes to a greater motor recruitment or an increase in the frequency of motor unit activation [46], an enhanced sympathetic activation and reduced perception of pain and effort [47, 48]. A reduction in subjective measures observed with caffeine in the current investigation is in accordance with previous research [8, 41, 42, 49, 50]. This may also be linked to the CNS facilitation, enabling participants to exercise for greater durations at potentially higher intensities for longer. A meta-analysis [51] conducted found a reduction in RPE of almost 6% during steady state exercise with caffeine doses ranging from 4-10 mg/kg BM^{-1} and may partially account for performance gains. This agrees with the current investigation that highlighted a decreased RPE throughout the protocol following Caf trial (overall mean for Caf trial 11.7 $\pm$ 0.9; 12.8 $\pm$ 1.3 for Pl trial). Examining individual responses within the current investigation may also support more of a central effect of caffeine rather than peripheral metabolic alterations. For the two participants that performed worse on the Caf trial, no metabolic differences were identified to explain this reduction in performance.

Those effects may be supported by specific actions of caffeine in skeletal muscle, although their relevance during exercise remains unclear. Caffeine has been shown to attenuate the potassium imbalance that reduces membrane excitability during exercise and increases sarcoplasmic reticulum calcium release and myofilaments sensitivity to calcium, improving excitation-contraction coupling [45]. Together with the observed decrease of RPE, these factors may have enabled the participants to run 12.8% further during the performance component of the investigation, but not to significant values, and may potentially provide a rationale for this improvement as no other variables measured illustrate sufficient differences between conditions.

The current investigation found no significant effects on fat mobilization or fat oxidation rates, which contrasts with some previous studies whereby caffeine was ingested after an overnight fast [8, 14, 52, 53]. Increases in fat oxidation have been attributed to an increased release of adrenaline, which can enhance FA mobilization [52] by stimulating hormone sensitive lipase. Also, the antagonistic effects of caffeine on adenosine receptors may reduce the antilipolytic effects of adenosine on adipose tissue, further increasing FA breakdown [45]. These increases could be supported by an increase in fat oxidation and CHO sparing. However, as highlighted in a review article [45], several studies show that caffeine does not affect respiratory exchange ratio, FA oxidation and the expected plasma FA increase during exercise, therefore providing further evidence against the so called "lipolysis theory" as a potential suggestion for caffeine's ergogenesis. Clearly, the data from our study do not support this theory either.

The evidence with regard to a possible glycogen sparing effect of caffeine are also inconsistent. The additional CHO feeding in our study prior to the protocol as well as the consumption of a CHO drink during the half time period is likely to have blunted any potential increase in fat oxidation, most likely through elevated insulin concentrations [54, 55]. However, the current investigation did not measure insulin levels and so we are unable to reflect on this further except that it is worthy to note that there are several reports in murine models and cell culture showing that adenosine receptors may affect insulin secretion, skeletal muscle insulin sensitivity and liver glycogenolysis/gluconeogenesis, in addition to the already mentioned effects of adenosine on adipose tissue lipolysis [56].

Previous reports have observed that when caffeine is co-ingested with CHO there is a blunted effect of FA concentration and fat oxidation [11, 57]. It has been highlighted that a high CHO diet combined with a high CHO meal pre-exercise prevented an expected rise in FA concentration following caffeine ingestion and the authors concluded that nutritional factors influence the response of FA to caffeine ingestion [57]. In addition, the current investigation failed to observe an increase in the rate of CHO oxidation or a significant difference in blood glucose between conditions. This is in contrast with previous research whereby caffeine alone is provided [20, 21, 22, 23, 45].

Differences between study designs may be accountable for some of the conflicting findings due to variations in exercise modalities, intensities and durations. Previous investigations have mainly used steady state protocols that do not elicit intensities that may require increased energy contribution

from the anaerobic system and durations ranging from approximately 60 – 120 minutes, unlike the soccer specific protocol that includes periods of high intensity running and maximal sprints.

Despite the absence of effects on CHO and FA oxidation, there was a significant difference observed for plasma lactate following the Caf trial. Significant increases in lactate have been observed in previous research [13, 58, 59], although the exact mechanism for this increase is not entirely known. It has been suggested that the increased lactate response is attributed to the increased anaerobic work performed with caffeine during their sprinting periods. However, this would not be the case with the current investigation as the lactate was collected following the controlled soccer simulation by which the participants had covered the same distance. In addition, researchers [60] have concluded that there was no evidence that caffeine or epinephrine could increase muscle lactate release or decrease blood lactate clearance following their investigation which resulted with an increased lactate response.

In conclusion, the additional caffeine supplementation following a CHO meal may reduce the perceived effort (RPE) undertaken during a soccer simulated protocol and enhance endurance capacity somewhat following a simulated soccer match. However, there was no evidence that those effects were associated to a change in energy metabolism and substrate partitioning during exercise, which suggests that central and/or other effects (possibly mediated via adenosine antagonism) could be responsible for the observed improvements. Nevertheless, caution should be exercised when interpreting these findings due to a lack of statistical power.

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Table 1 – Energy and macronutrient breakdown of test meal.

Food	Amount	CHO (g)	Protein (g)	Fat (g)	Energy (kcals)
Apple juice	590 ml	65.5	0.6	0	248.0
Basmati rice	100 g	71.1	9.7	1.3	317.1
Chicken breast	100 g	0.3	26.1	5.8	157.7
Tomato based sauce	300 g	23.1	5.1	17.1	260.9
Total	**-**	**160.0**	**41.5**	**24.2**	**983.7**

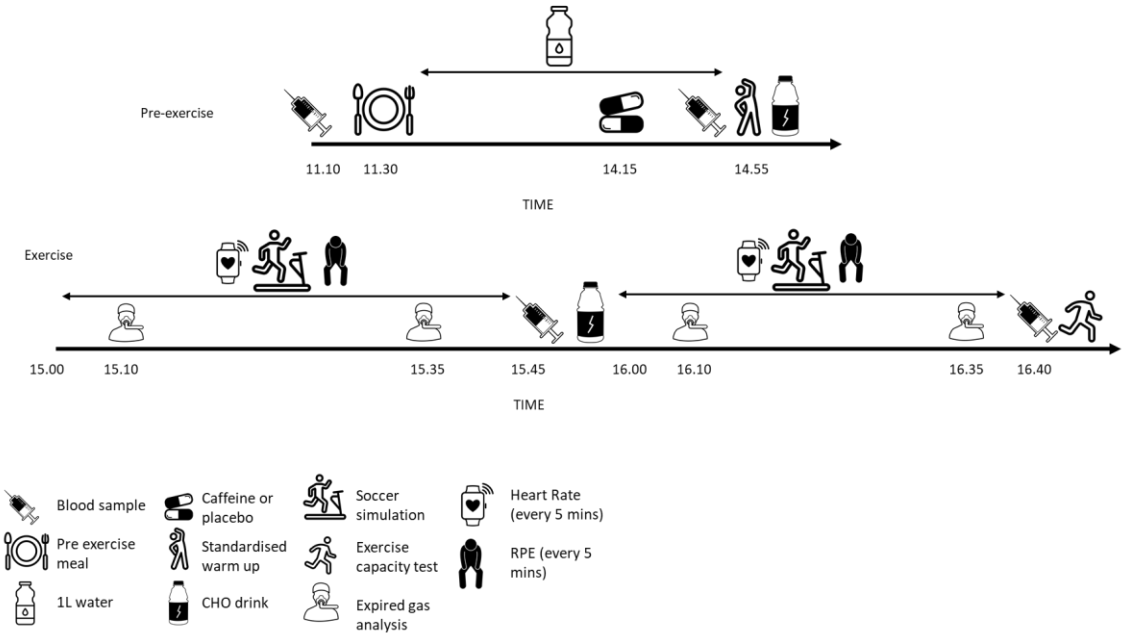

Figure 1 – Schematic representation of protocol.

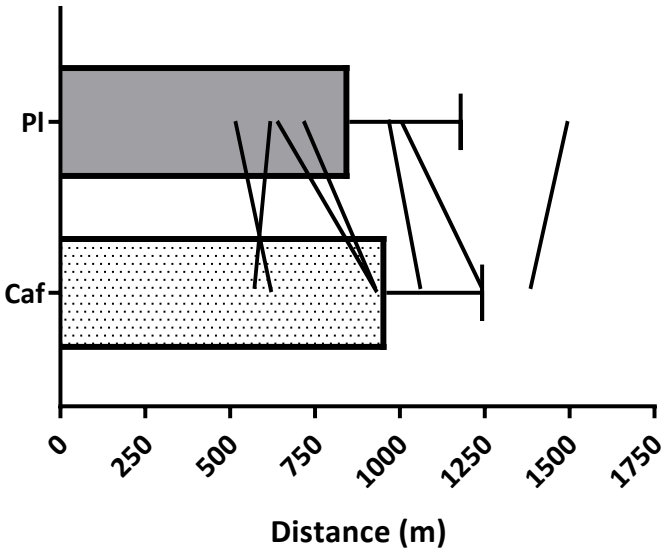

Figure 2: Performance results from the YYIE2 for both Caf and Pl trials. Bars illustrate the mean ± SD for the group, with lines indicating individual responses.

Table 2 – Mean± SD CHO and fat oxidation between conditions during the first and second half of the protocol.

	CHO Oxidation (g.min^{-1})		**Fat Oxidation (g.min^{-1})**	
	1st half	**2nd half**	**1st half**	**2nd half**
Pl	2.95 ± 0.39	2.79 ± 0.64	0.41 ± 0.22	0.41 ± 0.23
Caf	2.48 ± 0.54	2.92 ± 0.74	0.54 ± 0.27	0.50 ± 0.21

Table 3 – Mean ± SD results from plasma metabolites throughout the trials.

	Pre-meal	Pre-exercise	Half time	Post-exercise	
		Pl			**Effect**
Glucose (mmol/L)	5.84 ± 0.57	5.53 ± 0.68	6.49 ± 0.67	6.42 ± 0.45	
FA (mmol/L)	0.29 ± 0.17	0.17 ± 0.07	0.46 ± 0.10	0.84 ± 0.27	
Glycerol (mmol/L)	23.29 ± 20.73	12.81 ± 10.56	59.93 ± 19.75	118.13 ± 45.03	
β-hydroxybutyrate (mmol/L)	0.040 ± 0.013	0.030 ± 0.009	0.041 ± 0.012	0.054 ± 0.022	
Lactate (mmol/L)	1.62 ± 0.63	1.88 ± 1.27	3.16 ± 1.02	2.26 ± 0.89	
		Caf			**Effect**
Glucose (mmol/L)	5.31 ± 0.52	5.48 ± 0.69	6.77 ± 1.22	6.96 ± 1.18	b
FA (mmol/L)	0.18 ± 0.06	0.26 ± 0.11	0.53 ± 0.22	0.69 ± 0.39	b
Glycerol (mmol/L)	13.81 ± 6.69	8.56 ± 4.48	68.25 ± 38.09	111.38 ± 56.01	b
β-hydroxybutyrate (mmol/L)	0.035 ± 0.016	0.034 ± 0.017	0.062 ± 0.021	0.058 ± 0.034	b
Lactate (mmol/L)	1.75 ± 0.68	1.51 ± 0.45	3.95 ± 1.52	3.55 ± 1.21	a, b, c

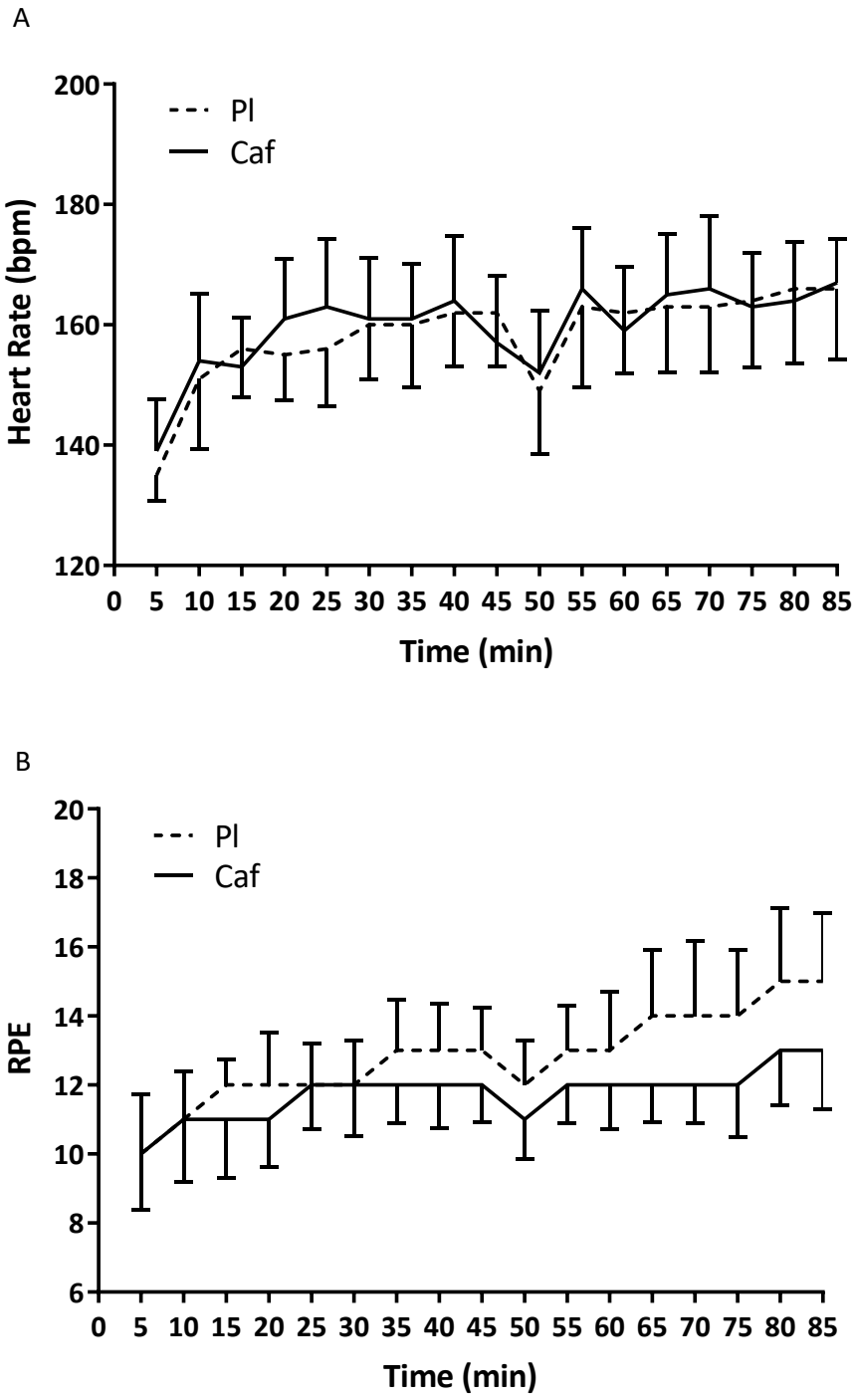

Figure 3: Mean ± SD for HR (A) and RPE (B) throughout the high intensity intermittent protocol for both Pl and Caf treatments. Significant condition effect of observed for RPE ($p<0.001$).