University of Bath

Alternative formats
If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

Title page:

Full title:

Effect of combined carbohydrate-protein ingestion on markers of recovery following simulated rugby union match-play.

Running title:

Carbohydrate-protein intake and rugby recovery.

Key words:

Sports nutrition, supplements, muscle damage, team sport.

Authors:

Simon P. Roberts, Keith A. Stokes, Grant Trewartha, Patrick Hogben, Jenny Doyle, & Dylan Thompson.

Institutions:

Department for Health, University of Bath, Claverton Down, Bath, BA2 7AY, UK

Corresponding Author:

Simon P. Roberts

Department for Health

University of Bath

Claverton Down

Bath

BA2 7AY

Tel:　　(+44) (0)1225 384531

Fax:　　(+44) (0)1225 383275

Email: s.p.roberts@bath.ac.uk

Effect of combined carbohydrate-protein ingestion on markers of recovery following simulated rugby union match-play.

Abstract

This study investigated the effect of ingesting carbohydrate alone or with protein, on functional and metabolic markers of recovery from a rugby union-specific shuttle running protocol. On three occasions, at least one week apart in a counterbalanced order, nine experienced male rugby union forwards ingested placebo, carbohydrate (1.2 g·kg body mass·hr^{-1}) or carbohydrate with protein (0.4 g·kg body mass·hr^{-1}) before, during and after a rugby union-specific protocol. Markers of muscle damage (creatine kinase, pre, 258 ± 171 vs 24 hours post, 574 ± 285 U·l^{-1}; myoglobin, pre, 50 ± 18 vs immediately post, 210 ± 84 nmol.l^{-1}, $P<0.05$) and muscle soreness (1, 2 and 3 [maximum soreness = 8] for pre-, immediately post- and 24 hours post-exercise, respectively) increased. Leg strength and repeated 6s cycle sprint mean power were slightly reduced post-exercise (93 and 95% of pre-exercise, respectively, $P<0.05$), but were almost fully recovered after 24 hours (97 and 99% of pre-exercise, respectively). There were no differences between trials for any measure. These results indicate that in experienced rugby players, the small degree of muscle damage and reduction in function induced by the exercise protocol were not attenuated by the ingestion of carbohydrate and protein.

Introduction

Rugby union match-play elicits muscle damage as evidenced by elevated systemic markers of damage such as plasma creatine kinase (CK) and myoglobin (Mb) (Gill, Beaven, & Cook, 2005; Mashiko, Umeda, Nakaji, & Sugawara, 2004; Smart, Gill, Beaven, Cook, & Blazevich, 2008; Takarada, 2003). It is likely that this damage occurs as a result of physical impacts as well as the mechanical and metabolic stresses associated with exercise (Cunniffe et al., 2010; Smart et al., 2008; Takarada, 2003) and the resulting pain and reduction in muscle function might be considered a limitation to subsequent training and match performance. In order to maintain physical and skill attributes throughout a season, it is important that interventions are identified that allow players to recover as quickly as possible (French et al., 2008).

Carbohydrate ingested after exercise is important for replenishing post-exercise glycogen reserves (Betts, Williams, Duffy, & Gunner, 2007) but has not been found to influence recovery from exercise-induced muscle damage (Cockburn, Hayes, French, Stevenson, & St Clair Gibson, 2008; Green, Corona, Doyle, & Ingalls, 2008; Miles, Pearson, Andring, Kidd, & Volpe, 2007). In contrast, ingestion of protein during or after exercise has been reported to reduce perceived muscle soreness and systemic markers of muscle damage (Greer, Woodward, White, Arguello, & Haymes, 2007) and to expedite post-exercise recovery of muscle function following prolonged exercise in untrained men (Etheridge, Philp, & Watt, 2008). Co-ingesting carbohydrate and protein is therefore an intuitively attractive strategy for post-exercise recovery. Several studies have reported that the co-ingestion of carbohydrate and protein before, during or after exercise can attenuate the rise in CK during recovery (Baty et al., 2007; Romano-Ely et al., 2006; Saunders et al., 2004; Saunders et al., 2007), while others have found no effect (Betts et al., 2009; Breen, Tipton, & Jeukendrup, 2010; Green et al., 2008; White et al., 2008; Wojcik et al., 2001). However, muscle function probably offers

the most valid assessment of muscle damage (Clarkson & Hubal, 2002) and is also more relevant than CK in an applied setting. Cockburn et al. (2008) reported recovery of peak torque of the knee flexors to be improved following co-ingestion of carbohydrate and protein compared with carbohydrate alone but other studies suggest that the inclusion of protein to carbohydrate had no effect on recovery of muscle function (Betts et al., 2009; Breen, Tipton, & Jeukendrup, 2010; Green et al., 2008; White et al., 2008; Wojcik et al., 2001). The total protein and carbohydrate ingested in these studies has varied from 23 and 75 g, of protein and carbohydrate, respectively (White et al., 2008) up to 165 and 492 g (Betts et al., 2009). The influence of carbohydrate and protein co-ingestion on recovery from rugby-specific exercise, when upper and lower body work is carried out and muscle damage might result from impact as well as metabolic and mechanical stress, has not been studied.

Changes in cortisol and testosterone concentrations have been reported following rugby match-play (Cunniffe et al., 2010). These changes might reflect recovery status in as much as high cortisol and low testosterone concentrations have been suggested to be indicative of a catabolic hormonal environment (Cunniffe et al., 2009; Elloumi, Maso, Michaux, Robert, & Lac, 2003). Carbohydrate and protein ingestion may increase post-exercise testosterone (Rowlands, Thorp, Rossler, Graham, & Rockell, 2007) and reduce cortisol concentrations which in turn might impact post-exercise protein metabolism (Bird, Tarpenning, & Marino, 2006a). The impact of circulating hormone concentrations on muscle protein synthesis is, however, open to question (West et al., 2009).

To date, one study has investigated the effect of a nutritional intervention on post-rugby match muscle damage and hormonal responses (Minett, Duffield, & Bird, 2010) but this involved a multinutrient supplement and the interaction of ingredients may have masked the

individual effects. Although using match-play maximises ecological validity, the lack of experimental control, for example in the amount of work performed, limits the detection of changes in performance outcomes. Therefore, the purpose of the present study was to examine the effects of carbohydrate and combined carbohydrate-protein ingestion on systemic indices of muscle damage, recovery of performance and salivary cortisol and testosterone concentrations in trained rugby players following a simulated rugby union match-play protocol.

Methods

Participants

Nine experienced male rugby union players (mean $\pm$ *s*: age = 21.8 $\pm$ 3.3 years, height = 183 $\pm$ 5 cm, body mass = 93.3 $\pm$ 13.7 kg, playing experience = 12 $\pm$ 4 years) volunteered and gave written informed consent to take part in this study which had institutional ethics committee approval. All participants played to University standard and testing was performed in-season when participants were involved in competitive match-play and regular training and therefore accustomed to rugby specific exercise.

Experimental Trial/Treatments

Participants completed three main trials in a random order with at least 7 days between trials: Placebo (Plac), Carbohydrate (CHO) and Carbohydrate with Protein (CHO-P). In the Plac trial, participants consumed a low-calorie orange (0.3 g carbohydrate per 100 ml) flavoured concentrate (Robinsons, Britvic, Chelmsford, UK) with water and aspartame artificial sweetener (Hermesetas, Zurich, Switzerland). In the CHO trial, participants consumed a carbohydrate-electrolyte solution (Glaxo-Smithkline, UK) containing 1.2 g.kg body mass.hr^{-1} in a 9% solution (mean total carbohydrate ingestion of 261 $\pm$ 38 g). In the CHO-P trial, participants consumed the same amount of carbohydrate combined with 0.4 g.kg body mass.hr^{-1} (Betts et al, 2009) whey protein isolate (Myprotein, Cheadle, UK) in a 9% solution, resulting in a mean total ingestion of 87 $\pm$ 13 g. This amount of carbohydrate has been reported to optimise muscle glycogen synthesis (Jeukendrup & Jentjens, 2000), while the addition of the protein dose has been suggested to enhance whole body net protein balance in recovery (Howarth, Moreau, Phillips & Gibala, 2009). An initial bolus of 500 ml was consumed 1 hour prior to exercise with the remaining solution (mean volume 1157 $\pm$ 235 ml)

divided equally into three separate opaque drinking bottles and consumed during the exercise protocol rest periods between blocks 1 and 2, 2 and 3 (half time) and 3 and 4. One hour after completing the exercise protocol, participants were provided with additional Plac, CHO or CHO-P solution calculated to be the equivalent of that ingested over one hour of exercise (mean volume = 1128 ± 416 ml). This drink was consumed within the following 2 hours.

Participants were asked to record their diet for 48 hours prior to their first main trial and for the remainder of the trial day after exercise, and then to replicate this for trials 2 and 3. Normal diets were maintained during this 48-hour period but participants were asked to refrain from ingesting any protein supplements. Participants were asked to refrain from strenuous exercise and any resistance training exercise the day before each main trial and for the remainder of the day of a trial. For the remaining days between trials, a normal training and playing schedule was carried out.

Preliminary testing

At least 7 days prior to their first main trial, participants attended a habituation session during which they performed the BURST (see below for description) for 30 min. On a separate occasion, participants undertook a habituation session for the vertical jump, cycle ergometer repeated sprint test and strength tests. During all habituation sessions, participants were instructed on appropriate techniques for each test.

Exercise protocol

Bath University Rugby Shuttle Test (BURST)

The BURST is a rugby union-specific match-play protocol derived from physical demands data for elite rugby union forwards (Roberts et al., 2008). The exercise requirements have

been described in detail elsewhere (Roberts, Stokes, Weston, & Trewartha, 2010). Briefly, the protocol comprises 16 x 315-s exercise periods grouped into 4 x 21-min blocks (Figure 1). Blocks 1 and 3 are followed by 4-min breaks, with 2 min allocated each to standing and walking. A 10-min 'half time' break follows block 2, comprising 7 and 3 min of sitting and walking, respectively. For each identical 315-s exercise period, participants perform shuttles of walking, cruising and jogging interspersed with bouts of simulated scrummaging, rucking and mauling. A Performance Test (a sustained high-intensity test of speed and agility lasting approximately 17 s) and 15-m sprint are also performed within the 315-s block. Timing is maintained by audible signals from a specifically pre-recorded compact disc. The BURST has been previously shown to induce mean values of 160 beats.min^{-1} for heart rate and 4.6 mmol.l^{-1} for blood lactate while distance travelled (7078 m) has been validated against match play demands derived from time-motion analysis (6418 m). The time spent performing contact events was the same for both match play and the BURST (9.9%) (Roberts et al., 2010). The coefficient of variation over two trials for heart rate, mean Performance Test times and 15-m sprint times during the BURST were 2.2, 1.3 and 0.9%, respectively (Roberts et al., 2010).

FIGURE 1 NEAR HERE

Repeated sprint test (RST)

The RST was performed against a load equivalent to 7.5% of the participant's body mass on a friction loaded cycle ergometer (Peak bike, Monark, Sweden) interfaced to a computer. Saddle height was standardised for each participant and feet were securely fastened to the pedals. The test consisted of 6 x 6 s sprints, each separated by 30 s of recovery. From a rolling start at 60 rpm with no resistance, the weight was applied to the flywheel and the

participant sprinted for 6 s in a seated position after which they stopped for 20 s and then cycled for 10 s with no resistance on the flywheel in preparation for the next sprint. Power output (Watts) was calculated for every 1-s interval of each 6-s sprint. Calculations were made for Peak Power (PP) and Mean Power (MP). Coefficients of variation for mean power and peak power, derived from a previous pilot study were 2.4 and 3.5%, respectively.

Strength tests

Bench press, bench pull and leg press were performed on a Concept II Dyno (Concept 2 Inc, Morrisville, USA). Each exercise set started with a warm-up of 3 repetitions, aiming for approximately 50% of the maximum achieved during familiarisation, after which the participant performed one set of 3 maximal efforts. During recovery between repetitions, the participant was required to return pull/push handle to the start position in 5 s in order to standardise flywheel deceleration. The mean of the three efforts was recorded. Coefficients of variation determined in a pilot study for the bench press, bench pull and leg press were 2.6, 2.6 and 3.8%, respectively.

Experimental procedure (Figure 1)

All main trials were performed in the morning to control for any diurnal variation. On waking, participants were asked to ingest 500 ml of water to help ensure euhydration. On arrival in the laboratory, participants provided a 3 ml saliva sample, before sitting quietly while a resting 600 µl capillary blood sample was obtained via a fingertip. They were then asked to rate their whole body active soreness (Thompson, Nicholas & Williams, 1999) and soreness for individual muscle groups on a diagram using a scale ranging from 0 (not sore) to 8 (very, very sore) (Figure 2). Following this, a warm-up was performed consisting of 3 min

of cycling at a pedal cadence of 60 rpm with a flywheel resistance of 1 kg, followed by 30 s of passive recovery, 30 s at 80 rpm, 30 s of passive recovery and 30 s at 110 rpm with flywheel resistance of 2 kg. Participants then performed the strength tests. Three countermovement vertical jumps with hands placed on hips, were then carried out using a contact mat (SportyCo, Manchester UK), and the highest jump recorded. Coefficient of variation for vertical jump was 3.0%. Immediately after the vertical jumps, the RST was performed. Following this, participants ingested a 500 ml solution of either Plac, CHO or CHO-P. One hour after the first blood sample, a further 600 µl capillary blood sample was obtained and another saliva sample collected. Participants then began a standardised 10-min warm-up consisting of jogging and sprinting for 5 min and one 315-s period of the BURST protocol excluding the Performance Test. After 5 min of recovery, the participants then began the BURST protocol during which heart rate (HR) was sampled every 5 s and the mean recorded for every 315-s period. A 300 µl capillary blood sample was collected approximately 30 s after the completion of every 20-min exercise block and a 600 µl capillary sample and a saliva sample was obtained at the end of exercise. Participants then completed the muscle soreness questionnaire before performing the warm-up, strength tests, vertical jumps and RST in the same manner as pre-exercise. Participants returned to the laboratory between 20-24 hours after the completion of the post-exercise tests. Samples for saliva and blood were obtained, followed by the warm-up, strength tests, vertical jump and RST.

FIGURE 2 NEAR HERE

Blood and saliva sampling and analysis

Capillary blood samples were obtained from a fingertip and stored on ice in EDTA prepared collection tubes (Microvette 500, Sarstedt, Numbrecht, Germany). Within 20 min of collection all samples were analysed for blood lactate and glucose (YSI 2300 Stat Plus, YSI, Ohio, USA). For the samples taken 60 min pre-, 10 min pre-, immediately post- and 24 hours post-exercise, the remaining whole blood sample was centrifuged at 10,000 rpm for 10 min after which, the plasma was dispensed into a microtube and stored at -20ºC until further analysis. Plasma was analysed for CK activity and Mb concentration using commercially available kits (CK110 and MY2127, respectively; both Randox Laboratories, Crumlin, Northern Ireland) on a Cobas Mira 'N' spectrophotometer (Roche Diagnostics, Switzerland). Saliva samples were obtained via passive drool into a sterile polypropylene collection tube. Participants rinsed their mouth with water thoroughly a few minutes prior to providing the sample to negate the possibility of contamination from the solutions consumed throughout the test. Samples were immediately placed on ice and then stored at -20ºC until analysis. On the day of analysis, samples were thawed, mixed using a vortex and centrifuged at 3,000 rpm for 15 min. Following this, the clear sample was removed and analysed for testosterone and cortisol concentrations by a competitive ELISA method, using commercially available kits (Salivary testosterone enzyme immunoassay kit; Salivary cortisol enzyme immunoassay kit, both Salimetrics, USA). The coefficients of variation for 20 replicates of CK, Mb, salivary testosterone and salivary cortisol, were 1.6%, 1.4%, 3.2% and 4.3%, respectively.

Statistical analysis

Data for Performance Tests, 15-m Sprints, Rating of Perceived Exertion and Heart rate are presented as the single 'baseline test' and thereafter, the means of 21-min blocks. Results are reported as the mean $\pm$ standard deviation of the mean (*s*). Data were analysed using a two-way (treatment x time) analysis of variance (ANOVA) with repeated measures. When the

assumption of sphericity was violated, the Greenhouse-Geisser correction was used. In the event of a significant outcome, a Bonferroni *post hoc* test was applied to reveal where the difference lay. Significance was accepted at $P < 0.05$ for all analyses. When ANOVA was used to test for trial order effects for performance measures, no differences were found. Descriptive statistics are used for discrete data (muscle soreness ratings) with values presented as median and interquartile ranges. Effect sizes were calculated according to Cohen's *d* statistic with 0.2, 0.5 and 0.8 considered as small, moderate and large effects, respectively.

Results

Heart rate, blood lactate and glucose

No difference was found between mean heart rates or changes over time for Plac (160 ± 11), CHO (161 ± 8) or CHO-P (158 ± 11 beats·min^{-1}) trials. There was no difference between conditions for blood lactate but there was a main effect of time ($P < 0.001$; Figure 3A) with *post-hoc* analysis showing lower concentration samples at -60 min and 0 min compared with all other time-points ($P < 0.05$). For blood glucose, there was a main effect between conditions ($P = 0.047$) with *post hoc* analysis revealing Plac to be lower than CHO ($P < 0.05$). There was a main time effect ($P = 0.006$) with *post-hoc* analysis revealing a difference between 21 and 42 min ($P < 0.05$) as well as an interaction effect ($P = 0.005$; Figure 3B).

FIGURE 3 NEAR HERE

Vertical Jump

There was no main effect of trial for vertical jump height between Plac (37.7 ± 1.1 cm), CHO (37.8 ± 0.8 cm), or CHO-P (38.5 ± 1.2 cm). There was a main effect of time ($P = 0.03$), although *post-hoc* tests did not identify where any differences existed. The effect size for the difference between pre- and immediately post-exercise was large for Plac ($d = 0.8$) and small or moderate for Plac ($d = 0.4$) and CHO ($d = 0.5$) (Figure 4). There was no interaction effect.

FIGURE 4 NEAR HERE

Strength Tests

There was no difference between conditions for mean values achieved for bench press, bench pull or leg press (Table 1). There were main effects of time for the bench pull ($P = 0.040$) and

leg press ($P < 0.001$). *Post-hoc* testing did not identify where any differences existed for bench pull (pre- to immediately post-exercise; $d = 0.2$), but revealed a reduction in strength immediately post-exercise compared with pre for leg press ($P = 0.001$, $d = 0.5$).

TABLE 1 NEAR HERE

Repeated Sprint Test

No differences were found between conditions for mean MP and mean PP over the RST performed pre-, post- and 24 hours post-exercise (Table 2). There was a main effect of time for MP ($P = 0.004$), with *post-hoc* analysis showing immediately post-exercise MP to be lower than pre-exercise ($P = 0.018$, $d = 0.4$) There was no time effect for PP ($P = 0.058$), with a small effect when comparing pre- and immediately post-exercise ($d = 0.3$).

TABLE 2 NEAR HERE

Muscle Soreness

There were no differences between conditions in the median values for either whole, lower body or upper body muscle soreness. On the 0-8 scale for whole body soreness, there was an increase of one point collectively for all conditions, with pre-exercise values of 1 (1-2), rising to 2 (2-3) immediately post-exercise. These values remained elevated above pre-exercise at 24 hours post-exercise (Plac: 3; CHO: 3 and CHO-P: 2). Lower body soreness increased following exercise with median values of 0 (0-1), 2 (1-3) and 2 (2-2) pre-, immediately post- and 24 hours post-exercise, respectively. Upper body soreness increased to a lesser extent with values of 0 (0-1), 1 (0-2) and 1 (0-1) pre-, immediately post- and 24 hours post-exercise, respectively.

Plasma creatine kinase and myoglobin

There was no main effect of trial for either Mb or CK. There was a main effect of time for both Mb (Figure 5A) and CK (Figure 5B) ($P < 0.001$) with *post-hoc* analysis for CK and Mb showing increased values immediately post- compared with pre-exercise and at 24 hours post- compared with pre-exercise. Mb was also significantly lower at 24 hours post- compared with immediately post-exercise. There was a large effect for CK, from immediately post- to 24 hours post-exercise in Plac ($d = 1.2$) and small effects for CHO ($d = 0.1$) and CHO-P ($d = 0.2$). There was no interaction effect for either Mb or CK.

FIGURE 5 NEAR HERE

Cortisol and testosterone

There was no difference between conditions for either salivary cortisol or testosterone. There was an effect of time for both hormones ($P < 0.001$; Figure 6) with *post ho*c analyses showing lower cortisol at 24 hours post-exercise compared with all other time points ($P < 0.05$) and lower testosterone at 24 hours post-exercise compared with -60 min pre- and immediately pre-exercise ($P < 0.05$). There was no interaction effect for either cortisol or testosterone.

FIGURE 6 NEAR HERE

Performance Test and 15-m Sprint

No main effect of trial was found for time taken to complete the Performance Test (Plac, 17.59 ± 1.25 s; CHO, 17.42 ± 0.92 s; CHO-C, 17.45 ± 1.02 s; $P = 0.617$). There was a main effect of time ($P < 0.01$), with *post-hoc* analysis revealing shorter times at baseline compared

with blocks 3 and 4 ($P < 0.05$). There was no main effect of trial for 15-m sprint times (Plac, 2.78 ± 0.17 s; CHO, 2.77 ± 0.18 s; CHO-P, 2.77 ± 0.17 s; $P = 0.961$). There was a main effect of time ($P = 0.05$) with *post-hoc* analysis revealing shorter times for baseline and block 1 compared with the block 4 ($P < 0.05$). No interaction action effect was found for either the Performance Test or 15-m sprint test.

After each trial, participants were asked to attempt to identify which treatment they had received. Of the 27 trials completed, the correct treatment was only identified for 11 trials. Three participants identified all three trials correctly while a further two participants only identified the placebo trial. All others guessed incorrectly for all three trials.

Discussion

The purpose of this study was to determine the effect of carbohydrate and carbohydrate co-ingested with protein on the recovery of selected performance parameters and markers of muscle damage following a rugby union specific exercise protocol (BURST). Modest elevations in blood CK and Mb concentrations offer evidence of some muscle damage. However, strength and power indices returned to near pre-exercise values after 24 hours of recovery in all trials, indicating that the trained players taking part in the current study did not exhibit impaired muscle function as a result of the BURST protocol. Ingestion of either CHO or CHO-P did not attenuate the increase in systemic muscle damage markers, and there was no difference in recovery of muscle function in the following 24 hours compared with placebo.

Strength and power (RST) were lower and muscle soreness was increased immediately post-exercise compared with pre-exercise values. Compared with the current study, larger reductions in mean peak power output in cycle ergometry were shown immediately after plyometric exercise (Byrne and Eston, 2002; Twist and Eston, 2005) and in contrast to almost complete recovery of peak power after 24 hours in the current study, these authors also reported reductions in peak power of 18% (Byrne and Eston, 2002) and 11% (Twist and Eston, 2005) compared with pre-exercise. However, participants in the current study were habituated to the type of activity performed, whereas those in the study of Byrne and Eston (2002) were not. In agreement with the current study, Semark et al. (1999) showed that despite an increase in muscle soreness, there was no reduction in 30-m sprint performance 24 hours after eccentric lower limb exercise in trained games players. It is likely that the participants in the current study had a developed protection against exercise-induced muscle damage through previous training and match-play, therefore blunting the effect of damage caused during the BURST (Howatson & van Someren, 2008; Newham et al., 1987). This may

also in part explain the finding that participants only rated their whole body as 'slightly sore' 24 hours post-exercise.

The BURST induced some muscle damage as shown by modest elevations in plasma CK, Mb and muscle soreness immediately and 24 hours post-exercise compared with pre-exercise levels. Although these changes are modest, reflecting the trained nature of the participants, 24 hour post-exercise values (mean: ~580 $U \cdot l^{-1}$) are comparable with the post match-play values of 637 and 645 $U \cdot l^{-1}$ reported by Suzuki et al. (2004) and Minett et al. (2010), respectively. Others have reported slightly higher values following match-play (1081 $U \cdot l^{-1}$, Takarada, 2003; 1182 $U \cdot l^{-1}$, Cunniffe et al., 2009), although these changes are also modest. Although the frequency and total time spent performing contact work in the BURST was the same as that in match-play, the slightly lower CK values in the current study may be the result of the relatively controlled nature of the contact situations in which high impact collisions were minimised for participant safety. It is interesting that in studies using match-play as the exercise model, pre-exercise CK values were greater than those in the present study, possibly because participants trained the day before the match (Cunniffe et al., 2009).

Relative to the placebo, ingestion of carbohydrate or carbohydrate with protein did not reduce markers of muscle damage immediately and 24 hours following exercise. This finding concurs with a number of studies involving exercise with a large eccentric component (Betts et al., 2009; Etheridge et al., 2008; Green et al., 2008; Wojcik et al., 2001). Others have reported that co-ingestion of carbohydrate and protein attenuates the rise in CK 24 hours post-exercise (Romano-Ely et al., 2006; Rowlands et al., 2007; Saunders et al., 2007), although these studies employed cycle ergometry. Interestingly, the magnitude of the increase in CK in the current study is similar (<1000 $U \cdot l^{-1}$) to studies reporting an attenuating effect following carbohydrate-protein ingestion. One reason why the current study did not find an

effect of carbohydrate-protein ingestion might be because the participants were accustomed to the particular mode of exercise employed. The implication of these findings is that accustomed individuals do not gain any benefit from co-ingestion of carbohydrate and protein in terms of reducing muscle damage or improving recovery of muscle function.

In the current study there was no difference between pre- and immediate post-exercise salivary testosterone and cortisol concentrations. Other studies showed significant increases in post-match cortisol (Elloumi et al., 2003; Cunniffe et al., 2009), which might reflect the psychological stress of competition in contrast to laboratory-based exercise. In the present study there was a reduction in cortisol 24 hours post-exercise compared with pre-exercise, which agrees with the findings of both Elloumi et al. (2003) and Cunniffe et al. (2009) following match-play. However, both of these studies also reported testosterone concentration to be recovered to pre-match levels, one day after the match, while the present findings showed testosterone to be significantly lower than pre-exercise levels 24 hours post-exercise. Nutritional intervention did not alter testosterone or cortisol concentrations at any time point which is in contrast to evidence that ingestion of carbohydrate with protein during exercise can lower cortisol after rugby match-play (Minett et al., 2010) and resistance exercise (Baty et al., 2007; Bird, Tarpenning, & Marino, 2006b) and increase testosterone after endurance exercise (Rowlands et al., 2007). That these results were not present in the current study might be due to the fact that the aforementioned studies all used different exercise modes as the stimulus compared with the BURST which is composed mainly of high-intensity intermittent running. Although Minett et al., (2010) used rugby match play as the exercise model, the authors suggested that post-exercise reductions in cortisol may have been due, at least in part, to comparisons with elevated pre-exercise levels caused by pre-competition anxiety.

Conclusion

In conclusion, the current study has shown that a rugby union match-specific simulation (BURST) induced small increases in markers of muscle damage and muscle soreness in trained rugby players. Leg strength and mean power output were reduced immediately following exercise but recovered to close to pre-exercise levels after 24 hours of recovery. The ingestion of carbohydrate and carbohydrate co-ingested with protein did not influence recovery of strength or power or markers of muscle damage post-exercise. These findings suggest that individuals accustomed to a given exercise do not gain any benefit from co-ingestion of carbohydrate and protein in terms of reducing muscle damage or improving recovery of muscle function.

References

Baty, J.J., Hwang, H., Ding, Z., Bernard, J., wang, B., Kwon, B., & Ivy, J.L. (2007). The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage. *Journal of Strength and Conditioning Research*, 21, 321-329.

Beaven, C.M., Gill, N.D. & Cook, C.J. (2008). Salivary testosterone and cortisol responses in professional rugby players after four resistance exercise protocols. *Journal of Strength and Conditioning Research*, 22, 426-425.

Betts, J., Williams, C., Duffy, K., & Gunner, F. (2007). The influence of carbohydrate and protein ingestion during recovery from prolonged exercise on subsequent endurance performance. *Journal of Sports Sciences*, 25, 1449-1460.

Betts, J.A., Toone, R.J., Stokes, K., & Thompson, D. (2009). Systemic indices of skeletal muscle damage and recovery of muscle function after exercise: effect of combined carbohydrate–protein ingestion. *Applied Physiology, Nutrition and Metabolism*, 34, 773-784.

Bird, S.P., Tarpenning, K.M., & Marino, F.E. (2006a). Liquid carbohydrate/essential amino acid ingestion during a short-term bout of resistance exercise suppresses myofibrillar protein degradation. *Metabolism*, 55, 570-577.

Bird, S.P., Tarpenning, K.M., & Marino, F.E. (2006b). Independent and combined effects of liquid carbohydrate/essential amino acid ingestion on hormonal and muscular adaptations

following resistance training in untrained men. *European Journal of Applied Physiology*, 97, 225-238.

Breen, L., Tipton, K.D., & Jeukendrup, A.E. (2010). No Effect of carbohydrate-protein on cycling performance and indices of recovery. *Medicine and Science in Sports and Exercise*, 42, 1140-1148.

Byrne, C., & Eston, R. (2002). Maximal intensity isometric and dynamic exercise performance following eccentric muscle actions. *Journal of Sports Sciences*, 20, 951-959.

Clarkson, P.M., & Hubal, M.J. (2002). Exercise-induced muscle damage in humans. *American Journal of Physical Medicine and Rehabilitation*, 81, S52-69.

Cockburn, E., Hayes, P.R., French, D.N., Stevenson, E., & St Clair Gibson, A. (2008). Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. *Applied Physiology, Nutrition and Metabolism*, 33, 775-783.

Cunniffe, B., Hore, A.J., Whitcombe, D.M., Jones, K.P., Baker, J.S., & Davies B. (2010). Time course of changes in immuneoendocrine markers following an international rugby game. *European Journal of Applied Physiology*, 108, 113-122.

Elloumi, M., Maso, F., Michaux, O., Robert, A., & Lac, G. (2003). Behaviour of saliva cortisol [C], testosterone [T] and the T/C ratio during a rugby match and during the post-competition recovery days. *European Journal of Applied Physiology*, 90, 23-28.

Etheridge, T., Philp, A., & Watt, P.W. (2008). A single protein meal increases recovery of muscle function following an acute eccentric exercise bout. *Applied Physiology, Nutrition and Metabolism*, 33, 483-488.

French, D.N., Thompson, K.G., Garland, S.W., Barnes, C.A., Portas, M.D., Hood, P.E., & Wilkes, G. (2008). The effects of contrast bathing and compression therapy on muscular performance. *Medicine and Science in Sports and Exercise*, 40, 1297-1306.

Gill, N.D., Beaven, C.M., & Cook, C. (2006). Effectiveness of post-match recovery strategies in rugby players. *British Journal of Sports Medicine*, 40, 260-263.

Green, M.S., Corona, B.T., Doyle, A., & Ingalls, C.P. (2008). Carbohydrate-protein drinks do not enhance recovery from exercise-induced muscle injury. *International Journal of Sport Nutrition and Exercise Metabolism*, 18, 1-18.

Greer, B.K., Woodard, J.L., White, J.P., Arguello, E.M., & Haymes, E.M. (2007). Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 17, 595-607.

Howarth, K.R., Moreau, N.A., Phillips, S.M., & Gibala, M.J. (2009). Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *Journal of Applied Physiology*, 106, 1394-1402.

Howatson, G., & van Someren, K.A. (2008). The prevention and treatment of exercise-induced muscle damage. *Sports Medicine*, 38, 483-503.

Mashiko, T., Umeda, T., Nakaji, S., & Sugawara, K. (2004). Position related analysis of the appearance of and relationship between post-match physical and mental fatigue in university rugby football players. *British Journal of Sports Medicine*, 38, 617-621.

Miles, M.P., Pearson, S.D., Andring, J.M., Kidd, J.R., & Volpe, S.L. (2007). Effect of carbohydrate intake during recovery from eccentric exercise on interleukin-6 and muscle-damage markers. *International Journal of Sport Nutrition and Exercise Metabolism*, 17, 507-520.

Minett, G., Duffield, R., & Bird, S.P. (2010). Effects of acute multinutrient supplementation on rugby union game performance and recovery. *International Journal of Sports Physiology and Performance*, 5, 27-41.

Newham, D.J., Jones, D.A., & Clarkson, P.M. (1987). Repeated high-force eccentric exercise: Effects on muscle pain and damage. *Journal of Applied Physiology*, 63, 1381-1386.

Roberts, S.P. Trewartha, G., Higgitt, R. J., El-Abd, J., & Stokes, K.A. (2008). The physical demands of elite English rugby union. *Journal of Sports Sciences,* 26,825-833.

Roberts, S.P., Stokes, K.A., Weston, L., & Trewartha, G. (2010). The Bath University Rugby Shuttle Test (BURST): a pilot study. *International Journal of Sports Physiology and Performance*, 5, 64-74.

Romano-Ely, B.C., Todd, M.K., Saunders, M.J., & St Laurent, S. (2006). Effect of an isocaloric carbohydrate-protein-antioxidant drink on cycling performance. *Medicine and Science in Sports and Exercise*, 37, 1608-1616.

Rowlands, D.S., Thorp, R.M., Rossler, K., Graham, D.F., & Rockell, M.J. (2007). Effect of protein-rich feeding on recovery after intense exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 17, 521-543.

Saunders, M.J., Kane, M.D., & Todd, M.K. (2004). Effects of a carbohydrate-protein beverage on cycling endurance and muscle damage. *Medicine and Science in Sports and Exercise*, 36, 1233-1238.

Saunders, M.J., Luden, N.D., & Herrick, J.E. (2007). Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. *Journal of Strength and Conditioning Research*, 21, 678-684.

Semark, A., Noakes, T.D., St Clair Gibson, A., & Lambert, M.I. (1999). The effect of a prophylactic dose of flurbiprofen on muscle soreness and sprinting performance in trained subjects. *Journal of Sports Sciences*, 17, 197-203.

Smart, D.J., Gill, N.D., Beaven, C.M., Cook, C., & Blazevich, A. (2008). The relationship between changes in interstitial creatine kinase and game related impact in rugby union. *British Journal of Sports Medicine*, 42, 198-201.

Suzuki, M., Umeda, T., Nakaji, S., Shimoyama, T., Mashiko, T., & Sugawara, K. (2004). Effect of incorporating low intensity exercises into the recovery period after a rugby match. *British Journal of Sports Medicine*, 38, 436-440.

Takarada, Y. (2003). Evaluation of muscle damage after a rugby match with special reference to tackle plays. *British Journal of Sports Medicine*, 37, 416-419.

Twist, C., & Eston, R. (2005). The effects of exercise-induced muscle damage on maximal intensity intermittent exercise performance. *European Journal of Applied Physiology*, 94, 652-658.

West, D.W., Kujbida, G.W., Moore, D.R., Atherton, P., Burd, N.A., Padzik, J.P., De Lisio, M., Tang, J.E., Parise, G., Rennie, M.J., Baker, S.K., & Phillips, S.M. (2009). Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. *Journal of Physiology*, 587, 5239-5247.

White, J.P., Wilson, J.M., Austin, K.G., Greer, B.K., St John, N., & Panton, L.B. (2008). Effect of carbohydrate-protein supplementation timing on acute exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition*, 5, 5.

Wojcik, J.R., Walber-Rankin, J., Smith, L.L., Gwazdauskas, F.C. (2001). Comparison of carbohydrate and milk-based beverages on muscle damage and glycogen following exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 11, 406-419.

Table 1. Mean strength scores achieved for bench press, bench pull and leg press, pre-, post- and 24 hours post-exercise for each condition (mean ± s, n = 9). [#]$P < 0.05$ *vs* Pre.

	Strength score (kg)		
Treatment	Pre	Post	24 hours post
Bench Press			
Plac	97 ± 8	95 ± 11	94 ± 11
CHO	98 ± 6	97 ± 8	96 ± 9
CHO-P	97 ± 8	94 ± 9	96 ± 6
All Trials	97 ± 8	95 ± 9	95 ± 9
Bench Pull			
Plac	93 ± 9	91 ± 9	91 ± 9
CHO	93 ± 9	92 ± 9	91 ± 7
CHO-P	92 ± 7	91 ± 8	89 ± 8
All Trials	93 ± 9	91 ± 9	90 ± 8
Leg Press			
Plac	221 ± 35	206 ± 34	214 ± 26
CHO	221 ± 33	206 ± 31	214 ± 28
CHO-P	226 ± 30	213 ± 30	219 ± 29
All Trials	223 ± 32	208 ± 32[#]	216 ± 28

Table 2. Mean and Peak Power in repeated sprints pre-, post- and 24 hours-post exercise (mean ± *s*, n = 9) *$P < 0.05$ *vs* Pre.

	Power (Watts)		
Treatment	Pre	Post	24 hours post
Mean Power			
Plac	914 ± 151	853 ± 153	902 ± 161
CHO	928 ± 140	867 ± 151	913 ± 140
CHO-P	947 ± 119	897 ± 140	938 ± 148
All Trials	930 ± 136	872 ±145*	918 ± 138
Peak Power			
Plac	1037 ± 189	980 ± 177	1022 ± 183
CHO	1072 ± 152	997 ± 164	1053 ± 150
CHO-P	1075 ± 150	1039 ± 168	1077 ± 183
All Trials	1061 ± 161	1005 ±167	1050 ± 169
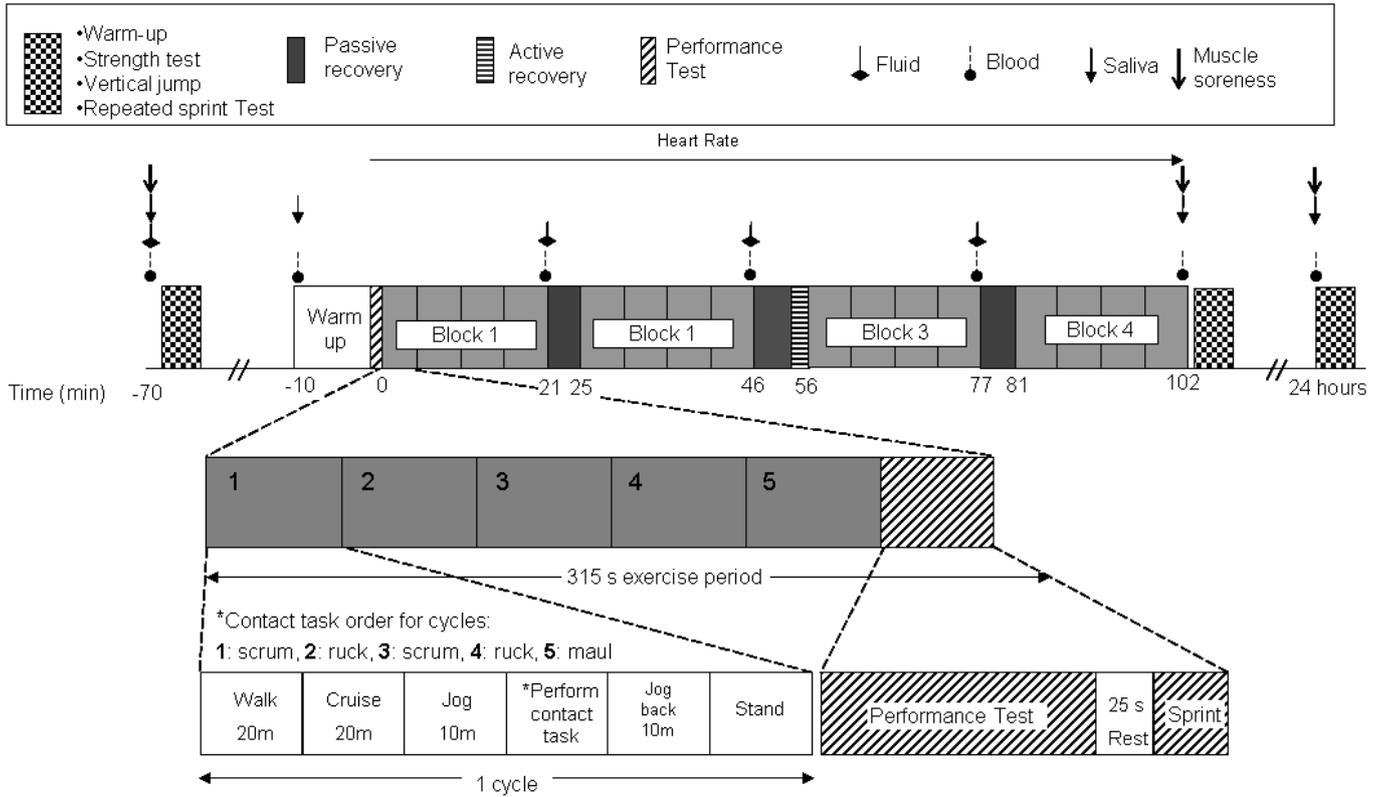
Figure 1. A schematic representation of the study protocol
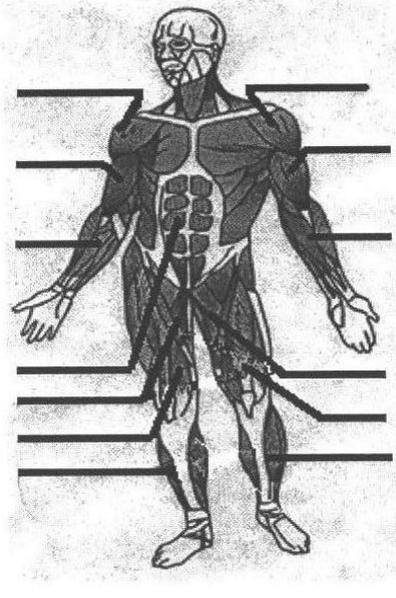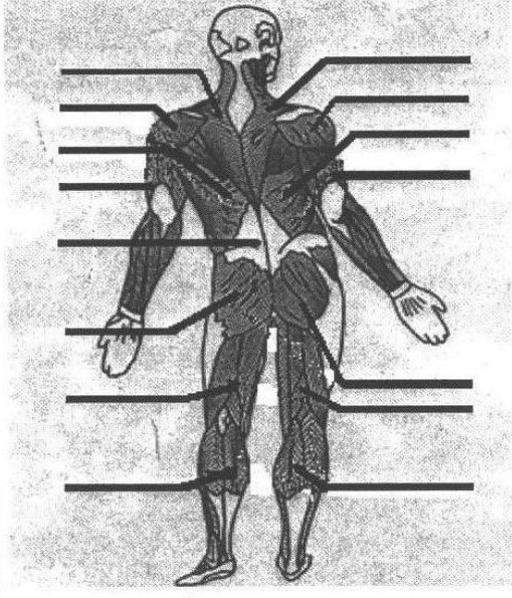
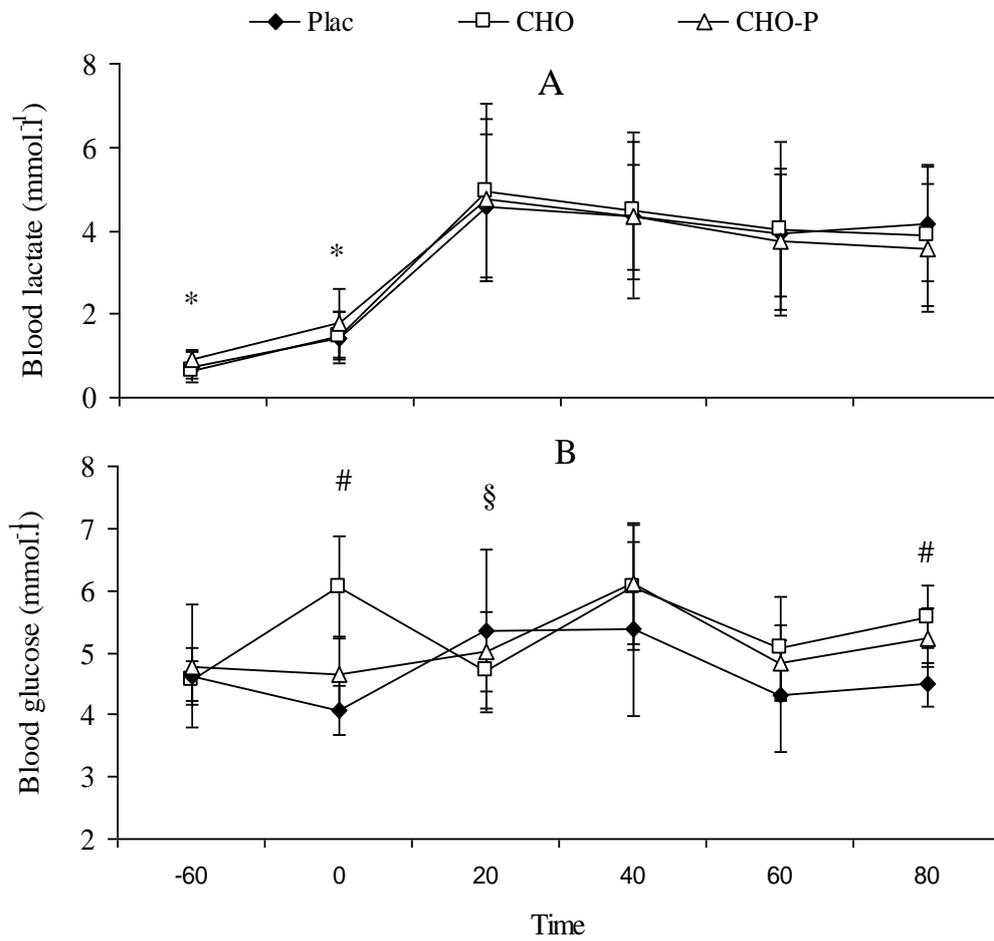**Figure 2**. Diagrams of the muscle groups rated for muscle soreness

Figure 3. Blood lactate (A) and glucose (B) pre-, during and post-exercise (mean ± *s*). Blood lactate: Time effect (*$P < 0.05$ *vs* all other time points). Blood glucose: Time effect ($\S P < 0.05$ *vs* 40 min); [#]Interaction effect (0 min: Plac less than CHO; 80 min: Plac less than CHO and CHO-P, both $P < 0.05$).
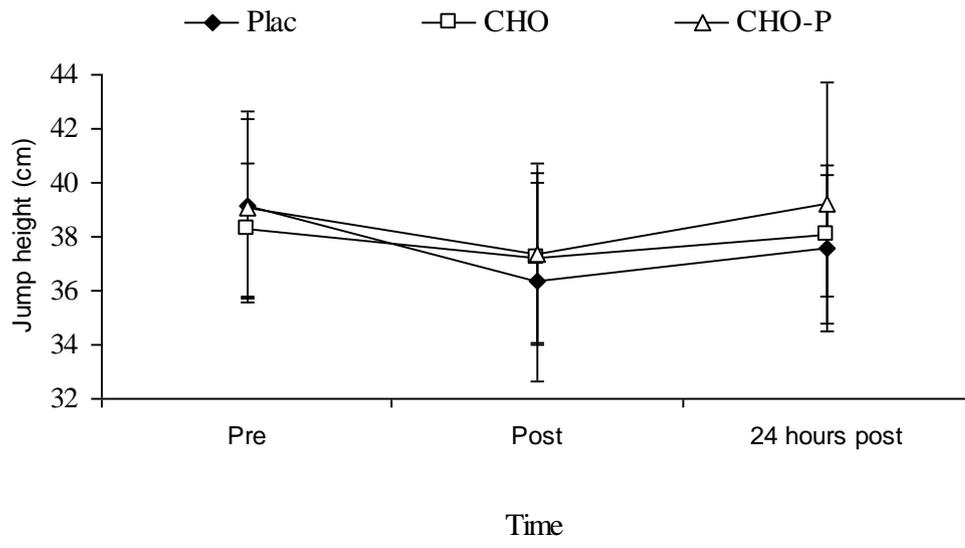
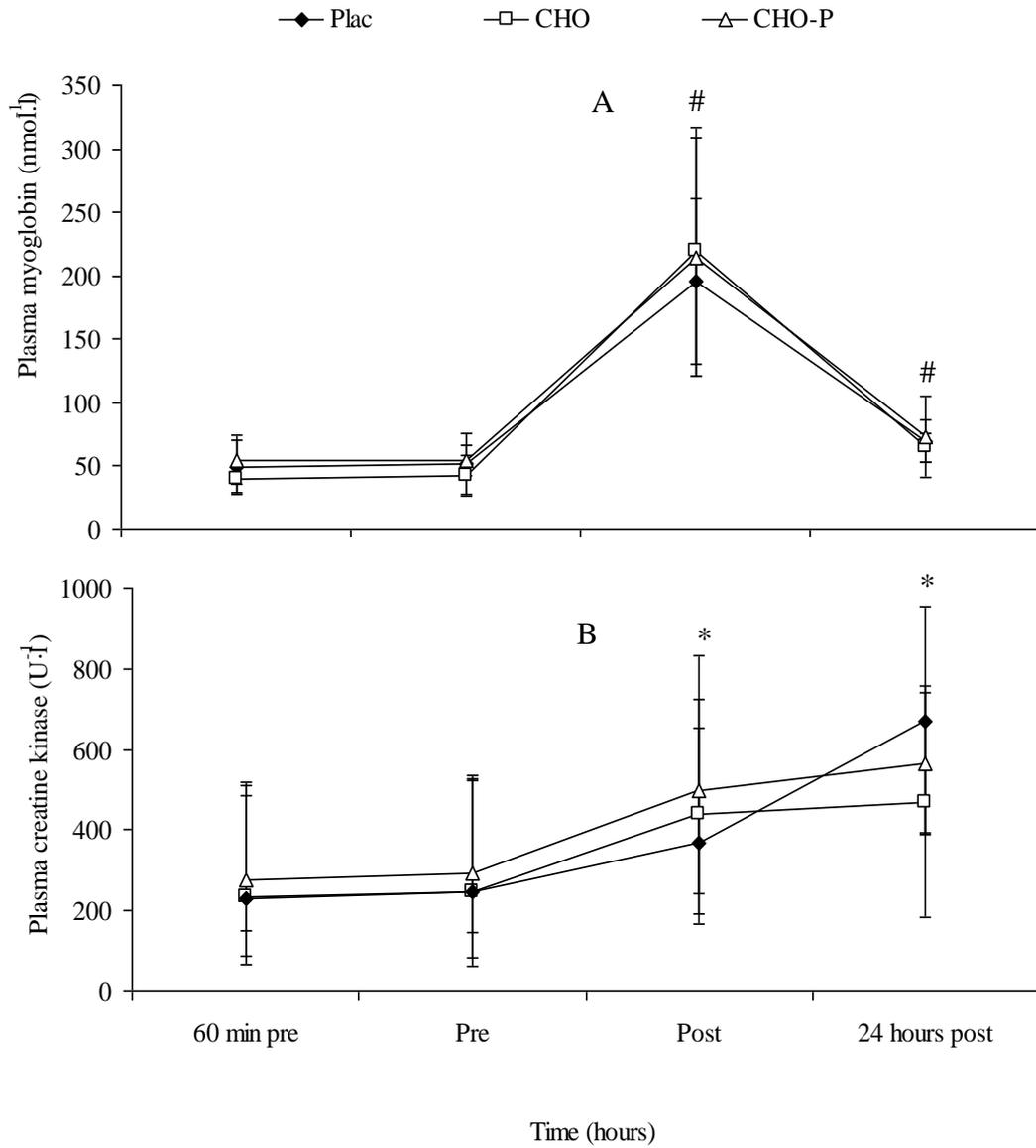**Figure 4**. Vertical jump height at Pre, Post and 24 hours post exercise (mean ± *s*).

Figure 5. Plasma myoglobin concentration (A) and creatine kinase activity (B) (mean ± *s*). Mb: [#]*P* < 0.05 *vs* all other time points. CK: *P < 0.05 *vs* -60 min pre and Pre.
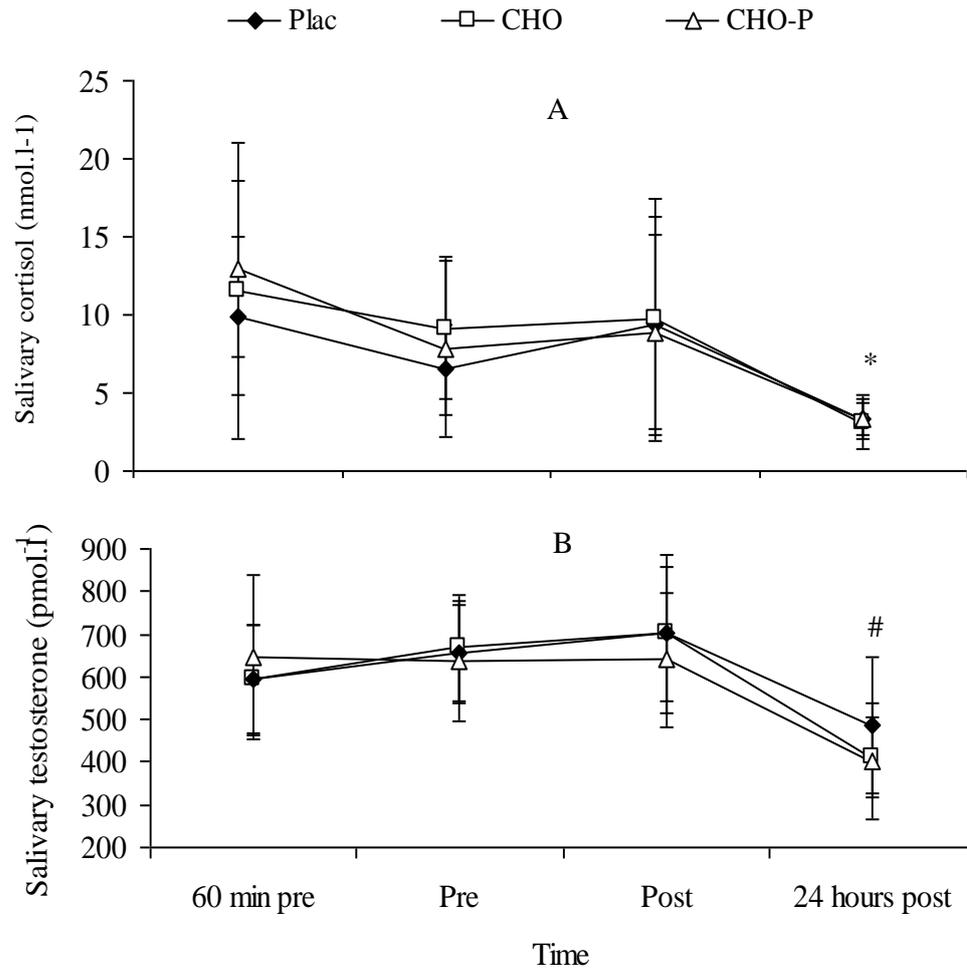
Figure 6. Salivary cortisol (A) and testosterone (B) responses to the BURST for Plac, CHO and CHO-P (mean ± *s*). *$P < 0.05$ *vs* all other time points. #$P < 0.05$ *vs* -60 min pre and Pre.