Title: Carbohydrate and caffeine improves high intensity running of elite rugby league interchange players during simulated match play
Abstract

The study examined the effects of carbohydrate and caffeine ingestion on simulated rugby league interchange performance. Eight male elite rugby league forwards completed two trials of a rugby league simulation protocol for interchange players seven days apart in a randomized crossover design, ingesting either carbohydrate (CHO; 40 g·h⁻¹) or carbohydrate and caffeine (CHO-C) (40 g·h⁻¹ + 3 mg·kg⁻¹) drink. Movement characteristics, heart rate, ratings of perceived exertion (RPE), and countermovement jump height (CMJ) were measured during the protocol. CHO-C resulted in likely to very likely higher mean running speeds (ES 0.43 to 0.75), distance in high intensity running (ES 0.41 to 0.64) and mean sprint speeds (ES 0.39 to 1.04) compared to CHO. Heart rate was possibly to very likely higher (ES 0.32 to 0.74) and RPE was likely to very likely lower (ES -0.53 to 0.86) with CHO-C. There was a likely trivial to possibly higher CMJ in CHO-C compared to CHO (ES 0.07 to 0.25).

The co-ingestion of carbohydrate with caffeine has an ergogenic effect to reduce the sense of effort and increase high intensity running capability that might be employed to enhance interchange running performance in elite rugby league players.

Keywords: Interchange; collision sport; sprinting; effort perception
INTRODUCTION

Rugby league is characterised as a high intensity intermittent collision sport, with movement characteristics determined by playing position (36, 37). While backs are typically whole match players, forwards are strategically rotated as one of 10 permitted interchanges to each play two ~20 min bouts during the 80 minute match. Interchange players adopt a high initial running intensity during their first bout that declines rapidly, followed by a more steady exercise intensity in their second bout (36). This is in contrast to whole match players, who perform less high intensity running, which decreases subtly as the match progresses. Interchange players are therefore often used by coaches as ‘impact’ players and introduced at pre-determined points of a match to increase the intensity and expose fatigue in opponents.

Despite clear reductions in players’ high intensity running capacity towards the end of a rugby league match (32, 36), no studies have yet investigated the role of nutritional interventions to maximise match performance in this group of athletes. Reductions in high intensity running for team sport athletes can be explained by reduced muscle glycogen (19) that is likely to be blunted with the ingestion of carbohydrate before and during exercise (1, 40, 42). Caffeine supplementation (~4-6 mg·kg⁻¹) taken ~1 hour before exercise also has positive stimulating effects on sprint performance, force production and skill during intermittent, high intensity exercise (27, 28, 31). As an adenosine antagonist (11), caffeine moves freely across the blood-brain barrier, with the central nervous system (CNS) its primary target (12). Effects include reduced perceptions of effort and pain (9, 27), increased central drive (25) and, to a lesser extent, increased muscle force and endurance (39).

The co-ingestion of caffeine with carbohydrate is known to decrease muscle glycogen utilisation in the early stages of exercise such that it is preserved for later use and delays
fatigue (8,10). Indeed, based on existing evidence in team sports that shows maintenance of blood glucose in the early stages of match play and a concomitant improvement in sprint performance (18), the co-ingestion of caffeine with carbohydrate is appealing to rugby players. In the only study to date, Roberts et al. (27) observed improvements in sprint performance (3.6%) in a rugby union-specific protocol after co-ingestion of CHO (1.2 g·kg⁻¹ body mass h⁻¹) and caffeine (4 mg·kg⁻¹) when compared to carbohydrate alone and a placebo. A lower rating of perceived exertion (RPE) suggested that improvements in performance were mediated by CNS stimulation rather than increased fat oxidation. An increased availability of CHO within the skeletal muscle might also have contributed to improved sprint performance in these rugby players. However, despite supporting the potential of caffeine to enhance rugby performance, several limitations exist. Firstly, distinct differences in the match characteristics between rugby league (33) and rugby union forwards (6) mean the findings are not easily transferable. Similarly, the use of sub-elite rugby players prevent generalizing the findings and contribute little to our understanding of how caffeine enhances performance in more highly trained team sport athletes (5). This is particularly important given that the benefits of caffeine supplementation on high intensity exercise performance are shown to be more effective in highly trained athletes (7). Finally, the use of caffeine doses above the suggested benchmark of 3 mg·kg⁻¹ (13) means our understanding of how lower doses affect rugby performance remain unknown. Such ‘low’ doses can easily be consumed (~1-2 cups of coffee) and yet are associated with few, if any, side-effects (30). Accordingly, the purpose of this study was to examine the effects of carbohydrate-caffeine (CHO-C) compared to carbohydrate (CHO) on high intensity running performance of elite players during a rugby league interchange simulation protocol. It was hypothesized that consuming CHO-C would improve high intensity running performance during a simulated match protocol when compared to CHO alone.
METHODS

Experimental Approach to the Problem

All subjects were tested at the same outdoor synthetic surface, at the same time of the day (± 2 h) with the same environmental condition between trials (mean temperature 23.2 ± 2.1°, humidity 56.8 ± 9.2%). To accommodate the players’ training and match commitments, testing was conducted the morning after the participant’s rest day, after an overnight ~10 h fast. Using a randomized, double blind crossover design, subjects completed two trials of the Rugby League Match Simulation Protocol relative for interchanged players (RLMSP-i; 35) consuming carbohydrate (CHO) or carbohydrate plus caffeine (CHO-C). Carbohydrate ingestion was included in the investigation into the efficacy of caffeine because previous studies have been criticised for failing to account for the metabolic conditions associated with ingestion of carbohydrate before and during exercise (5). Trials were conducted seven days apart, with subjects completing the simulation in the same group of four on each occasion (i.e. two consuming CHO-C and two CHO). Subjects then completed a standardized 10-min warm-up, comprising walking, jogging, running, sprinting, stretching and a single cycle of the RLMSP-i. Thereafter, subjects commenced the RLMSP-i, during which running, heart rate and ratings of perceived exertion (RPE) were recorded at specific points during the simulation (see Figure 1). Countermovement jump (CMJ) performance was also measured at baseline and throughout the simulation.

Subjects

The sample size was estimated using the data from a previous study (27) in which changes in running performance during a rugby simulation were compared for carbohydrate and caffeine versus caffeine alone. Using an α level of 0.05, a power (1 − β) of 0.8 and an effect size of 0.52 for differences in running performance between conditions, we calculated that at least
seven subjects were necessary for this study. After consent from the club and institutional ethics approval, eight elite male players (age 21.4 ± 2.4 y, stature 189.2 ± 7.2 cm, body mass 94.9 ± 11.4 kg, sum of seven skinfolds 59.5 ± 14.7 mm) from an English Super League club consented to participate in the study. All players were contracted to the club with the study taking place during the players’ in-season period. Subjects recorded their diet 48 h before the first trial and then replicated this on the next trial. A list of foods and beverages containing caffeine were provided to the subjects and they were asked to avoid these foods for 48 hours beforehand. Subjects were asked to consume 500 ml of water on waking to ensure euhydration, with urine osmolality measured on arrival at the training facility.

Procedures

Supplement composition and ingestion schedules
In the CHO and CHO-C trials, subjects consumed a 6.9% carbohydrate-electrolyte solution (Lucozade Sport, glucose syrup and maltodextrin, 6.4 g per 100 ml), dosage 40 g·h⁻¹, in a initial bolus of 500 ml 1 hour before exercise and then 130 ml feedings immediately before, midway and at the end of the first 23 min block and then at the start and midway through the second 23 min block. The presented dosage results in improvements in team sports performance (41). The CHO-C trial replicated this protocol, with the caffeine (3 mg·kg⁻¹) added to the initial 500 ml bolus in the form of a crushed tablet (Proplus, Bayer PLC, UK). Subjects and the researcher were blinded to the colour/look of the drink and were not be informed which condition they had received.
Urine Osmolality

Subjects were asked to provide a urine sample in a 30 ml container (Sterilin universal, Sterilin, UK) upon arriving at the training ground. Samples were analysed for osmolality measured in mOsm·kg⁻¹ using a handheld osmometer (Osmocheck, PerformBetter, UK), which has previously been validated (29).

Countermovement jump (CMJ)

All jumps were recorded using a jump mat (Just Jump Meter, Probotic Inc, Huntsville) interfaced to a hand held monitor. Subjects performed the CMJ starting in an upright position after which they were required to rapidly flex the knees to approximately 90° before jumping for maximal height. The researcher monitored all jumps visually to ensure that they were conducted appropriately, with any jumps that did not conform to the required technique removed from the analysis and repeated. The CMJ with the greatest jump height from three attempts was taken for analysis. Players were familiar with the CMJ procedures as they formed part of their daily monitoring processes for the club.

Rugby League Match Simulation Protocol for interchanges (RLMSP-i)

All players were familiarised with the protocol. Before commencing the protocol, subjects completed a standardized 10-minute warm up comprising varied intensities of running and dynamic stretching. Subjects then performed the RLMSP-i in groups of four matched by position on an artificial grass pitch according to the procedures described previously (35). In brief, subjects ran back and forth at varying speeds between cones positioned at various points along a 28.5 m linear track, controlled by an audio signal. Contact was simulated with
subjects tackling a soft, cylindrical-shaped tackle bag. The movements are based on the mean locomotive speeds and activities of interchanged players established during senior elite rugby league matches (36, 37). The RLMSP-i lasted 42.86 min (2 x 21.43 min separated by 20 min), replicating the average time that a forward spends on the pitch during a match (36). Between bouts players were seated to replicate their normal activity during match play, performing a 5 min re-warm-up before the second bout. A schematic of the RLMSP-i and accompanying measurements is shown in Figure 1.

**Movement demands, heart rate and RPE during the RLMSP-i**

Subjects were pre-fitted with an appropriately sized vest housing the portable GPS unit (SPI-Pro; 5 Hz, GPSports, Canberra, Australia) between the scapulae. The GPS device sampled at a rate of 5 Hz and was integrated with a 6-g tri-axial accelerometer sampling at 100 Hz, with the participant wearing the same GPS unit for each trial. All devices were activated 30 minutes before data collection to allow acquisition of satellite signals, and synchronise the GPS clock with the satellite’s atomic clock. Movement data included: total distance (m), mean speed (km·h⁻¹), distance in high-intensity running (>14.0 km·h⁻¹), and mean sprint speed (km·h⁻¹). The test-retest reliability coefficient of variation for the measurements of distance and speed by the GPS devices ranged from 1.8-2.1% and 1.9-2.1%, respectively (38). The subjects’ heart rate (HR) was collected using a HR monitor (Polar Electro Oy, Kempele, Finland). Heart rates were later calculated as a percentage of each participant’s peak heart rate (%HRpeak), defined as the highest heart rate achieved throughout all testing visits. Both movement and HR data were downloaded using SPI Ezy V2.1 (GPSports, Canberra, Australia) and analyzed using Team AMS V2.1 software (GPSports, Canberra, Australia).
Australia). A digital watch was synchronized with Greenwich Mean Time and used to record the start and end of the protocol, as signalled by the CD player. These times were later used to truncate the raw GPS data file into quartiles of the first and second playing bouts. Rating of perceived exertion (RPE; 4) was also recorded during walking intervals after every quartile (5.36 min) in the first and second bout (each lasting 21.43 min) of the RLMSP-i. In house determined test-retest reliability coefficient of variation for the measurement RPE was 2.4%.

**Statistical Analysis**

All data were log transformed to reduce bias due to non-uniformity of error and analysed using the effect size (ES) statistic with 90% confidence intervals (CI) and % change to determine the magnitude of effects. Magnitude-based inferential statistics were employed to provide information on the size of the differences allowing a more practical and meaningful explanation of the data (2). Thresholds for the magnitude of the observed change for each variable was determined as the within-participant standard deviation (SD) in that variable x 0.2, 0.6 and 1.2 for a small, moderate and large effect, respectively (16). Threshold probabilities for a meaningful effect based on the 90% CI were: <0.5% most unlikely, 0.5–5% very unlikely, 5–25% unlikely, 25–75% possibly, 75–95% likely, 95–99.5% very likely, >99.5% most likely. Effects with 90% CI across a likely small positive or negative change were classified as unclear. All calculations were completed using a predesigned spreadsheet (15).
RESULTS

Differences in urine osmolality for the CHO-C (605.0 ± 153.1 mOsm·kg⁻¹) and CHO (603.4 ± 124.3 mOsm·kg⁻¹) trials were unclear (ES -0.04 ± 0.87), suggesting that values were similar between trials.

External load during RLMSP-i with CHO-C and CHO

There were likely reductions in mean running velocity across quartiles for both CHO-C and CHO trials during Bout 1 (ES -0.79±0.75; and -0.94±1.08, respectively) but changes were unclear at the end Bout 2 (ES 0.34±0.61; and 0.20±0.83, respectively). Mean running velocity was likely higher in the CHO-C trial for Quartiles 1-4 during Bout 1 (ES 0.75±0.49; 0.68±0.8; 0.63±0.81; and 0.72±0.70, respectively), but during Bout 2 unclear for Quartiles 1, 2 and 4 (ES 0.11±1.12; 0.07±0.78; and 0.54±0.82, respectively) and likely higher for Quartile 3 (ES 0.43±0.51) (Figure 2A). Total distance covered in CHO-C trial (4374 ± 294 m) was likely higher than CHO trial (4088 ± 350 m; ES 0.73±0.72).

Changes in high intensity running for both CHO-C and CHO trials were unclear during Bout 1 (ES -0.79±1.12; and 0.66±1.25, respectively) and Bout 2 (ES -0.29±0.79; and 0.23±0.79, respectively). However, distance covered in high intensity running was likely higher in the CHO-C trial for Quartiles 1, 2 and 4 during Bout 1 (ES 0.64±0.54; 0.67±0.53; and 0.58±0.68, respectively) and Quartile 3 and 4 during Bout 2 (ES 0.41±0.55; and 0.50±0.56, respectively). All other differences in high intensity running between trials were unclear (Figure 2B).

Changes in mean sprint speed for both CHO-C and CHO trials were unclear during Bout 1 (ES 0.26±1.16; and 0.24±0.55, respectively) and Bout 2 (ES -0.48±0.86; and 0.14±0.78,
respectively). However, during Bout 1, mean sprint speed was very likely higher at Quartile 1 (ES 1.04±0.68) and likely higher at Quartiles 2-4 (ES 0.83±0.64; 0.55±0.61; and 0.54±0.72, respectively) in CHO-C trial. During Bout 2, mean sprint speed was likely higher in Quartile 1 (ES 0.57±0.38) and 3 (ES 0.39±0.33), unclear in Quartile 2 (ES 0.23±0.44) and very likely higher in Quartile 4 (ES 0.75±0.49) during the CHO-C trial (Figure 2C).

***** Figure 2A-C here*****

**Internal load during RLMSP-i with CHO-C and CHO**

During Bout 1, heart rate was possibly higher in CHO-C trial for Quartiles 1 and 3 (ES 0.32±0.48; and 0.37±0.56, respectively), unclear for Quartile 2 (ES 0.16±0.64) and likely higher for Quartile 4 (ES 0.46±0.54). During Bout 2, heart rate was likely higher for Quartiles 1, 2 and 4 (ES 0.48±0.51; 0.56±0.54; and 0.61±0.56, respectively) and very likely higher for Quartile 3 (0.74±0.52) (Figure 3A). During Bout 1, differences in RPE were unclear for Quartile 1 (ES 0.27±0.67) but were likely lower in CHO-C for Quartile 2 (ES -0.53±0.41) and very likely lower in Quartile 3 and 4 (ES -0.83±0.49; and -0.86±0.43, respectively). During Bout 2, differences in RPE were unclear for Quartile 2 (ES -0.18±0.47) but were likely lower in CHO-C for Quartile 1 (ES -0.74±0.66) and very likely lower in Quartile 3 and 4 (ES -0.73±0.43; and -0.89±0.66, respectively). (Figure 3B).

***** Figure 3A-B here*****
There were *unclear* changes in CMJ height during Bout 1 (ES -0.34±0.54) but *likely* decreases in CMJ height during the entire RLMSP-i for CHO (ES -0.73±0.53). For the CHO-C trial, *possible* decreases in CMJ height occurred during Bout 1 (ES -0.33±0.47) that were likely decreased during the entire protocol (ES -0.61±0.58). During Bout 1 there was a *likely trivial* difference in CMJ height between trials at Time 1 (ES 0.07±0.25) and *possibly higher* CMJ height in CHO-C at Time 2 (ES 0.16±0.31). In Bout 2, CMJ height was *possibly higher* in CHO-C at Time 1 (ES 0.25±0.36) but *unclear* at Time 2 (ES 0.19±0.41) (Table 1).

***** Table 1 here*****

**DISCUSSION**

This is the first study to examine the effect of co-ingestion of CHO-C compared to CHO alone on the external and internal responses of elite rugby league players to an interchange-specific match protocol. The co-ingestion of CHO-C compared to CHO resulted in small to moderate improvements in mean running speed, high intensity running, and sprint performance. These improvements in external capability were accompanied by small to moderate increases in heart rate despite players reporting a lower perceived exertion. All of these effects were greater than the typical variation associated with reliability (35), and therefore were likely to be due to the effect of caffeine ingestion rather than measurement error.

When players consumed CHO-C there were moderate increases in mean running speed during simulated interchange match play when compared to CHO alone. This was most likely because players performed more high intensity running (i.e. >14 km·h⁻¹) after the co-
ingestion of CHO-C. These improvements were more noticeable in Bout 1 of the simulation, although small increases in both mean velocity and high intensity running were observed in the final two quartiles of the CHO-C trial. Indeed, the ingestion of caffeine appeared to markedly alter the pacing profile adopted by subjects, such that high-intensity running was up-regulated from the outset of the protocol, and an end-spurt that was more pronounced than the CHO trial was evident at the end of each exercise bout. Such an effect on pacing is likely due to the well-established reduction in RPE associated with caffeine ingestion (9). An individual’s RPE is considered to be fundamental to setting an appropriate exercise intensity during team sports, such that the match can be completed at a sufficient intensity without seriously compromising homeostasis (34). Furthermore, RPE is thought to play a significant role in the commonly observed ‘end-spurt’ toward the end of exercise, where an individual will tolerate a high RPE providing the end of the exercise bout is near (34). Taken together, our data suggests that co-ingestion of carbohydrate with a low caffeine dose is better than consuming carbohydrate alone to ensure high intensity running is increased and maintained across two ~20 min bouts of intermittent exercise.

These findings are practically meaningful for rugby league interchange players. Reductions in high intensity running during a rugby league match are often used to indicate fatigue (32, 36). For example, Waldron et al. (36) reported reductions in high intensity running for in interchange players of ~56% and 40% for the first and second bout of a match. While the lack of real physical collisions probably meant simulated match activity resulted in much smaller reductions in high intensity running compared to matches, the pattern of fatigue for elite players is similar to that shown in a match. Accordingly, co-ingestion of CHO-C could be consumed by interchange players to enhance high intensity running in their first bout and in later stages of their second bout.
The unclear changes in sprint velocity during the protocol are different to the large changes observed in rugby league match play (~50%, 32) and are probably explained by the lack of a true collision within the simulation (21, 24). Using a standard tackle bag (mass ~23 kg) rather than a heavier object similar in mass to a player is likely to have resulted in a decreased internal load and less neuromuscular fatigue in the lower limbs (24). Less fatigue from physical collisions would therefore mean players would be more capable of preserving sprint performance during the accompanying running activity. This notwithstanding, mean sprint velocity was ~11-25% higher in Bout 1 and ~10-16% higher in Bout 2 when the subjects co-ingested carbohydrate with caffeine compared to carbohydrate alone. The present study reaffirms previous observations of the potential improvements in sprint performance during prolonged intermittent exercise after consuming carbohydrate and caffeine (27, 31). However, the relative increases in sprint performance observed in this study are higher than those reported previously (~1-4%). The benefits of caffeine ingestion on maximal anaerobic capacity are more pronounced in those with specific training (7). That we used specifically trained elite compared to sub-elite rugby players (e.g. 27, 31) might therefore explain the larger increase in sprint performance in our study. Our use of GPS to measure mean peak sprint speed throughout the protocol rather than a single sprint using electronic timing gates (27, 31) might also have contributed to the discrepancies between studies.

We observed that total distance covered during the protocol was ~7% higher during the CHO-C trial when compared to CHO alone. This is of particular interest given that the RLMSP-i is a fixed distance protocol. Examination of the accelerometer data from the GPS indicated that, on average, more impacts occurred in the high impact zone (>9 G) for the CHO-C trial when compared to CHO alone. Combined with the faster running speeds in the CHO-C trial, we propose that subjects were sprinting faster into the contact (tackle bag),
producing higher impacts and therefore making the tackle bag travel further. This would have meant them having to travel a greater distance in retrieving the tackle bag and replacing it in its start position. Furthermore, analysis of the individual sprint data showed an increase of ~2.4 m per sprint after co-ingestion of CHO-C compared to CHO. We attribute this to the faster sprints with CHO-C requiring greater time and distance to decelerate, culminating in an increased total distance during the trial.

Our data provide clear evidence that caffeine co-ingestion had a centrally-mediated effect that increased high intensity running and mean sprint speed throughout the protocol. The small to moderate reductions in RPE alongside increased running and heart rate during the CHO-C compared to the CHO trial supports this and is consistent with the findings of Roberts and colleagues (27). Reductions in RPE are caused by caffeine restricting adenosine from binding to the adenosine receptors in the brain, thus blocking the inhibitory action of the adenosine on the central nervous system (12, 31). Caffeine also increases motor unit recruitment and drive to the muscle that enhances muscular force production (25). A greater force production facilitated by caffeine ingestion might therefore have contributed to improved sprint performance in the CHO-C trial. Albeit differences between trials were small, CMJ height was preserved and remained ~2% higher in CHO-C trial and supports the potential role of caffeine ingestion on muscle force production. Caffeine intake is also known to lower interstitial potassium during repeated high intensity exercise because a greater catecholamine response increasing activity of the sodium-potassium pump (20). While a single mechanism to fully explain the impact of caffeine on performance is unlikely, further work is needed to understand its role during short duration, high intensity collision exercise.

Our study is not without limitations. That we employed a simulation protocol means that
players were not exposed to the true loads imposed during match play, most notably physical collisions and the cognitive challenges associated with the performance of team sports. With the ingestion of a substrate designed to modify peripheral and extracellular metabolism, it would also have been beneficial to include some biochemical analysis to understand whether the ingested product caused an increase in performance or not. However, such measures were not possible with elite athletes who were reluctant to provide such invasive measures. Similarly, the challenges of working with elite athletes who are engaged in a full time training programme meant it was not possible to employ an additional placebo trail in this study. We were also constrained by having to run four players together with different experimental conditions applied between them. It is therefore possible that players were motivated by the performance of others and thus altered their running performance. While every effort was made to match beverage taste, we cannot discount the possibility that performance improvements in the CHO-C trial were partly attributed to players believing they were taking the stimulant. The use of the placebo effect to evoke improved running performance might, however, be acceptable in the context of the real world (14). Finally, despite showing the positive effects of caffeine supplementation on rugby league performance, there are also potential consequences for sleep (3). This is particularly pertinent when matches are played late evening, after which ~53% athletes have previously reported deteriorations in sleep quality (17). The consumption of caffeine to improve match performance might therefore present unintended consequences that lead to players adopting inappropriate strategies to induce sleep after evening matches.
Practical Applications

The current study presents valuable information for rugby coaches planning to maximise the high intensity running performance of their interchange players. Where interchange players are required to have impact, the co-ingestion of carbohydrate with caffeine rather than carbohydrate alone is recommended to enable players not only to start at a higher intensity but also to maintain that intensity for longer. The ability of interchange players to maintain a higher running intensity is likely to provide the team with an advantage in both attack and defence that enables them to be more successful.

Conclusion

Co-ingestion of carbohydrate with caffeine compared to carbohydrate alone enables improvements in high intensity running performance of professional rugby league interchange players during simulated match play. Enhanced running performance seems to be mediated by alterations in a player’s sense of effort that have a direct effect on the working muscle. The data presented in this study can be used to specifically aid rugby league interchange forward pre-match preparation.
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Tables

Table 1. Changes in CMJ (cm) during RLMSP-i for CHO-C and CHO trials. Values expressed as mean ± SD.

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<thead>
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Figure legends

**Figure 1.** Schematic of the RLMSP-i.

**Figure 2.** Mean velocity (km·h\(^{-1}\), panel A), high intensity running (m, panel B) and mean sprint speed (km·h\(^{-1}\), panel C) in CHO-C (black squares) and CHO (white triangles) during Bouts 1 and 2 of RLMSP-i. Q1-Q4 indicates Quartile number of Bout. ♯ Denotes a small difference; * denotes moderate difference. Values expressed as mean ± SD.

**Figure 3.** Heart rate (%maximum, panel A) and RPE (panel B) in CHO-C (black squares) and CHO (white triangles) during Bouts 1 and 2 of RLMSP-i. Q1-Q4 indicates Quartile number of Bout. ♯ Denotes a small difference; * denotes moderate difference. Values expressed as mean ± SD.