Post-exercise ingestion of a carbohydrate and casein hydrolysate supplement reduces perceived muscle soreness but not fatigue in Sevens Rugby Players

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Objectives. This study investigated the effects of prolonged use of a protein (casein hydrolysate) supplement on perceived muscle soreness and fatigue, in international level Sevens rugby players (n=23) during a pre-season training camp.
Methods. A randomised, double-blind, placebo-controlled design was used. Players were assigned to a carbohydrate-only or an isocaloric carbohydrate and protein supplement and ingested the assigned supplement after each training session (17 field training sessions over a 23-day period). Before each training session a questionnaire and 10-point visual analogue scale was completed that assessed muscle soreness and muscle fatigue in the calf, hamstring, and quadriceps.
Results. No significant differences were observed in leg fatigue in any of the muscle groups. However, muscle soreness was significantly lower in the experimental group in the quadriceps at Week 3, Day 5 ($p=0.04$) and calves at Week 4, Day 2 ($p=0.02$) and Day 3 ($p=0.04$). Additionally, no significant differences were found during the Heart rate Interval Monitoring System Test ($p=0.350$) used to determine training load recovery.
Conclusion. The results suggest that prolonged use of a protein hydrolysate supplement may be beneficial in reducing muscle soreness, but not muscle fatigue, during periods of continuous high training loads.
Keywords. Supplement, protein feeding, recovery

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With the increased emphasis on achieving success in both recreational and professional sport, nutritional strategies to improve performance and enhance recovery have been the focus of considerable research. Previous studies have shown that protein supplementation during or post exercise positively affects several factors related to recovery, such as a reduction in the subjective rating of muscle soreness and fatigue.[1–3] Delayed Onset Muscle Soreness (DOMS) can compromise performance and training due to pain and a reduction in joint range of mostion, shock attenuation and peak torque.[4] Muscle fatigue following hard training is common, and if not managed correctly, can result in overreaching or overtraining syndromes.[4] A period of reduced training load between hard training sessions is therefore necessary to allow recovery. This, however, limits the number of key workouts that an athlete can perform each week. Enhancing recovery through nutritional means by reducing either DOMS or muscle fatigue, or both, would permit an athlete to recover faster and be able to train at a high intensity more frequently.

Previous studies have shown that protein supplementation either during or post exercise positively affects several factors related to recovery, including a reduction in the subjective rating of muscle soreness.[5,6] On the other hand, a number of studies have failed to show any potential beneficial effect on muscle soreness or muscle fatigue.[3,7,8] Despite these inconsistencies in findings related to DOMS and fatigue, when performance has been investigated, several studies have shown that post-exercise protein supplementation improves subsequent performance[9-11] although, as with DOMS and fatigue, some studies have shown no positive effect.[3,7]

Importantly, the majority of studies have only assessed the effect of protein ingestion on recovery after an initial or second bout of exercise, or over a relatively short period of time. Training is a long-term process in which athletes gradually adapt to progressively higher training loads and physical demands. Thus assessing the long-term effects of protein supplementation appears logical. However, there are only a handful of studies investigating long-term effects of protein supplementation on post-exercise recovery.[5,12–14] Flakoll et al.[13] assessed post-exercise protein supplementation in US marine recruits during basic military training over 54 days. Both at day 34 (6 mile full gear hike) and day 54 (final physical fitness test), protein supplementation resulted in a significant reduction in muscle soreness as well as other outcomes, such as a decreased total number of medical visits.

Furthermore, a study by Witard et al.[14] examined the effect of increased protein intake on short-term decrements in endurance performance during a block of high-intensity training. Well-trained cyclists completed two 3-week trials in which participants were divided equally into normal, intensified or recovery training. Cyclists received either a high-protein (Protein; 3 g protein·kg^{-1} body mass (BM) d^{-1}) or a normal diet (Control; 1.5 g protein·kg^{-1} BM d^{-1}) during intense training and recovery. Increased dietary protein intake led to a possible attenuation (4.3%; 90% confidence limits ×/÷5.4%) in the decrement in time trial performance after a block of high-intensity training compared with normal (Protein = 2639 ± 350 s; Control = 2555 ± 313 s). Restoration of endurance performance during recovery training possibly benefited (2.0%; ×/÷4.9%) from additional protein intake. Additional protein intake reduced symptoms of psychological stress and may have resulted in a worthwhile amelioration of the performance decline experienced during a block of high-intensity training. Furthermore, Goh et al.[15] found no difference in the perception of muscle fatigue and muscle soreness between different compositions of carbohydrate (CHO) and protein (PRO) drinks during prolonged cycling exercise. However, these studies are limited due to their short duration. Thus, the evidence regarding the effect of prolonged protein supplementation on recovery and performance remains limited.

The current study therefore aimed to determine the effects of prolonged ingestion of a CHO + casein hydrolysate (CHO + PRO) supplement post-exercise on perceived levels of DOMS and muscle fatigue, in international level Sevens rugby players participating in a 23-day pre-season training camp. The authors hypothesised that CHO + PRO supplementation would result in reduced levels of

DOMS and perceived muscle fatigue, as compared to CHO-only supplementation.

Methods

Study participants

The entire training group of the international level Sevens rugby players, who were attending a pre-season training camp, agreed to participate in the study. Players were randomly divided from a list of names into either a control (n=10) or experimental (n=13) group, in a double-blind, placebo-controlled design. Additionally, all players in the two groups were matched by playing position and fitness level. There were no significant differences between the two groups in mass, height, percentage body fat or lean mass (Table 1). All the players participating in the study lived at a facility on-site (thus the same meal choices were provided to all the players for the duration of the study). Although the macronutrient intake of the meals was not controlled (i.e. the players were free to choose from the available foods served at each meal), prior to the start of the training camp all the players were given a detailed lecture by a qualified sports dietician on guidelines to achieve good sports nutrition, including the meeting of adequate carbohydrate and protein requirements. Although no dietary records were completed by the players, players received strict instructions not consume any additional supplements during the training camp.

Table 1. No significant differences were found in mass, height, body fat percentage and lean mass between the experimental and control groups

	Experimental (n=13)	Control (n=10)	
Mass (kg ± SD)	85.7 ± 8.4	89.9 ± 11.8	p=0.368
Height (cm ± SD)	179.1 ± 4.0	180.3 ± 5.8	p=0.632
Body fat (% ± SD)	13.5 ± 2.82	12.7 ± 2.8	p=0.499
Lean mass (kg ± SD)	77.7 ± 7.9	76.72 ± 10.1	p=0.795

Before the study, all participants received a detailed outline of the study procedure and were required to sign an informed consent form in accordance with the Declaration of Helsinki (Seoul, October 2008) before entry into the study, which was approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. All information collected during this study was kept confidential and anonymity was ensured via a participant coding system. All data collected were stored on a password-protected system. All the participants received a detailed report of the overall study findings but did not receive their individual results.

Training

The training load (Fig. 1) was standardised by the coach for all the players taking part in the training camp. All players in both groups completed identical workouts (resistance and field training) consisting of a programme designed by the coaching staff and was strictly controlled (Table 1). Resistance training comprised strength exercises (including squat variations, vertical push, vertical pull, horizontal push, horizontal pull, power clean, push press, chest press, bicep and triceps exercise variations) and field training (including skills training) comprised of attack and defensive patterns, general rugby skills (lineouts, scrumming, ball handling and passing) and rugby-specific fitness conditioning (40 m sprints, ruck-specific conditioning and tackling). During each training session, details of time, intensity, and type (resistance or field training) were recorded to give an overall numerical value (session value) for each of the total of 17 sessions over the 23-day period. No data were collected during the weekends as these were rest days assigned by the coaches, during which no training took place.

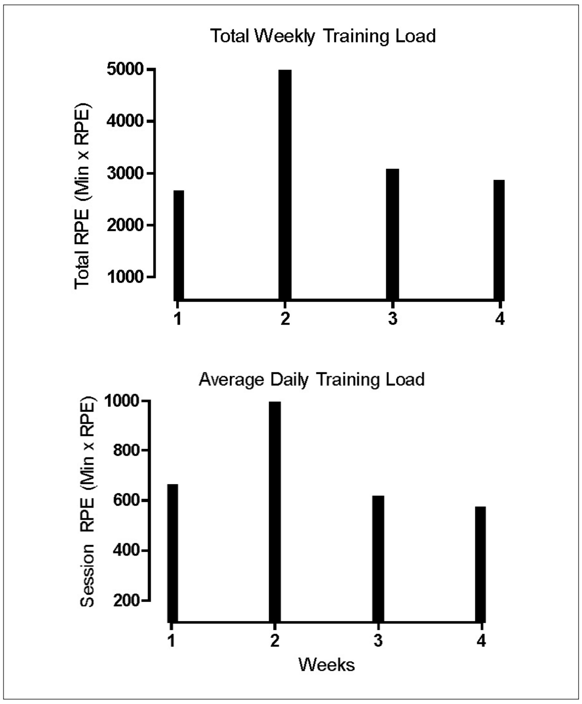

Fig. 1. Total weekly training load and average daily training load for each week as calculated by session RPE (min x RPE) over the duration of the training camp.

Initial measurements: Anthropometry

The standard anthropometric data of weight (kg), stature (cm) and skinfolds (triceps, bicep, subscapular, suprailiac) were measured by the same qualified biokineticist using the ISAK technique (International Society for the Advancement of Kinanthropometry[16]). The skinfold measurements were subsequently used to calculate lean body mass and percent body fat.[16]

Protein supplement/Intervention

Immediately after each field and resistance training session the players ingested either a carbohydrate (0.8 g/kg body mass) plus protein (casein hydrolysate) (0.4 g/kg body mass; containing di- and tripeptides) supplement (PeptoPro, DSM, The Netherlands), or an isocaloric placebo carbohydrate-only drink (1.2 g/kg body mass), dissolved in 500 ml water. Both drinks contained the same carbohydrate base consisting of maltodextrin (Table 2). Additionally, each batch of the supplement was tested for contaminants by an independent laboratory. The drinks were mixed individually for each player by a research assistant who was blinded to the drink's content, and intake by the players was monitored by the coach. No other supplements were used for the duration of the training camp.

Table 2. Nutritional content of the test beverages

	Protein (PRO + CHO/100 g)	Placebo (CHO/100 g)
Energy (KJ)	1656	1658
Protein (casein hydrolysate)	18.5 g	nil
Carbohydrate (maltodextrin)	36.4	53 g
Total Fat	nil	nil
Sodium Chloride	205 mg	205 mg

Testing protocol/Measurements

Data were collected via a self-administered questionnaire including a 1-10 visual analogue scale targeting perception of muscle soreness and muscle fatigue as has been used in previous research[17,18] and shown to be valid and reliable. Specifically, perceived muscle soreness and muscle fatigue were assessed in three different muscle groups (quadriceps, hamstrings, and gastrocnemius), under four different conditions in this study. The conditions were as follows; (1) at rest (i.e. when the muscle is not active), (2) during daily activity (such as walking to and from rooms or field), (3) when a stretch was applied (the player completed a stretch of the muscle group and reported immediately the pain or fatigue experienced) and (4) before the commencement of training. The questionnaire and analogue scale were completed at the same time of day, before each field training session, and before any other activities (i.e. warm-up or stretching). Both DOMS and muscle fatigue were rated on a scale of 1-10 (10-point scale using 1 unit intervals) with 1 being none, and 10 being severe.[19]

Heart rate Interval Monitoring System Test (HIMS)

The HIMS[19] is a standardised multistage submaximal test used to quantify the variation in heart rate under controlled conditions so that training-induced changes in submaximal heart rate can be interpreted more precisely. Heart rate recovery is the rate at which heart rate decreases, usually in the first minute or two, after moderate to heavy exercise and is a consequence of parasympathetic reactivation and sympathetic withdrawal.[19] After a standardised stretching warm-up, all participants were fitted with a heart rate transmitter and wrist monitor recorder (Polar Accurex, Polar Electro, Kempele, Finland) before the start of the test. The heart rate monitor measured the heart rate every five seconds during the test which consisted of a submaximal shuttle run test of four increasing intensity stages, interspersed with one minute recovery periods. The participants were required to run between two lines that were 20 m apart, the pace of running within each of the four stages (8.4, 9.6, 10.8, and 12.0 km h^{-1}, respectively) being set by a pre-recorded auditory signal. Each stage lasted two minutes, followed by the one minute of recovery. The participants then rested for two minutes after the fourth stage, during which HR was recorded. This test was designed to be submaximal and nonaversive for the participants so that from a practical perspective the test could be administered frequently during different phases of training without influencing the training outcome. The recorded HR was transferred to a computer using an interface (Polar Electro). The heart rate for each stage was recorded for the final 15 seconds. The heart rate for each recovery period was determined in the same way. During the recovery period after the fourth stage, the heart rate was recorded at one and two minutes, respectively. These heart rates were expressed as a percentage of the heart rate measured during the fourth stage and defined as recovery percentage (%) first minute and recovery percentage (%) second minute, respectively.[19]

Data analysis

To test the significance of the treatment effect (post-exercise supplementation with a CHO + PRO drink vs. an isocaloric CHO-only drink) on each muscle group and each outcome (DOMS and fatigue), a mixed model was fitted with random effects for player, player x week and player x day. A mixed model approach was used due to the multiple data for the same players over the 17 days and to accommodate any missing data. For each muscle group, the 4-way interaction model including "condition" (rest, daily activity, stretch, pressure) was used. Supplement (casein hydrolysate and placebo), week, and day showed that the 4-way interaction with "condition" was not significant (quadriceps: p=0.953, hamstrings: p=0.967, calf: p=0.997), indicating that the treatment effect was similar for all conditions. This resulted in the fitting of a 3-way mixed model for each outcome and each muscle group. The Type 3 Tests of Fixed Effects (F value, p) were considered for overall significance of the treatment effect. Conditional on the overall test being significant, suitable contrasts were set up to test the significance of the treatment effect over the 17 testing days individually. Least squares means were used to summarise the size of the treatment effects. For all assessments the level for statistical significance was set at $p<0.05$. In addition, a second mixed model was fitted in which the measurements at baseline (day 1 of each week), were subtracted out at each subsequent day for that week.

Results

Analysis of muscle fatigue data (Figs. 2-4) showed that the overall 3-way interactions of week x day x drink for all three muscle groups were not significant (quadriceps, p=0.48; hamstrings, p=0.53; calves, p=0.67; legs, p=0.18) at any time during the experimental period.

Analysis of muscle soreness data (Figs. 5-7) showed that the 3-way interaction of week x day x drink was significant for two muscle groups (quadriceps, $p<0.0001$; calves p=0.016). Various contrasts (post-hoc tests) were set up to compare the two groups for effect of drink type. These contrasts compared the muscle soreness score for groups at each of the 17 time points (Week 1, Days 1-4; Week 2, Days 1-5; Week 3, Days 1-5; Week 4, Days 1-3). Significant differences were observed for the calves (Fig. 4) at Week 4, Day 2 (estimated effect=1.2; SE=0.50; p=0.02) and Day 3 (estimated effect=1.0; SE=0.49; p=0.04) and quadriceps at Week 3 (Fig. 5), Day 5 (estimated effect=1.0; SE=0.49; p=0.04). Each mean value in the figures represents 52 observations for the experimental group and 40 for the control group.

No significant difference (p=0.350) was found between the type of drink ingested and heart rate recovery at each at each of the three HIMS testing points (Fig. 8).

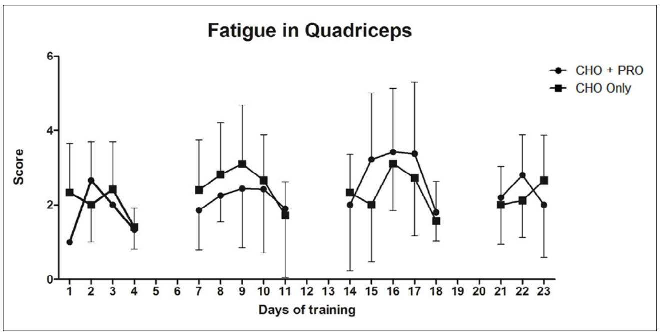

Fig. 2. *Fatigue scores in the control and experimental groups in the quadriceps. There were no significant differences (n=10 in the experimental group and 13 in the control group).*

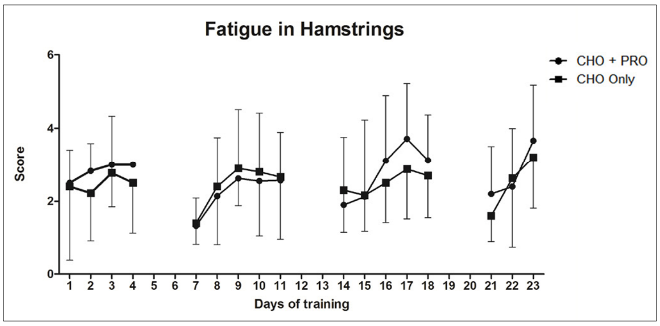

Fig. 3. *Fatigue scores in the control and experimental groups in the hamstrings. There were no significant differences (n=10 in the experimental group and 13 in the control group).*

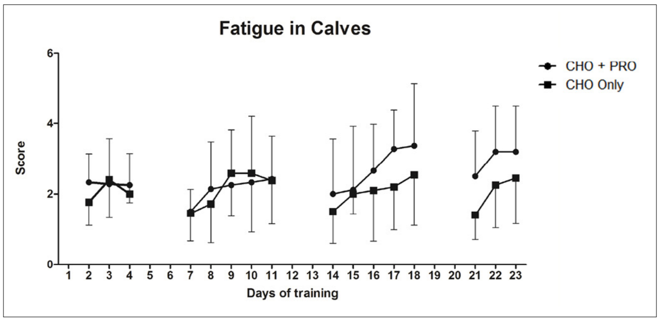

Fig. 4. *Fatigue scores in the control and experimental groups in the calves. There were no significant differences (n=10 in the experimental group and 13 in the control group).*

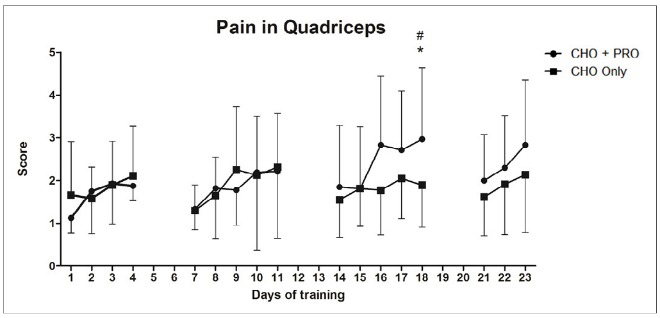

Fig. 5. *Pain scores in the control and experimental groups in the quadriceps. Significant differences were observed at Day 18 (p=0.04) *=p<0.05. Significant differences from baseline were found at Day 18 (p=0.0063) #=p<0.05.*

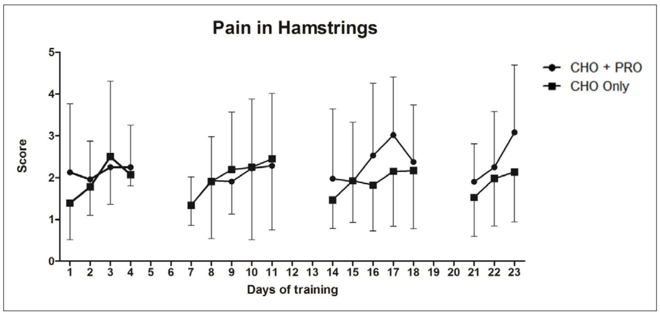

Fig. 6. *Pain scores in the control and experimental groups in the hamstrings. There were no significant differences.*

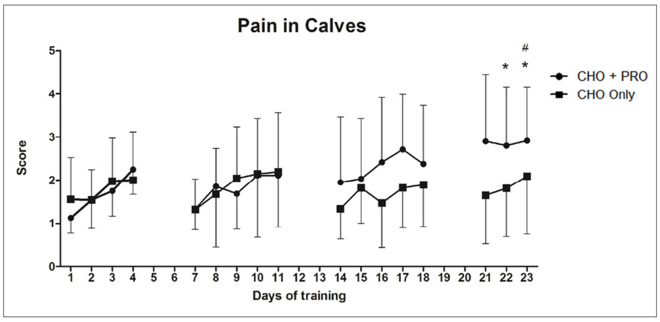

Fig. 7. *Pain scores in the control and experimental groups in the calves. Significant differences were observed at Day 22 (p=0.02) and Day 23 (p=0.04) *=p<0.05. Significant differences from baseline were found at Day 23 in the calves (p=0.0011) #=p<0.0.*

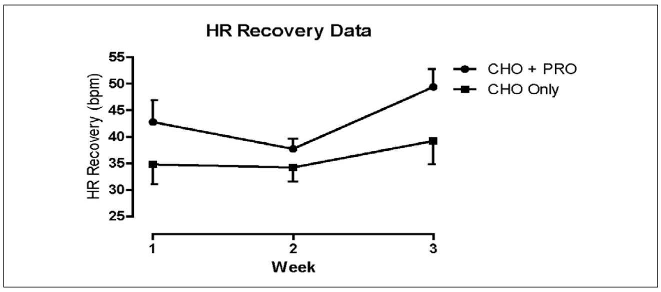

Fig. 8. *Heart rate recovery (bpm) for the experimental and control groups. No significant differences were detected between the different beverage groups during each of the HIMS testing periods (p=0.350). However, HR recovery tended towards significance at Week 1 (p=0.095) and at Week 3 (p=0.0665). Error bars indicate SEM.*

Discussion

The first important finding of this study was that towards the end of the study period, DOMS was perceived to be lower in the quadriceps and calf muscles of the players who ingested the CHO+PRO supplement, compared to those players who ingested an isocaloric CHO-only control drink (Figs. 5-7). The effect was estimated to be about one unit (on the 1-10 point scale[17,18]), which could potentially have short-term practical implications from a training perspective, as well as long-term implications in the adaptation of muscle to training load. This effect was first noticeable at the end of the third week of camp when the cumulative training load may have reached a critical level.

To the best of the authors' knowledge, there are only a few studies investigating long-term effects of protein supplementation on post-exercise recovery.[12–14] The study by Flakoll et al.[13] assessed the effect of post-exercise protein supplementation in US marine recruits during basic military training over 54 days. As in Luden et al.,[5] protein supplementation resulted in a significant reduction in muscle soreness. Unfortunately, in both of these studies the experimental treatment was not isocaloric with the control, making it impossible to determine whether the observed treatment was due to the protein supplement or additional energy supplied by the protein-containing drink. In a more carefully balanced study by Witard et al.,[14] the effect of increased protein intake on short term decrements in endurance performance during a block of high-intensity training was examined. Well-trained cyclists completed two 3-week trials sequentially consisting of one week of normal training, one week of intensified training and one week of recovery training. Cyclists were prescribed either a high-protein or a normal diet during intensified and recovery training periods. Increased dietary protein intake led to a possible attenuation (4.3%; 90% confidence limits ±5.4%) in the decrement in time trial performance (time to complete set amount of work) after a block of high-intensity training compared with a normal (control) diet (Protein = 2639 ± 350 s; Control = 2555 ± 313 s). Restoration of endurance performance during recovery training possibly benefited (2.0%; ±4.9%) from additional protein intake.

In the current study, the positive effect of the CHO + PRO supplement was noticeable after a much longer period of supplementation use than in previous studies, in which a CHO + PRO supplement has often been ingested on only one occasion post-exercise. Interestingly, the reduction in DOMS in specific muscle groups within the experimental group could be aligned to preceding training sessions. Reduced DOMS in the quadriceps on day 5 in week 3 (Fig. 5), was preceded by two training sessions that specifically targeted the quadriceps on days 1 and 2 of that week. Similarly, reduced DOMS in the calf muscles was observed on days 2 and 3 of week 4 (Fig. 7), after two training sessions in which the calves were particularly targeted. Perceived muscle soreness scores did not increase significantly from baseline in the players who ingested the CHO + PRO supplement, whereas those players who ingested the CHO-only supplement, had a significant increase in DOMS above baseline over the study duration. A reduction in DOMS in athletes who train rigorously and are at an elite level is critically important from a training perspective. Previous research has shown that muscle soreness can severely impair athletic performance.[17] Twist et al.[20] showed that a prolonged increase in perceived muscle soreness and fatigue have critical implications on the quality of training performed by players in the 48 h after a rugby league match. The authors found significant changes in creatine kinase concentrations, perceptual measures of fatigue, muscle soreness, attitude to training, and countermovement jump height flight time of the players 24 h and 48 h post-match. Consequently, perceptual muscle soreness and fatigue is a significant modifying factor in a player's exercise tolerance and attitude towards training.[21] In this study, where players were required to exercise rigorously for an extended period of time (23-day pre-season camp), the perception of muscle soreness increased in players ingesting the placebo.

The second notable finding of this study was that there was no significant effect observed on muscle fatigue (Figs. 2-4) between the two groups despite the differences in DOMS. Muscle fatigue therefore appears to not be affected by protein ingestion, at least under the current experimental conditions, and suggests that other factors may play a more important role in relation to muscle fatigue. Recently, Goh et al.[15] found no difference in perceptions of muscle fatigue and muscle soreness between different compositions of CHO + PRO drinks during prolonged cycling exercise. Furthermore, Gilson and colleagues[6] found no effects on myoglobin concentration, muscle soreness, fatigue ratings and isometric quadriceps force between participants who ingested either a carbohydrate drink or isocaloric chocolate milk drink as a recovery beverage. The lack of treatment effect on fatigue observed in this present study and in previous research[7,8] may be, in part, due to investigations being conducted on elite-level athletes. Therefore, in the current study, the lack of difference in perception of muscle fatigue may be due to the high level of conditioning of the players.[22] It is possible that DOMS and perceived levels of muscle fatigue are governed by different mechanisms.

Finally, to accurately monitor changes in training load and heart rate recovery of the players during the 23-day training camp, the HIMS test was implemented as it is easy to administer, noninvasive and sensitive to change.[19] The final finding of this study was that there were no differences found between the experimental and control groups with regards to heart rate recovery (Fig. 8) as measured by the HIMS test, although HR recovery tended towards significance at week 1 (p=0.095) and at week 3 (p=0.0665). This may be attributed to the increase in training load over the course of the camp being carefully monitored by the coaching staff, thus leading to appropriate changes in load to prevent over training.[4]

Limitations

To the best of these authors' knowledge, the participants in this study did not consume additional macronutrients or supplements, as they were strictly instructed not to do so. However, it is possible that some may have consumed additional macronutrients without the authors' knowledge. Additionally, the daily macronutrient intake of the meals provided at the facility was not strictly controlled, with the players able to choose from the available foods served at each meal. Also, the players did not keep a dietary record. The authors therefore had to interpret the results with caution. Furthermore, due to the nature of this study, where the research participants were elite international Sevens rugby players, they were not willing to consent to the taking of blood samples, and the authors were unable to obtain any biochemical data, such as CK concentrations. Future research needs to be conducted whereby the parameters of the study are more strictly controlled so that the risk of confounding variables is reduced.

In conclusion, the results of this study suggest that during a period of increasing cumulative training load, post-exercise ingestion of a CHO + PRO supplement reduces DOMS, but not fatigue. Thus the effect of protein supplementation on DOMS and fatigue appears to be different. Post-exercise ingestion of CHO + PRO may therefore have a long-term beneficial effect by reducing DOMS and perceived muscle soreness as a consequence of cumulative training load.

Practical applications

- Coaches can better inform players about the benefits of protein supplementation and improved nutritional standards for peak performance.
- Supplementation of CHO+PRO following exercise may reduce perceived symptoms of pain in subsequent exercise.
- Long-term supplementation of CHO+PRO may improve exercise ability as more high intensity and demanding training sessions can be completed in a shorter amount of time.

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