Title: Pre-sleep casein protein ingestion accelerates functional recovery in professional soccer players

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Abstract

Purpose: This study examined whether consuming casein protein (CP) before sleep would enhance recovery after a night-time soccer match in professional players. **Methods:** In a randomized, crossover design, ten professional soccer players from the reserve squad of a team in the highest tier of English soccer consumed 40 g of CP or 40 g of carbohydrates (CON) 30 min pre-sleep after a soccer match (kick off 19:00). To assess recovery, countermovement jump (CMJ) height, reactive strength index (RSI), muscle soreness (MS), and the adapted Brief Assessment of Mood Questionnaire (BAM+) were measured before, 12, 36 and 60 h after each match. Dietary intake across the testing period was also recorded. **Results:** There were *unclear* differences in external load in the matches and dietary intake between CON and CP. CP had a *most likely* and *likely beneficial* effect on CMJ recovery at 12 and 36 h post-match (CP -1.6; ±1.2% vs. CON -6.6; ±1.7%; -4.1; ±2.3% vs. -0.4; ±1.1%, respectively). RSI recovery was *most likely* enhanced with CP at 12 and 36 h post-match and muscle soreness, as measured with a visual analogue scale (mm), was *most likely* greater in CON vs. CP at 12 h post (72; ±17 vs. 42; ±20 mm). BAM+ was *possibly lower* in CON at 36 h post but unaffected at other time points. **Conclusions:** Pre-sleep CP accelerates functional recovery in professional soccer players and therefore provides a practical means of attenuating performance deficits in the days after a match.

Key words: Muscle damage, exercise, protein, muscle soreness.

Introduction

A number of studies report that soccer players often suffer from prolonged decrements in physical function following matches [1-3]. Not only is strength, power and sprint performance affected, but complaints of muscle soreness and psychometric disturbances are also prevalent [2]. Although the time it takes for each component to fully recover varies, a recent meta-analysis established that most of the aforementioned variables are not restored to pre-match values 72 h following a match [1]. This would suggest that performance might not be optimal in the 48 - 72 h period following competitive matches.

The functional deficits outlined above are ostensibly due to muscle damage, a consequence of the high strain of the muscle contractions and eccentric activity associated with soccer [1, 2]. Attempts to alleviate their symptoms often focus on mitigating the initial muscle damage or trying to accelerate the remodelling process [3]. One of the most frequently recommended strategies to stimulate the latter is immediate protein feeding [3, 4]. Indeed, a number of recent reviews on the topic recommend that players consume 20 – 40 g or 0.25 - 0.4 g/kg of high quality protein as soon as conveniently possible after a match [4-6]. The theory is that protein feeding augments exercise induced muscle protein synthesis (MPS), creating the positive net protein balance required to repair and remodel the proteins needed for normal muscle function [7]. This, in turn, might attenuate the decrements in force observed in the days following a match, and perhaps also reduce muscle soreness, by modulating the inflammatory response and accelerating ultrastructural repair [8]. In support of such effects, a recent meta-analysis indicated that whey protein feeding post-exercise has small to positive benefits for muscle function recovery in the days following strenuous exercise [9].

Nonetheless, the majority of the studies assessing the effects of immediate protein feeding on exercise recovery are performed in the morning, when there are several opportunities for additional protein feeding and, thus, further stimulation of MPS throughout the day [9, 10]. Such effects might not translate to scenarios whereby exercise is performed in the evening, and there are fewer opportunities to further promote MPS before sleep — typically a 7 – 10 h post-absorptive period. Indeed, it has been shown that a single dose of 20 – 30 g of protein after an evening exercise bout is not sufficient to elevate overnight MPS [11, 12] probably because MPS rates typically only remain elevated for 1-3 h after a single protein feeding [10]. To overcome this, studies have found that providing a large dose (≥40 g) of casein protein (CP) before sleep can augment overnight MPS. The benefits of this was best demonstrated in the study by Res and colleagues [12] in which participants were fed 20 g of protein after a bout of resistance exercise in the evening before ingesting 40 g of CP or a placebo 30 min before bed. The results indicated that CP improved overnight MPS by ~22%, indicative of enhanced muscle remodelling following the exercise bout [12, 13]. These effects have been replicated in subsequent studies [14-16] and suggest that perhaps by improving MPS, CP supplementation might enhance recovery when exercise is performed late in the evening or at night time.

In the context of soccer, several matches are played in the evening. In the UK, not only are several league and cup matches are played at night (19:45 – 20:05), but all of the league matches played by the under 23 and reserve teams have an evening kick off time (19:00). Given the aforementioned benefits of pre-sleep CP ingestion on MPS, it would be reasonable to assume that after night matches an additional dose of CP pre-sleep (on top of a post-match protein feeding) could enhance the players recovery, by ensuring the damaged muscles have a favourable protein balance during the early stages of the remodelling process. Soccer players are actually already being recommended to consume CP pre-sleep for such purposes [4,-6]; however, the effects on acute functional recovery, which is of most practical relevance to the players, has never been experimentally tested. Thus, the aim of this study was to examine whether pre-sleep supplementation with CP after a competitive match could accelerate acute functional recovery in professional soccer players. We hypothesized that consuming CP before bed would accelerate the recovery of muscle function and reduce muscle soreness as compared to a carbohydrate only control.

Methods

Participants

Ten male soccer players (Age, 19 ± 1 yrs; height, 1.80 ± 0.73 m; mass, 76.3 ± 9.5 kg) from the under-23 and reserve squad of a professional soccer team playing in the English Premier League gave written informed consent for this study. The study received institutional ethical approval from Newcastle University. Players were instructed to avoid the use of any putative recovery modalities (e.g., compression garments) throughout the testing period.

Experimental design

This study employed a randomised, single-blind, placebo controlled, crossover design. After two separate night matches (kick off: 19:00) participants consumed either CP or a carbohydrate control (CON) 30 minutes before going to bed. Their habitual diet was not altered but they did keep a 3-day food diary to record their dietary intake. The order in which the players received the supplements was randomly generated (www.randomizer.org) by the senior author who was not involved in data collection. The following measures were taken at baseline (BL; 84 h after their last match), 12, 36 and 60 h following each match to assess recovery: muscle soreness, countermovement jump (CMJ) height, reactive strength index (RSI) and the adapted brief assessment of mood scale (BAM+). External load during the matches was calculated from GPS units (OptimEye S5B, Version 7.18; Catapult Innovations, Melbourne, Australia) worn during each match. These variables were used to assess for differences in external load between the two matches/conditions (Table 3). There were two players that played less than 60 minutes in one of the matches and these were excluded from the analysis. We used a ≥60 min cut off in a previous study to match the external load in each condition [17].

Counter movement jump height

As in previous studies [17, 18], CMJ was recorded from flight time using an optoelectric system (Optojump, Bolzano, Italy). Players were instructed to jump vertically with maximal force, landing in the same position as take-off. Hands remained on hips throughout the movement. The best effort from 3 attempts (separated by approximately 60 s of recovery) was used for data analysis. CMJ was already used at the club to monitor recovery so the players were well familiarised with the procedure. The inter-day coefficient of variation (CV) was 1.4%.

Reactive strength index

The Optojump optoeletric system was also used to collect RSI; calculated as height/contact time*100 [19]. Participants stood on a 30 cm box with feet shoulder width apart and, after a verbal cue, dropped off the box before jumping vertically with maximal effort. Participants were instructed to minimise their contact time on the floor to <200 ms and to jump with maximal effort. The best of 3 attempts was used for data analysis. The players were familiarised with this procedure prior to data collection; the inter-day CV was 6.8%.

Muscle soreness

Muscle soreness (MS) was recorded using a 0 – 200 mm visual analogue scale (VAS) [18]. The VAS had “no soreness” at 0 mm and “unbearably painful” at 200 mm; the players were asked to mark a vertical line between these two anchors, which was subsequently recorded with a ruler.

BAM+

The BAM+ contains 10 questions relating to subjective wellbeing, each of which is scored by marking a line on a 100 mm VAS anchored with “not at all” and “extremely” at opposing ends. The BAM+ score is calculated by dividing the 4 positively associated questions (e.g., How confident do you feel?) by the 6 negatively associated questions (e.g., How angry do you feel?). For a full list of the questions see Shearer et al., [20].

Dietary analysis

Participants recorded their habitual dietary intake on the day of each match and in the two days following the match (3 days in total) using electronic food diaries. These were subsequently analysed using the online dietary analysis software, Intake24 (Newcastle University, UK). We also analysed the players intakes after each match separately, which encompassed their post-match meals eaten at the club and any snacks before they took the supplement pre-bed. They were not provided with any specific supplements after the match.

Supplements

The macronutrient composition of the CP supplement (Micellar Casein, Maxinutrition, London, UK) and the CON supplement (Pro Iso Elite, Performance Athlete Nutrition, Wales, UK) are displayed in Table 1. These supplements were chosen because they had been batch tested for prohibited substances and the players had not taken them previously. The latter was important for blinding purposes because the supplements were distinguishable by taste. We were unable to find two supplements that were batch tested for prohibited substances, taste matched, and had the necessary macronutrient composition. Thus, the study was single blind only — data collection was performed by someone blinded to which supplement the players had taken. Because we could not blind the players by taste, our next best option was to use two supplements that were unfamiliar to the players, and not tell the players which was the CON and with the CP. Thus, the players were never explicitly told which supplement was which, allowing us to leave them uncertain as to whether they had the CP or the CON supplement, despite the fact the two had different tastes (CP; caramel, CON; lemon). To test whether the players could identify which supplement was which, we gave them a brief questionnaire at the end of testing, asking them to identify whether they thought they had the CP or CON supplement after the two different matches. Only 6 of the 10 correctly identified the supplements they had after each, suggesting that our strategy did help to mitigate the potential influence of a placebo effect on the findings.

In terms of dose and timings, 40 g of CP was selected because 30 g does not seem to be sufficient to maximise overnight MPS [10]. The supplements were provided to the players in the same opaque bottles after each match with instructions to consume them within 30 minutes before going to sleep.

Data analysis

Data were analysed by making probabilistic magnitude-based inferences (MBI) about the true value of outcomes by expressing the uncertainty as 90% confidence limits [21]. The smallest worthwhile change was standardised as the smallest (Cohen) change (muscle function: 0.2 times the between-subject standard deviation; Perceptions: 10% of the scale range). To determine the effect of CP on each dependent variable a spreadsheet designed for the analysis of a crossover trial was used [22]. Change over time in each dependent variable was analysed using a published spreadsheet for the analysis of time series [22]. In order to reduce bias arising from non-uniformity error muscle function data was log-transformed [23]. Means of log-transformed data were back transformed to provide mean percentage change and 90% confidence limits. Qualitative magnitudes of standardised effects were assessed using the following scale: trivial <0.2, small 0.2 – 0.6, moderate 0.6 – 1.2, large 1.2 – 2.0 and very large >2.0 [24]. Clinical inferences were based on threshold chances of harm and benefit of 0.5% and 25%, respectively.

Statistical significance was set at $P < 0.05$. RSI, CMJ, MS and BAM+ values were analysed using a 2 x 4 time points repeated measures ANOVA. If an interaction effect occurred, post-hoc analysis with Bonferroni adjustments were performed. External load and dietary analysis data were analysed with paired student t-tests. These analyses were performed with IBM SPSS Statistics 23 for Windows (Surrey, UK).

Results

Table 2 displays the average macronutrient intake (g/kg) for the 3 day testing period and in the meal consumed post-match in each condition; differences between the dietary intakes were unclear in both analysis (*P*>0.05). The effect of the match and the impact of consuming CP before sleep on markers of muscle function, soreness and performance readiness are shown in Table 4.

Countermovement Jump Height

The competitive soccer match reduced CMJ height, however, the magnitude and time course of this response differed between conditions (Figure 1). Up to 12 h, decreases were moderate in CON but only small in CP. CMJ height remained below baseline at 60 h in CON (small decrease), whereas performance had returned to baseline by 36 h (trivial) in the CP condition. There were clear small to moderate benefits of consuming CP on limiting decreases in CMJ height up to 36 h, with no clear differences at 60 h. The *P* value for the main interaction effect was 0.011. Post analysis revealed significant differences in CMJ height at 12 and 36 h post-match (P = 0.001 and 0.046, respectively).

Reactive Strength Index

RSI was reduced (large) up to 36 h in the CON condition, returning to baseline by 60 h (trivial). There were unclear or trivial changes in RSI in the CP condition. There was a clear large benefit of consuming CP on limiting reductions in RSI up to 36 h, with no clear difference at 60 h. The *P* value for the main interaction effect was 0.001; however, with post-hoc analysis, differences were P = 0.125, 0.192 and 0.511 at 12, 36 and 60 h post-exercise.

Muscle Soreness

Muscle soreness was evident in both conditions up to 36 h (moderate to very large), with peak increases observed at 12 h (Figure 2). At 60 h muscle soreness had returned to baseline values (trivial). A large benefit of consuming CP was observed at 12 h, with unclear or trivial differences at 36 h and 60 h, respectively. The *P* value for the main interaction effect was 0.159.

BAM+

Following each match, the BAM+ score was reduced (moderate to large), indicating a compromised readiness to perform. BAM+ remained below baseline up to 60 h in CON (moderate) but had recovered at this time point in the CP condition (trivial). There were no clear differences in this measure between CON and CP except at 36 h were there was a small benefit in the CON condition. The *P* value for the main interaction effect was 0.309.

Discussion

The main finding of this study was that 40 g of casein protein consumed before sleep enhanced muscle function recovery in the 36 h following a soccer match played at night. Consistent with previous studies in soccer players muscle function was reduced in the 2-3 days following a match [1-3]; however, in the present study, the deficits in muscle function, specifically CMJ and RSI performance, were restored to pre-match values more rapidly when CP as opposed to a carbohydrate CON was ingested 30 min before sleep. These findings are in line with a recent meta-analysis that suggested post-exercise protein intake can attenuate markers of EIMD [9].

The most obvious mechanism by which pre-sleep CP ingestion might have attenuated EIMD is by increasing overnight MPS. This could have accelerated myofibrillar and connective tissue remodelling, which, in theory, would be expected to mitigate muscle force deficits following a damaging insult [7, 25, 26]. An accelerated remodelling process could have also influenced the post-exercise inflammatory response. Indeed, a muscle proteomic study showed that protein ingestion promotes an anti-inflammatory environment consistent with enhanced muscle re-modelling in the early stages of exercise recovery (+4 h) [8]. An attenuated local inflammatory response might also help to explain why muscle soreness was reduced in the CP group 12 h post-match. Although the aetiology of muscle soreness is still not precisely understood, the general consensus is that a local inflammatory response is likely involved [25]. It is possible that the CP helped minimise the early pro-inflammatory response to the muscle damage, which is more pronounced in the first 24 h [26], and this helped attenuated the perceptions of soreness.

Because of the nature of the study and the participants involved, we could not obtain muscle biopsy samples from the players to confirm or refute the aforementioned mechanisms. However, support for such effects are provided by studies showing pre-sleep CP ingestion after evening exercise, as in the present study, augments MPS and results in greater muscle protein accretion compared to a control [12, 14, 16]. Importantly, these effects are evident even if 20 g of protein is consumed immediately after the exercise bout [12] .This latter aspect is important, as in the present study, ingesting the CP before sleep accelerated the recovery of muscle function, despite the fact that the players had consumed ~0.5 g/kg after the match and had high habitual protein intakes of ~1.9 g/kg across the 3 days. These findings are therefore consistent with the idea that when performing exercise in the evening, an immediate protein feeding might not be sufficient to maximise muscular reconditioning and functional recovery [11].

It is important to note that with CP, players consumed an additional 40 g of protein. Given that absolute protein intake over a 24 h period is thought to have the greatest impact on MPS and therefore muscle re-conditioning [10], it would be reasonable to assume that it was this greater absolute protein intake as opposed to the timing of the intake that improved recovery in this study. In partial support of this, a recent study found that when absolute daily protein intake was kept consistent (1.8 g/kg), resistance training induced adaptations in strength over a 10-week period were similarly improved regardless of whether 35 g of CP was consumed during the day or pre-bed [29]. In contrast, when participants received an additional 27.5 g of daily protein for 12 weeks, in the form of a pre-bed CP supplement, strength gains over a 12-week training period were greater [30]. Taken the findings from these studies into consideration, it could be argued that the results of the present study are because CP increased total protein intake in the 24 h following the match, and the pre-bed timing was not a critical factor. Because habitual protein intakes were high (~1.9g/kg), this suggest perhaps even higher intakes are beneficial for recovery after a match. Thus, what this study perhaps best demonstrates is that consuming a large bolus of CP before bed represents a practical and easy to implement strategy to enhance total protein intake following a night match and this appears to enhance functional recovery. Clearly, research that matches total daily protein intakes are needed to investigate whether it was the timing of CP intake or total protein that influenced recovery.

Whether the recovery benefits seen in this study (and others) are specific to CP or can be replicated with other high quality protein sources, such as whey, should also be examined in future studies. Some support for similar effects with whey was provided by West et al., [26] who found that consuming 25 g of whey protein after an exercise bout (21:00) and again 10 h later upon waking (07:00) accelerated the recovery of strength and CMJ performance at 10 and 24 h post-exercise. Whey protein only elevated whole body net protein balance (compared to control) at 24 h post-exercise though, with no effects seen at 10 h. This suggests that either CP is more beneficial than whey for maximising overnight MPS, or a higher dose of whey is required to elicit similar effects (e.g., 40 g). Also, given that full recovery is not always shown to occur within 72 h after a match [1], it is possible that by raising the players daily protein intake on these subsequent days (via pre-bed CP supplementation), muscle tissue remodelling could be even further accelerated. The most beneficial dosage strategy should be examined in future studies. Further studies are also needed to determine whether the benefits observed in this study are specific to night matches. Previous studies have shown that pre-sleep protein feeding augments the overnight MPS response when exercise is performed in the evening, suggesting the effects might be more pronounced after night matches [13.] However, this needs to be experimentally tested to refute.

A limitation of this study is the low sample size and future studies should look to include greater numbers. Also, that participants were male professional under 23 soccer players means that these findings might not be transferable to recreational players, those playing other sports, and females. However, the fact that this study was performed with professional soccer players is clearly an overall strength of this study, as the vast majority of studies examining any form of protein supplementation and exercise recovery are performed in recreationally active individuals, meaning that the findings are not necessarily applicable to well-trained soccer players.

Practical applications

From a practical perspective, these findings suggest that professional soccer players can accelerate functional recovery by ingesting 40 g of casein protein after matches played at night. Intriguingly, these benefits were evident despite the fact that the players consumed a high protein meal post-match. This would suggest that a protein-rich meal after a night match is not sufficient to optimise recovery following a night match, and therefore players should be encouraged to consume an additional bolus of protein before bed.

Conclusion

In conclusion, this is the first study to demonstrate that pre-sleep CP ingestion can enhance the recovery of muscle function after a soccer match played at night. Although the precise mechanisms remain to be elucidated, these findings suggest that the additional CP before sleep could have accelerated the morphological processes required to restore muscle function.

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Conflicts of interest

The authors declare no conflicts of interest.

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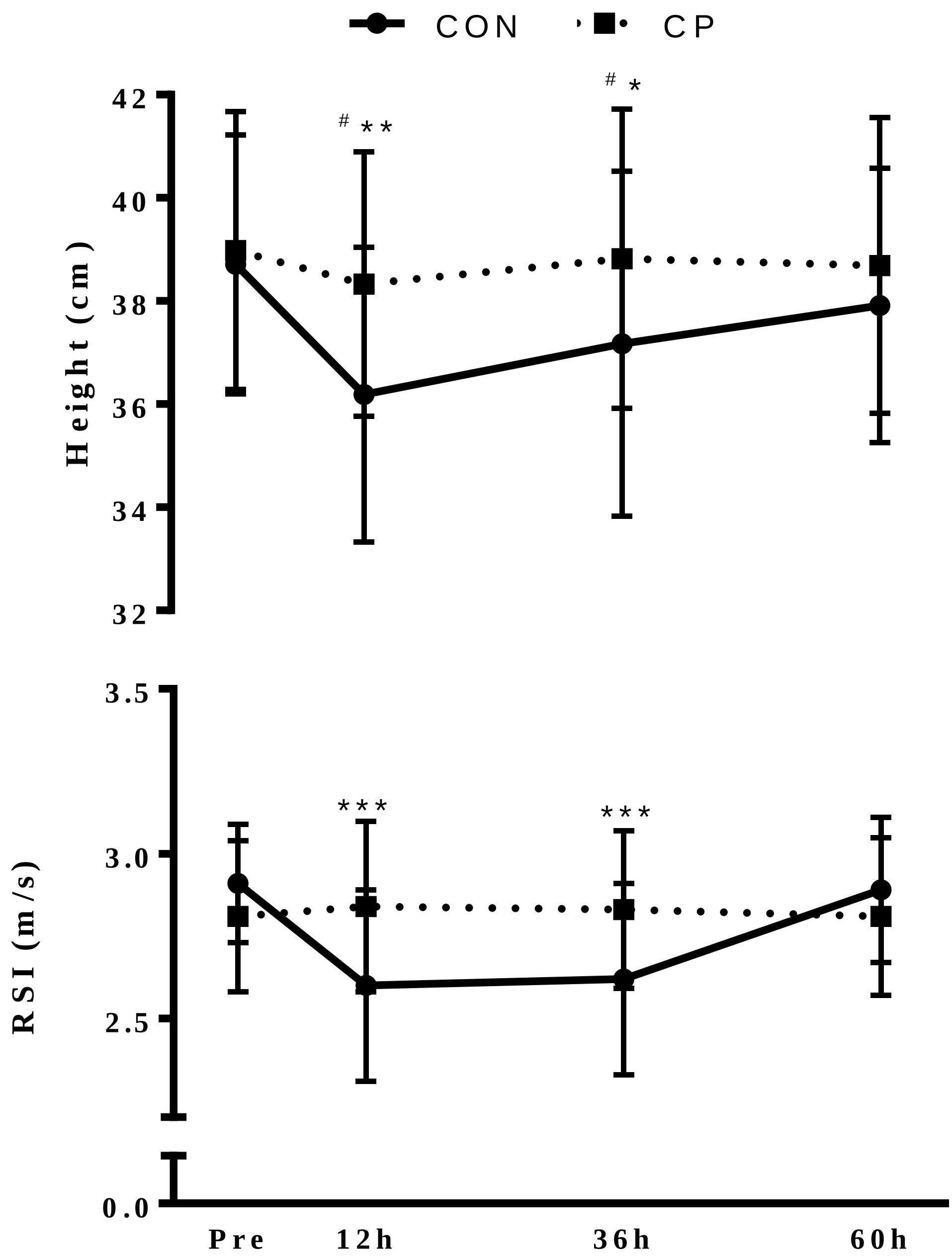

Figure 1. Changes in countermovement jump (CMJ) height and reactive strength index (RSI) before and up to 60h following a match. Clear differences represented by the number of asterisks (*) with *small, **moderate and ***large. #Represent P < 0.05.

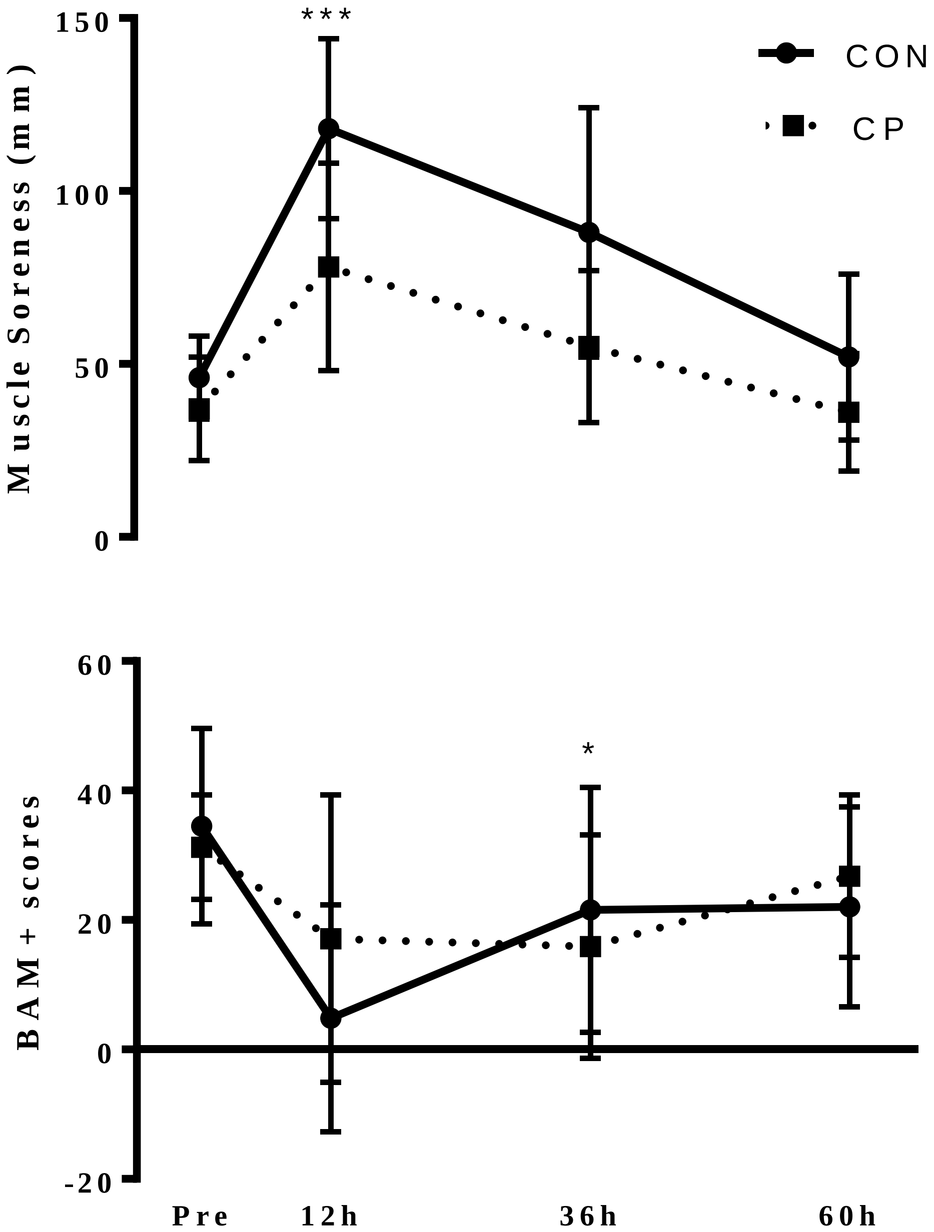

Figure 2. Changes in muscle function and BAM+ scores before and up to 60h following a match. Clear differences represented by the number of asterisks (*) with *small, **moderate and ***large.

Table 1. Energy and macronutrient content of the casein protein (CP) and control (CON) supplements.

Supplement	CP	CON
Energy (kcals)	188	160
Volume (ml)	350	350
Carbohydrate (g)	3.6	40
Protein (g)	40	0
Fat (g)	1.2	0

Table 2. Average energy and macronutrient intake (g/kg) across the 3-day testing period and after the match when casein protein (CP) or carbohydrate (CON) was consumed.

	Kcal	FAT	PRO	CHO
Average dietary intake				
CP	36.14 ± 5.59	0.98 ± 0.24	1.86 ± 0.22	4.97 ± 0.90
CON	36.67 ± 6.07	1.08 ± 0.31	1.93 ± 0.27	5.12 ± 0.92
Difference (%)	1.2; ±12.3	-8.6; ±19.2	-3.2; ±10.0	-2.7; ±14.9
Post-match dietary intake				
CP	9.24 ± 2.13	0.33 ± 0.08	0.51 ± 0.13	1.13 ± 0.28
CON	9.84 ± 2.39	0.34 ± 0.07	0.52 ± 0.12	1.24 ± 0.41
Difference (%)	-5.5; ±18.3	-5.7; ±18.2	-2.4; ±19.9	-6.1; ±24.7

These values do not include the CP or CON supplements and are therefore representative of their habitual intakes.
Difference reported as Mean (CP minus CON) ± 90% confidence limits (add and subtract this number to obtain 90% confidence limits for the true difference
There were no clear differences between conditions for any variable.

Table 3. A comparison of external load during match-play for the two conditions (PL vs. CP). Total distance is the total distance covered during the match; explosive distance refers to the distance travelled accelerating at $\geq$2 $m.sec^{-1}$ and decelerating at $\leq$2 $m.sec^{-1}$; sprint distance is the distance travelled at $\geq$60% of maximum speed ($km.h^{-1}$); and duration is the total number of minutes spent on the field of play

	PL	**CP**	**Difference** (Mean[a]; ±90% CL[b])
Total distance (m)	10605 ± 1701	11009 ± 889	4.8; ±11.2
Explosive distance (m)	679 ± 131	744 ± 116	10.2; ±15.8
Sprint distance (m)	566 ± 225	649 ± 289	9.9; ±50.2
Duration (min)	85 ± 11	89 ± 2	5.5; ±8.6

PL and CP values presented as mean ± SD; n =
There were no clear differences between conditions for any variable
[a]Mean represents CP minus PL; [b]add and subtract this number to the mean to obtain 90% confidence limits for the true difference

Table 4. The effect of casein protein on muscle function and perceptions following a competitive soccer match**.**

		Change Over Time				Effect	
		CON		CP		CON/CP	
		Change (Mean; ±90% CL)	Magnitude	Change	Magnitude	Difference (Mean[a]; ±90% CL[b])	Magnitude
CMJ (%)	B – 12h	-6.6; ±1.7****	Moderate	-1.6; ±1.2*	Small	5.3; ±2.1****	Moderate
	B – 36h	-4.1; ±2.3***	Small	-0.4; ±1.1**	Trivial	3.9; ±3.0**	Small
	B – 60h	-2.1; ±1.5**	Small	-0.8; ±1.6*	Trivial	1.4; ±2.4	Unclear
RSI (%)	B – 12h	-11.0; ±4.2****	Large	0.9; ±3.9	Unclear	13.3; ±7.9***	Large
	B – 36h	-10.2; ±4.0****	Large	0.8; ±4.0	Unclear	12.3; ±7.9***	Large
	B – 60h	-0.8; ±2.0*	Trivial	0.1; ±1.5**	Trivial	1.0; ±3.1	Unclear
DOMS (mm)	B – 12h	72; ±17****	Very Large	42; ±20***	Very Large	-30; ±30*	Large
	B – 36h	41; ±22*	Very Large	19; ±17*	Moderate	-23; ±32	Unclear
	B – 60h	6; ±13***	Trivial	-1; ±12***	Trivial	-6; ±21**	Trivial
BAM+ (mm)	B – 12h	-30; ±30***	Large	-14; ±16*	Large	16; ±27	Unclear
	B – 36h	-13; ±15*	Moderate	-15; ±10**	Large	-2; ±20*	Small
	B – 60h	-13; ±14*	Moderate	-5; ±10**	Trivial	8; ±18	Unclear

The likelihood that the true value will have the observed magnitude is represented by the number of asterisks (*) with *possibly, **likely, ***very likely and ****most likely.

Magnitude of effect size are assessed using the following criteria: <0.2 = Trivial, 0.2-0.6 = small, 0.6–1.2 = moderate, 1.2-2.0 = large and >2.0 = very large.

[a]Mean represents CP minus PL; [b]add and subtract this number to the mean to obtain 90% confidence limits for the true difference