Andreas Druzkowski

The impact of pre-sleep protein ingestion on sleep parameters and recovery markers

A pilot study in proffesional soccer players

Master's thesis in Physical Activity and Health - Exercise Physiology
Supervisor: Ulrik Wisløff
Co-supervisor: Arnt Erik Tjønna
May 2021

Master's thesis

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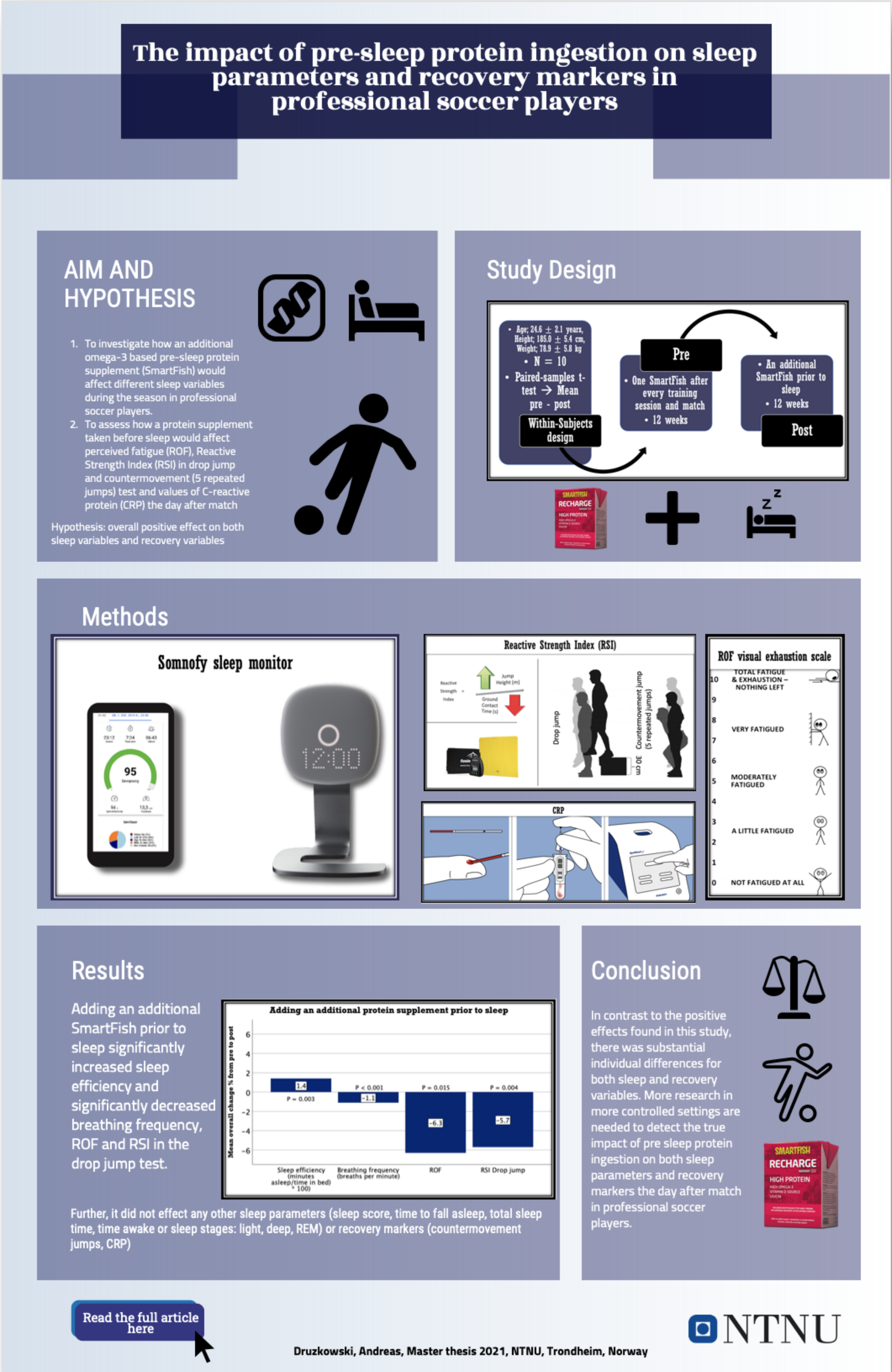

Abstract

Background & purpose: It has been demonstrated that protein ingestion before sleep increases muscles protein synthesis rates during overnight recovery after sessions with high physical exertion. However, it remains unestablished how a pre-sleep protein supplement would affect different sleep variables. The primary aim of this study was to investigate how an omega-3 based pre-sleep protein supplement would affect different sleep variables during the season in professional soccer players. Secondary aims were to assess how a protein supplement taken before sleep would affect perceived fatigue (ROF), Reactive Strength Index (RSI) and values of C-reactive protein (CRP) the day after soccer matches.

Methods: In a within-subjects design, ten male soccer players (Age; 24.6 ± 2.1 years, Height; 185.0 ± 5.4 cm, Weight; 78.9 ± 5.8 kg) from a male team playing in the second highest division system in Norway, consumed a 200 mL omega-3 whey protein-based drink (SmartFish, Oslo, Norway) after every training session and match for twelve weeks. Thereafter, an additionally 200 mL of the same protein supplement were taken before sleep for twelve weeks. Sleep was objectively measured using non-contact radar technology (Somnofy, VitalThings AS, Norway) and the following sleep variables were collected: sleep score (0-100 score, algorithm of all variables) sleep efficiency score (0-100 score, (minutes asleep/time in bed) * 100), time to fall asleep, total sleep duration, time in different sleep stages (REM, deep sleep, light sleep, awake) and respiratory breathing rate per minute (RPM). To assess following match day recovery ROF was measured using the Hooper-Index, a 0 to 10 visual exhaustion scale (where 0 referred as "not fatigue at all" and 10 referred as "total fatigue & exhaustion – nothing left"), while RSI (jump height/contact time) was measured in a drop-jump (DJ) and five repeated countermovement jump (CMJ) test performed on a contact math (Smart-Jump, Fusion Sport, Nottingham, United Kingdom). Capillary blood was collected to measure CRP concentrations and analyzed by the QuikRead go machine (Orion Diagnostica, Espoo, Finland). Due to multiple testing on sleep parameters the significance level on primary aims was set at $p < 0.01$, while a p-value of $p < 0.05$ was used for secondary aims and recovery markers.

Results: Adding an extra protein prior to sleep significant increased sleep efficiency by relative 1.4 % ($p = 0.003$) and significant decreased RPM by relative 1.1 % ($p < 0.001$), ROF by relative 6.3% ($p = 0.015$) and RSI in the DJ test by relative 5.7% ($p = 0.004$). All except two players increased sleep efficiency and decreased in RPM and ROF, while all players decreased RSI in the DJ test. No other variables significantly changed when taking an additional protein supplement prior to sleep.

Conclusion: Adding an additional protein ingestion prior to sleep increased sleep efficiency and decreased RPM, while both ROF and RSI in the DJ test decreased the day after match. There were substantial individual differences for both sleep and recovery variables. More research is needed to detect the true impact of pre sleep protein ingestion on both sleep parameters and recovery markers the day after match in professional soccer players.

Bakgrunn og formål: Det er vist at inntak av protein før søvn øker proteinsyntesehastigheten i muskler under restitusjon over natten etter økter med høy fysisk anstrengelse. Det er imidlertid ikke etablert hvordan et proteintilskudd før søvn vil påvirke forskjellige søvnvariabler. Hovedmålet med denne studien var å undersøke hvordan et omega-3-basert proteintilskudd før søvn ville påvirke forskjellige søvnvariabler i løpet av sesongen hos profesjonelle fotballspillere. Sekundære mål var å undersøke hvordan et proteintilskudd tatt før søvn ville påvirke opplevd utmattelse (ROF), Reaktiv Styrke Indeks (RSI) og verdier av C-reaktivt protein (CRP) dagen etter fotballkamper.
Metode: I et innen gruppe-design, ti mannlige fotballspillere (Alder; 24,6 ± 2,1 år, Høyde; 185,0 ± 5,4 cm, Vekt; 78,9 ± 5,8 kg) fra et mannlig lag som spiller i det nest høyeste divisjonssystemet i Norge konsumerte en 200 mL omega-3 myseproteinbasert drikke (SmartFish, Oslo, Norge) etter hver treningsøkt og kamp i tolv uker. Deretter ble ytterligere 200 ml av det samme proteintilskudd tatt før søvn i tolv uker. Søvn ble målt objektivt ved hjelp av berøringsfri radarteknologi (Somnofy, VitalThings AS, Norge) og følgende søvnvariabler ble samlet inn: søvnpoeng (0-100 poengsum, algoritme for alle variabler) søvneffektivitetspoeng (0-100 poengsum, (minutter) sovende / tid i sengen) * 100), tid til å sovne, total søvnvarighet, tid i forskjellige søvnstadier (REM, dyp søvn, lett søvn, våken) og respirasjonsfrekvens per minutt (RPM). For å vurdere restitusjon etter kampdag ble ROF målt ved hjelp av Hooper-indeks, en 0 til 10 visuell utmattelsesskala (hvor 0 referert til som "ikke utmattelse i det hele tatt" og 10 referert til som "total utmattelse - ingenting igjen"), mens RSI (hoppehøyde / kontakttid) ble målt i dropp-hopp (DJ) og fem gjentatte motbevegelseshopp (CMJ) -tester utført på en kontaktmatte (Smart-Jump, Fusion Sport, Nottingham, Storbritannia). Kapillærblod ble samlet for å måle CRP-konsentrasjoner og analysert av QuikRead go-maskinen (Orion Diagnostica, Espoo, Finland). På grunn av flere tester på søvnparametere ble signifikansnivået på primære mål satt til $p < 0,01$, mens en p-verdi på $p < 0,05$ ble brukt for sekundære mål og restitusjonsmarkører.
Resultat: Tilskudd av et ekstra protein før søvn økte søvneffektiviteten signifikant med relative 1,4% ($p = 0,003$) og reduserte RPM signifikant med relative 1,1% ($p <0,001$), ROF med relative 6,3% ($p = 0,015$) og RSI i DJ-test med relative 5,7% ($p = 0,004$). Alle unntatt to spillere økte søvneffektiviteten og reduserte i RPM og ROF, mens alle spillerne reduserte RSI i DJ-testen. Ingen andre variabler endret seg signifikant når et ekstra proteintilskudd ble konsumert før søvn.
Konklusjon: Å konsumere et ekstra proteintilskudd før søvn økte søvneffektiviteten og redusert RPM, mens både ROF og RSI i DJ-testen reduserte dagen etter kamp. Det var betydelige individuelle forskjeller for både søvn- og restitusjonsvariabler. Mer forskning er nødvendig for å bekrefte den virkelige effekten av proteintilskudd før søvn har på både søvnparametere og restitusjonsmarkører dagen etter kamp hos profesjonelle fotballspillere.

Introduction

Soccer involves many different demanding physical activities such as sprinting, high intensity running, accelerations and decelerations, in addition to technical aspects such as passing, dribbling and shooting. These requirements often lead to prolonged decrements in physical function following matches or high-intensity training sessions (1). Strength, power and sprint performance are usually negatively affected, in addition to psychometric disturbances and occurrence of muscles soreness (2). Generally, this decline in muscle performance associated with muscle activity is defined as fatigue (1). Although there are individual differences to the rebuilding of each component. A meta-analysis established that the mentioned parameters are not fully recovered up to 78 hours post-match (3), which clearly highlights the importance of adequate recovery for soccer players.

Sleep is a crucial body function that has not been giving enough attention in athletic populations. It provides a number of important physiological functions that may be fundamental to the recovery process (4), and is arguably the single most important factor in exercise recovery. Essentially, it is not just the total sleep time, but also time spent in each sleep cycle is of importance for recovery (5). Simplified, sleep can be categorized into two types of sleep, non-rapid eye-movement (NREM) sleep and rapid eye-movement (REM) sleep (6). NREM is further divided into four different stages after a continuum of relative depth, where each stage has unique characteristics which includes differentiations in eye movement, brain wave patterns and muscle tone etc. Sleep stage one and two is defined as light sleep which make about 50 % of total sleep during the night. Stage three is deep sleep and make about 20-25 % of total sleep time. At this stage brain waves and the respiratory system is at its lowest (7). In contrast REM sleep is characterized with rapid eye-movements, lack of muscle activity known as muscle atonia and being the dreaming-state of the different sleep cycles. This sleep cycle is important for emotional recovery and adaptions (8). The total sleep is made up of these stages going in repeated cycles. In a typical night, the total sleep is distributed into 4-6 cycles where each cycle last about 60-90 minutes (5).

It is well known that elite soccer players may experience constraints that can interference with their sleep patterns, like variable wake-up times and/or bedtimes as a results of variable match schedule, exposure of bright lights and engaging in exciting or emotionally stimulating actions etc. Further, there is a lack of scientific evidence on sleep characteristics and requirements in elite soccer players, mostly due to the inaccuracy of the use of actigraphy and wrist activity monitors for sleep tracking (4). The gold standard for sleep tracking is polysomnography, which records brain waves activity, oxygen level in blood, hearth rate, breathing, in addition to leg and eye movement (9). However, polysomnography is not feasible in real-life settings due to its compliance and costs.

Another field which has not been giving enough attention in athletic populations is the timing and effects of protein ingestions after training and matches. However, in the past years timing of protein consumption has been given more attention in the literature (2, 10–13). It is well known that muscle damage and inflammation after demanding physical activities increases the muscle protein breakdown, resulting in increased muscle protein synthesis (MPS) and muscle remodeling (10). A study done in professional rugby players showed that adding two daily 200 mL omega-3 whey protein-based supplements, containing a high number of eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) protein, after morning and afternoon training sessions during pre-season training resulted in reduced muscle soreness and better maintenance of explosive power compared to a placebo (14). A similar whey protein supplement study was performed in younger adults, where protein ingestion prior to sleep suggested an accelerated recovery in the first days after an evening soccer match (2). Overnight sleep is the longest post-period of the day, suggesting pre-sleep protein ingestion would improve muscle MPS rates and consequently an accelerated muscle recondition and recovery (12). More research is required to further establish the true impact of pre-sleep protein supplements may have on muscle reconditioning and recovery in more practical settings of soccer performance.

To the best of the authors´ knowledge, no study has yet conducted how such a pre-sleep protein supplement would affect different sleep variables in an elite soccer population. Partly explained by previous research the measure method for tracking sleep has been primarily wrist monitors, questioners, or a combination of both. This study is one of the first studies to implement a new non-contact radar technology and machine learning in elite soccer players, which has shown to have an accuracy up to 96 percent compared to polysomnography (15). Unlike previous research, this measurement device can register time under each sleep cycle, as well as bedroom analysis (air quality, light, noise, temperature). This new technology open up a wide range of opportunities for sleep research, thus making the practical aspect of sleep interventions feasible for such an athletic population.

The Hooper-Index is shown to be a promosing tool to measure subjective rate of fatigue (ROF) in soccer players (16), indicating an easy and precise measure to implement. The reacitve strength index (RSI) demonstrates a soccer player´s ability to quickly and effectively move trough the stretch-shortening cycle and their ability to change from an eccentric to a concentric contraction (17). This is of importance in various physical variables such as jump performance, running speed, agility, muscular endurance and maximal strength (18). By measuring RSI (jump height/contact time) on a force plate in both the drop jump (DJ) and five-repetead counter movement jump (CMJ) test it gives information about how well the central-nervous system has recovered. Furthermore, to assess the inflammatory response due to tissue damage blood samples where taken to measure the amount of C-reactive protein (CRP) (19).

The primary aim of this study was to investigate how a pre-sleep protein supplement would affect following sleep variables: sleep score, sleep efficiency, time to fall asleep, total sleep time, deep sleep, REM sleep, light sleep, time awake and breathing frequency per minute (RPM), during the season in professional soccer players. A secondary aim was to further establish the impact of a pre-sleep protein supplement would have on next day recovery markers ROF, RSI and CRP.

We hypothesize that an implementation of an additionally 200 mL omega-3 protein-based supplement prior to sleep would have an overall positive effect on both sleep parameters and recovery markers.

Methods

Participants

Ten male soccer players (Age; 24.6 ± 2.1 years, Height; 185.0 ± 5.4 cm, Weight; 78.9 ± 5.8 kg) from a professional male soccer team playing in the second highest division in Norway participated in the study. Goalkeepers were excluded from the study. The study received institutional ethical approval from the Norwegian University of Science and Technology and Norwegian Center for Research Data (project ID 139892). All players gave written permission to participate in the study, including sleep recordings and protein supplements. Further, players who played >60 minutes in matches were instructed to participate in testing of recovery markers (ROF, CRP, RSI) the following day, regardless of the game were played at home or away.

Experimental design

This study is a within-subjects experimental design. For twelve weeks, immediately after every training session and match the players consumed a 200 mL whey protein-based drink. In the following twelve weeks the players consumed an additional 200 mL of the same protein drink 30 minutes before going to sleep, in addition to the one received after every training and match. Their habitual diet was not altered. Sleep was objectively measured every night throughout the whole study intervention. The following measures were taken at baseline (defined as 84 hours after their last match), and 12-36 hours following each match: ROF, RSI (DJ and CMJ), and CRP. A cut off ≥60-minute for matches were used to match the external load. Additionally, players who played ≥60-minutes were instructed to participate in a cold-water exposure (12 degrees) for 15 minutes at all home-games throughout the season.

Sleep monitor

A non-contact sleep monitor named Somnofy (Somnofy, VitalThings AS, Norway) were used to objectively conduct sleep variables. Before the intervention, every player was handed out a sleep monitor and instructed how to correctly set up and use the device. The sleep monitor registrers which of each sleep cycle you are in with 30-seconds intervals, where radar emitted pulses is reflected by objects and returned to monitor (15). Sleep monitoring is carried out automatically when the players enter the bed. The following variables are registered in the Somnofy app; time in bed, time to fall asleep, time point when you fell asleep, time point when you wake up, total sleep duration, sleep score (0-100, algorithm of all variables), sleep efficiency score (0-100, minutes asleep/time in bed) * 100), sleep stages (REM, deep sleep, light sleep, awake), RPM. The data variables are processed in advanced algorithms which produces a sleep score and a sleep efficiency score. Also, it covers a bedroom analysis including air quality, light, noise and temperature, variables are not included in this study due to lack of relevance of the aim. Every player had access to their own data through the app, while the researchers had access to real-time overview of each players data. The data was observed closely throughout the intervention period to determine and solve any technical issues that could appear in relation to the sleep monitoring systems.

Supplements

The protein-based drinks contained multiple nutrients including 1400 omega-3 PUFA (440 mg EPA and 660 mg DHA), 15 g protein, 2,2 g leucin and 3µg vitamin D (SmartFish, Oslo, Norway). All drinks were handed out by equipment personnel in the club at every training and match.

Ratings of perceived fatigue

Rating of perceived fatigue was documented using a 0 to 10 visual exhaustion scale (20). The ROF scale consist of 11 numeric points ranging from 0 to 10, where 0 referred as "not fatigue at all" and 10 referred as "total fatigue & exhaustion – nothing left". This was retrieved from the students by verbal communication between the students and players during the days of testing.

Reactive Strength Index

RSI (jump height/contact time) were used as a measurement of neuromuscular fatigue and implemented in two different jump tests performed on a contact match (Smart-Jump, Fusion Sport, Nottingham, United Kingdom). Firstly, they performed a DJ test where the players were instructed to stand on a 30-cm box with the feet shoulder with apart, hands on the hips, drop slowly with one foot forward and jump vertically with maximal effort and as little contact time on the math as possible. Secondly, the CMJ test was performed on the math. Equally, the players were instructed to have hands on the hips, jump with maximal effort and with as little contact time as possible. Performing five repeated jumps, where the last four jumps were used in the data analysis. The RSI test provide a quick and reliable means of monitoring reactive strength capabilities of athletes (21).

C-reactive protein

CRP was measured using a quantitative analysis machine QuikRead go (Orion Diagnostica, Espoo, Finland). Capillary blood was collected from the fingers from the players, and an automated CRP value was given (mg/L). This is a valid and practical method to monitor inflammation, in addition to distinguish between viral and bacterial infections (22). Lower values specify less inflammatory response due to tissue damage, indicating a faster recovery (19).

Statistical analyses

A paired-samples t-test was used to determine whether there was a statistically significant difference within multiple sleep variables (total sleep time, time to fall asleep, sleep score, sleep efficiency, REM sleep, deep sleep, light sleep, awake time, RPM, respectively) and within recovery variables (ROF, RSI, CRP), when players imbibed one omega-3 based protein drink after training and matches compared to taking an additionally omega-3 based protein drink prior to sleep (IBM SPSS Statistics Version 27). Due to multiple testing on sleep parameters the significance level on primary aims was set at $p < 0.01$ to deal with the probability to find significant result due to chance. A p-value of $p < 0.05$ was used for secondary aims and recovery markers. Data are presented as mean $\pm$ 1SD. Normality was assessed by Shapiro-Wilik test of normality along with visual assessments boxplots and Q-Q Plots. Inspections of outlier values did not reveal any to be extreme and they were kept in the analysis. Due to missing data because of lack of playing time or not being present/not participating in the recovery test three players had to be excluded in ROF and five in RSI and CRP analysis, respectively.

Results

Sleep variables

When consuming one omega-3 protein-based supplement after every training and match following results were obtained for all players: sleep score 85.5 ± 6.0 (0-100), sleep efficiency 79.8 ± 6.5 (0-100), total sleep time 07:51 ± 00:54 (hours: minutes), time to fall asleep 00:47 ± 00:27 (hours: minutes), REM sleep 01:51 ± 00.26 (hours: minutes), deep sleep 01:24 ± 00:20 (hours: minutes), light sleep 04:37 ± 00:42 (hours: minutes), awake time 00:31 ± 00:23 (hours: minutes) and RPM 14.4 ± 1.7 (breaths per minute).

Adding an additional omeg-3 protein-based supplement prior to sleep significantly increased sleep efficiency by relative 1.4% (**Figure 2**, 80.9 ± 7.3, p = 0.003) and decreased RPM by relative 1.1% (**Figure 9,** 14.2 ± 1.6, p < 0.001), but did not impact sleep score (86.2 ± 9.2 (0-100)), total sleep time (07:50 ± 00:55 hours: minutes), time to fall asleep (00:47 ± 00:31 hours: minutes), REM sleep (01:50 ± 00.27 hours: minutes), deep sleep (01:24 ± 00:22 hours: minutes), light sleep (04:38 ± 00:42 hours: minutes) or awake time (00:31 ± 00:27 hours: minutes) taking all players in consideration.

All expect two players increased in sleep efficiency and decreased in RPM. No other variables significantly changed when taking an additional protein supplement prior to sleep. Individual variability is presented in **Figure 1-9**.

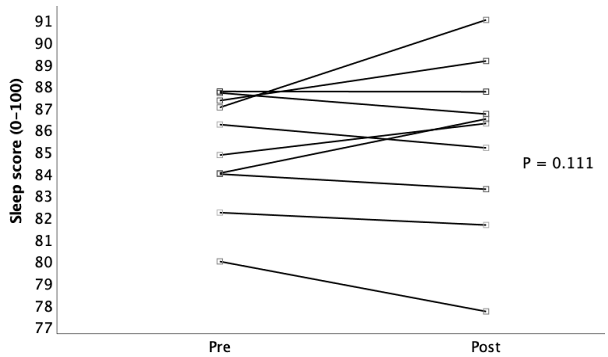

Figure 1. Individual variety for sleep score (0-100) shown as mean pre and post. Mean change pre to post (all players) 0.7 ± 10.5, p = 0.111.

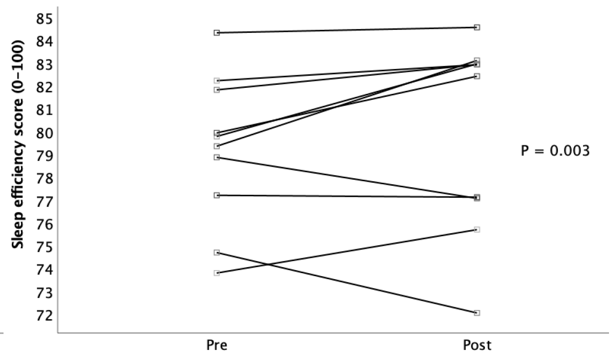

Figure 2. Individual variety for sleep efficiency (0-100) shown as mean pre and post. Mean change pre to post (all players) 1.1 ± 7.3, p = 0.003.

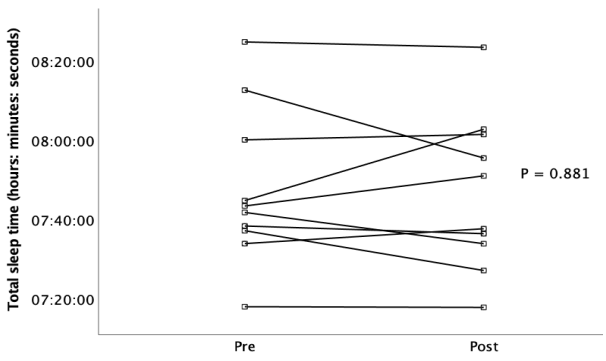

Figure 3. Individual variety for total sleep time shown as mean pre and post. Mean change pre to post (all players) 00:00 ± 01:16 (hours: minutes), p = 0.881.

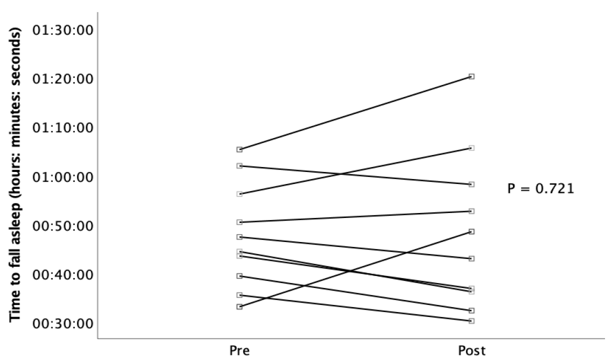

Figure 4. Individual variety for time to fall asleep shown as mean pre and post. Mean change pre to post (all players) -00:01 ± 00:38 (hours: minutes), p = 0.721.

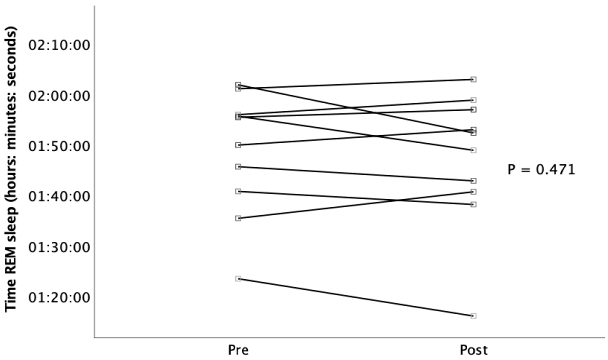

Figure 5. Individual variety for time in REM sleep shown as mean pre and post. Mean change pre to post (all players) -00:01 ± 00:35 (hours: minutes), p = 0.471.

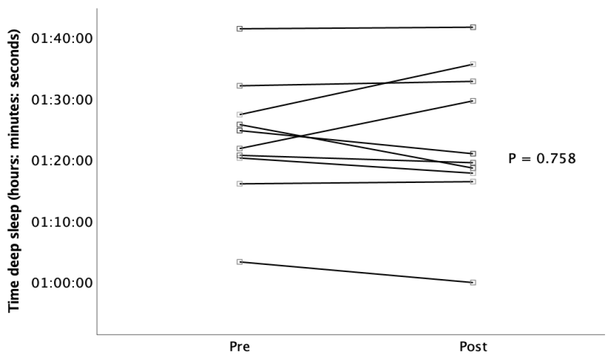

Figure 6. Individual variety for time in deep sleep shown as mean pre and post. Mean change pre to post (all players) 00:00 ± 00:26 (hours: minutes), p = 0.758.

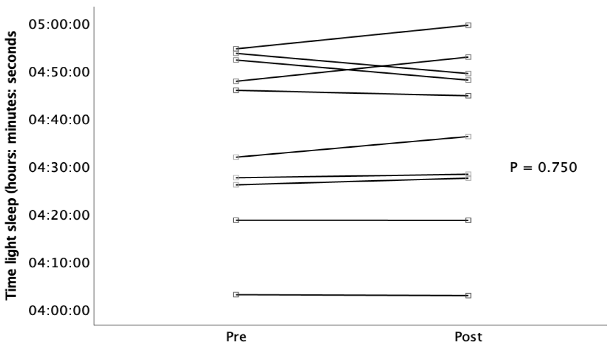

Figure 7. Individual variety for time in light sleep shown as mean pre and post. Mean change pre to post (all players) 00:01 ± 00:57 (hours: minutes), p = 0.750.

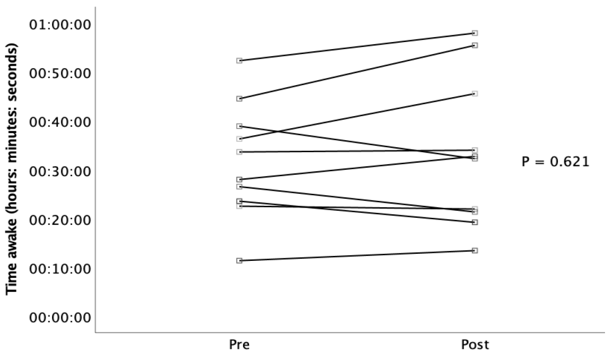

Figure 8. Individual variety for time awake shown as mean pre and post. Mean change pre to post (all players) 00:01 ± 00:31 (hours: minutes), p = 0.621.

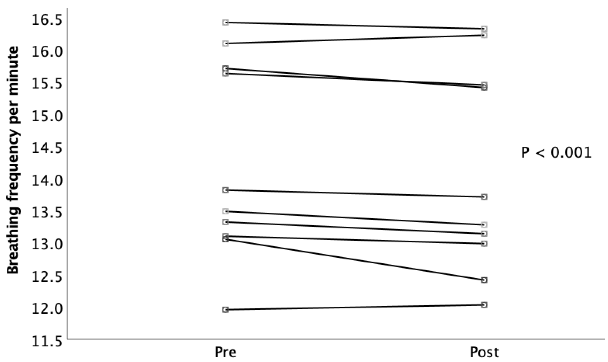

Figure 9. Individual variety for breathing frequency shown as mean pre and post. Mean change pre to post (all players) -0.1 ± 0.9 (breaths per minute), p < 0.001.

Recovery variables

When consuming one omega-3 protein-based supplement after every training and match following results were obtained for all players: ROF 6.6 ± 1.2 (0-10), DJ test 1.65 ± 0.32 (RSI), CMJ test 1.85 ± 0.32 (RSI) and CRP 4.0 ± 2.5 (mg/L). Adding an additional omega-3 protein-based supplement prior to sleep significantly decreased ROF by relative 6.3% (**Figure 10**, 6.2 ± 0.6, p = 0.015) and DJ performance by relative 5.7% (**Figure 11**, 1.56 ± 0.31, p = 0.004) taking all players in considerations. An additional omega-3 protein-based supplement prior to sleep did not impact CMJ performance (**Figure 12**, 1.88 ± 0.3, p = 0.504) or CRP concentrations (**Figure 13**, 4.2 ± 2.2, p = 0.673) the following match day. Five of seven players decreased ROF, while all players decreased DJ performance.

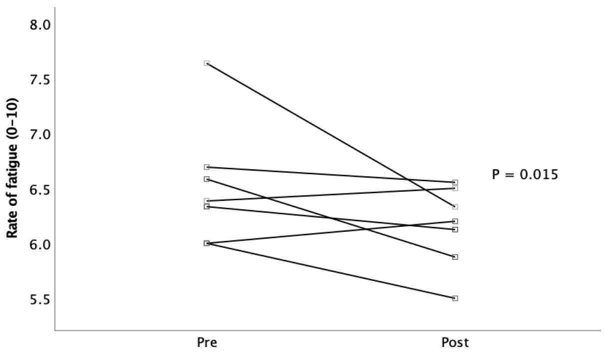

Figure 10. Individual variety for rate of fatigue (ROF) shown as mean pre and post. Mean change pre to post (all players) -0.4 ± 1.3 ROF, p = 0.015.

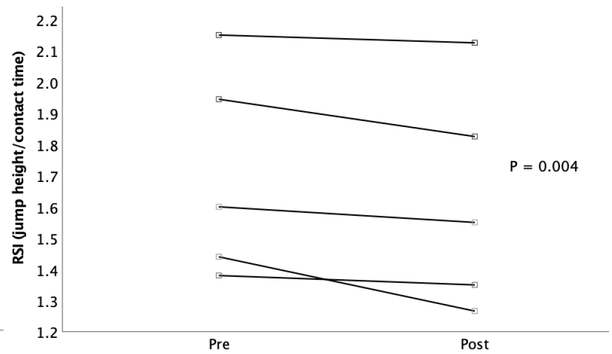

Figure 11. Individual variety for drop jump (DJ) performance (RSI) shown as mean pre and post. Mean change pre to post (all players) -0.1 ± 0.2 RSI, p = 0.004.

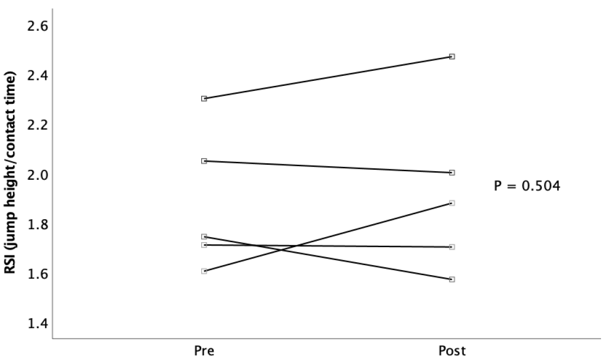

Figure 12. Individual variety for counter movement (CMJ) performance (RSI) shown as mean pre and post. Mean change pre to post (all players) 0.0 ± 0.3 RSI, p = 0.504.

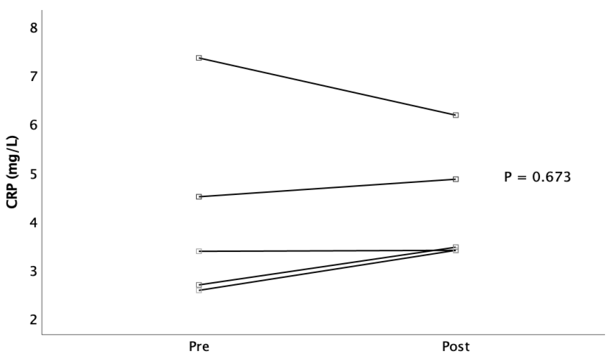

Figure 13. Individual variety for c-reactive protein (CRP) values shown as mean pre and post. Mean change pre to post (all players) 0.2 ± 3.0 mg/L, p = 0.673.

Discussion

The present study emphasizes the individual variety related to sleep in professional male soccer players. The main finding of this study was that adding an additionally protein supplement prior to sleep significantly increased sleep efficiency and decreased RPM, while ROF and RSI in the DJ test decreased the day after match. However, this was only significant when examining all players. There were substantial individual differences in both sleep and recovery variables.

Sleep variables

The overall increase in sleep efficiency and decrease in RPM were minor, making the clinical relevance questionable. Though, one player showed a relative 4.0% increase in sleep efficiency which could have a positive practical effect for this player. The same where found for one player with a 5.1% decrease in RPM, equaling 0.6 fewer breaths per minute. Further, RPM is shown to be a stable metric which has only slightly changes in the different sleep stages. Heart rate variability and resting heart rate may change meaninglessly from day to day, while RPM generally does not (23). Indicating that a change in RPM tends to be meaningful. Following the stress argument by Tipton et al (24) the respiratory ventilation will increase when individuals are faced with higher internal or external stressors. Therefore, even a slightly decreased RPM might indicate an overall better ability to cope with the total stress load in an overnight recovery period when consuming an additional protein supplement prior to sleep.

Also, one relative increase of 4.6% in sleep score were detected. Since sleep score is calculated by an algorithm of all variables taken together, it is reasonable to expect a better overall sleep for this player. When taking an additional protein supplement before sleeping this player also showed a relative 1.4% increase in sleep efficiency, relative 1.2% decrease in RPM, relative 6.5% less time to fall asleep and 24.3% less time awake, respectively. None of these variables were found significant, although this could provide an overall positive effect for this player. No other studies have investigated the effects of adding a pre-sleep protein on several sleep variables in an athletic population and therefore we have no data to compare with. Previous research indicates that adding a pre-sleep protein is not found to effect sleep (getting to sleep, quality of sleep, waking from sleep) in healthy untrained older men and women (25). Interestingly, one study investigated vitamin D and omega-3 fatty acids (EPA and DHA) supplementation in healthy young-middle aged men and women who did not habitually consume oily fish, found a significant increase in sleep efficiency when consuming DHA-rich oil compared to a placebo (26).

Taking this in considerations, it is reasonable to speculate that the effect seen in sleep efficiency and RPM in the present study was because of the high amount of omega-3 fatty acids in the protein supplement and not the amount of protein per se. More research on the effects of both omega-3 fatty acids and protein on sleep are needed to further detect the true impact omega-3 fatty acids and protein may have on different sleep parameters in athletes. It is also worth mentioning that the overall results could be of coincidences when interpreting a high number of paired samples data. Further, due to its compliance of measurement napping was not taken care of in this study, even though napping has been established to be an efficient recovery strategy for soccer players to cope with both cognitive and physical abilities decline following partial sleep deprivation nights (27–29). Still, minor changes over prolonged periods during in-season could be of importance in athletic populations. Sport science is characterized by looking for margins that can give real-life advantages for athletes, where these margins not necessary is easy to establish from a research perspective.

Recovery variables

The overall decreased ROF (**Figure 10**, p = 0.015) is in line with the work of Abbott et al. (2) which found reduced muscle soreness at 12 hours post-match after pre-sleep protein ingestion in professional soccer players. The same dose of the same omega-3 protein-based supplement were used in the study by Black et al (14), which found a reduced muscle soreness with protein ingestion compared to a placebo during pre-season training in rugby players. This study supports these findings, where five of seven players showed a lower mean ROF when taking an additional protein supplement prior to sleep. Thus, indicating a faster overnight recovery and a better physiological capability to overcome the load in upcoming training sessions and matches. However, the decrease of all players in RSI in the DJ test (**Figure 11)** indicates that the decreased ROF not necessary reflects physiological enhancements. Speculative, it may be explained by the Hawthorne-effect where players improved subjective feeling of fatigue only because they inhibited an additional protein supplement prior to sleep and not because of changes from the additional protein supplement.

The same omega-3 protein-based supplement were used in the study by Philpott et al (30) and did not detect changes in CRP concentrations following eccentric exercise in competitive soccer players and corresponds with the result found in this study (**Figure 13**). One possible explanation for the similar CRP concentrations between the two intervention periods may relate to the high carbohydrate content in the protein supplements. A high carbohydrate intake has previously been known to reduce inflammation (31). Since the players were taken protein supplements in both intervention periods it could impact the possibility to detect any effects from the additional protein supplement prior to sleep on CRP values in this study. Additionally, it could be beneficial to measure creatine kinase concentrations as a marker of tissue damage and recovery efficiency, especially since professional soccer players is found to have high resting creatine kinase values during in-season and are not in the same extent affected by the carbohydrate intake (32).

Somewhat surprisingly, the overall RSI in the DJ test the day after match decreased in the period of consuming an additional protein supplement prior to sleep (**Figure 11**, p = 0.004), where all five players had a lower mean RSI value when receiving an additional protein supplement prior to sleep. Whereas DJ performance decreased there was contradictorily differences for RSI in the CMJ test (**Figure 12**, p = 0.504), where two individuals showed a slightly increase, two individuals showed a slightly decrease and one individual showing no changes after implementation of an additional protein supplement prior to sleep. This is in contrast of the study by Black et al. (14) where two daily doses of the same omega-3 protein-based supplement increased overall peak force during three CMJ jumps (4.6% ± 5.9%) after 35 days into pre-season training in elite rugby players. Unlike the study by Black et al (14) the additional protein supplement prior to sleep period in our study was over twelve weeks in the last half of the season. Distance-based variables like total-distance, sprint, accelerations, and decelerations is found to progressively decrease across the season in professional soccer players (33), indicating a requirement for longer recovery time further out in the season. Thus, more controlled studies with more repeatedly post-match testing (10, 24, 48, 72 hours) on in-season periods are needed to further detect the true impact of an omega-3 protein-based supplement taken before sleep on recovery markers in professional soccer players.

Interestingly, a meta-analysis by Davies et al. (34) found a moderate effect for jump height to be greater with a whey protein supplementation after 10 hours of recovery, but not after 24 hours, suggesting a more rapid restoration of neuromuscular function in limited recovery durations (<10 hours). Further, there was a moderate beneficial effect of protein supplementation on the eccentric phase variables at both 10 hours and 24 hours after resistance exercise-induced damage, but the concentric phase was hindered at 24 hours. In this study post-match testing was performed at different time points depending on the team's training schedule and where mostly performed ~ 24 hours. The decreased RSI in the DJ test, which is calculated by jump height/contact time and measures the neuromuscular ability to go from an eccentric motion to a concentric contraction, could partly be explained by a hindered ability in the concentric contraction. Furthermore, the players only performed one attempt at both the DJ and the CMJ test, which perhaps failed to ascertain the true RSI value and influence the results in our study.

Protein dose-response relationship

Previous studies have indicated that there may exist a protein-dose relationship. Res et al. (13) revealed for the first time that 40g of casein protein ingestion before sleep increased MPS rates by relative 26% and improved whole-body protein balance (61 ± 5 vs -11 ± 6 $\mu mol \bullet kg^{-1}$ per 7.5 h) during postexercise overnight recovery compared to a non-protein placebo in healthy young men. During sleep the casein protein was effectively digested and absorbed, resulting in a rapid rise in amino acid levels. Further, Moore et. Al (35) found that 20g of whole egg protein to be sufficient to maximally stimulate MPS rates after resistance exercise in young men. Indicating a dose-response relationship between protein ingestion and MPS rates which reaches a plateau at ~ 20g.

Taking these findings in consideration, the protein supplement used in this study consisted of 15g whey protein per serving, making a total of 30g protein per day, which could consequently not fully maximize the overnight MPS rates and may contribute to explain the relatively small effect seen on outcome variables in the present study. However, previous studies in professional rugby and soccer players using the same amount of the same protein supplement has demonstrated reduced muscle soreness, fatigue, as well as maintaining of CMJ performance (14,30). Demonstrating that the protein supplement used in this study would contain enough protein to stimulate MPS rates enough to expect any possible changes in the sleep parameters or recovery variables, even though the dose is lower than the known dose to fully maximize MPS rates in younger persons (35).

Protein type and omega-3 fatty acids

The protein supplement used in this study contains of whey protein and has a high amount of omega-3 fatty acids. It has been demonstrated that supplementation of whey protein in resistance training that whey protein is absorbed quicker than other types of protein, resulting in an increased MPS rates (36). Thus, it is considered as a high-quality protein. The supplement also has a high level of the fatty acids EPA and DHA, where supplementation of these omega-3 fatty acids has shown to increase MPS rates in healthy men and women (31, 37). Further, it enhances the muscles sensitivity to protein, making the combo of omega-3 fatty acids and whey protein extra effective. However, the work of Mcglory et al. (38) in resistance-trained males did not detect any effect of adding omega-3 fatty acids on MPS rates, were a dose of 30g protein was used, achieving maximal MPS and leaving no further capacity for omega-3 fatty acids to confer anabolic influence.

In a meta-analysis by Thielecke and Blannin (39) focusing on effects of EPA and DHA supplementation on performance-related outcomes in athletes found EPA and DHA supplementation to reduce ROF in rugby players and reduced delayed onset muscle soreness in healthy males. Further, no study has found a change in either DJ or CMJ performance, power, or speed variables in athletes, but one study found an increase in CRP concentrations in male cyclist omega-3 fatty acids supplementation.

Limitations

A low sample size and the difficulty of dealing with missing data are the two major limitations of this study. High individual variability in the sleep variables, which is reflected by higher standard deviations in the results, may lead to bias when interpreting the results. Due to the sleep monitors were placed at the players homes it was not possible to conduct data related to accommodation in away matches or other situations where the players did not sleep at home. The within-subjects design is also at risk of bias regarding that there was no control group in our study and there were protein supplementations in both intervention periods. Ideally it would be beneficial with a randomized case-control study design were one group received a protein supplement before sleep and one group received a placebo. However, in this study it was not possible due to practical aspects and clarifies the difficulties of performing research in elite alethic populations. Furthermore, no dietary considerations were taken care of in this study and the total protein intake between the two intervention periods is likely not matched. Further, the protein supplementation was not matched for bodyweight. Consequently, heavier players received a smaller dosage of omega-3 fatty acids and protein while lighter players received a larger dose of omega-3 fatty acids and protein in terms of g/kg, which would provide different individual possibilities for the effect of an additional protein supplement ingested before sleep.

Lack of data is further the major weakness for the recovery variables. Because of the nature of the study, we could not influence the starting line-up or how much each player played in each match. Only players who played more than 60 minutes in matches were brought in to testing the day after matches. Furthermore, the players could not be present the day of testing or did not perform the RSI tests because of injuries of discomfort. Also, the players training load and daily stress load were not considered. Overall, this contributed to some reduced dataset and could make it difficult to detect any actual true differences between the two intervention periods for both the sleep parameters and the recovery markers.

Our study highlights the difficulties of studying elite athletic populations and creates the basis for further studies on the effects of protein ingestion before sleeping on sleep parameters and next day recovery markers in professional soccer players. Future studies should be performed in more controlled settings, perhaps in a junior elite soccer team where it can be controlled for total protein intake and external load, where one group is randomized to one omega-3 protein-based supplement before sleep and another group randomized to one placebo supplement over a prolonged in-season period.

Strengths

To the best of authors´ knowledge this is the first study to implement a new objective validated sleep measurement over a prolonged in-season period in professional soccer players. Most of the studies examining any form for protein supplements, sleep and recovery is performed on recreationally active individuals, meaning that results from those studies not necessary could be relevant for well-trained soccer players. Further, this is the first study to investigate how a protein ingestion prior to sleep would affect multiple sleep variables like time under different sleep stages (light, REM, deep) and RPM, where time under each stage and RPM is of importance for recovery (5). The protein supplement used in this study consist of whey protein, which is considered a high-quality protein and suitable protein source for post training and match in our population. Additionally, it contains high levels of omega-3 fatty acids which enhances the muscles sensitivity to protein, making the combo of whey protein and omega-3 fatty acids more effective.

Conclusion

Adding an additional omega-3 protein-based ingestion prior to sleep increased overall sleep efficiency and decreased RPM, while both ROF and RSI in the DJ test decreased the day after match. There were substantial individual differences for both sleep and recovery variables. It is unclear if these changes are of any practical importance and if these changes were caused by the additional protein or the omega-3 fatty acids implemented before sleep. More research in more controlled settings is needed to detect the true impact of such a pre-sleep omega-3 protein-based ingestion may have on both several sleep parameters and recovery markers the day after match in professional soccer players.

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