THE SCIENCE BEHIND

NIGHTTIME PROTEIN

Nighttime Protein

Key Points

- Casein is a slow release protein in comparison with whey.
- Casein is the protein of choice in research using a protein source before sleep.
- There is clear evidence that when casein is ingested before sleep, it is digested and absorbed and leads to increased amino acid availability for muscle recovery.
- A combination of casein and resistance exercise in the evening leads to an increase in muscle.
- A dose of 40g casein is deemed desirable before sleep in order to promote muscle protein accretion.
- A combination of tryptophan and magnesium could prove useful in aiding sleep duration and quality.
- NutritionX Nighttime Protein is an ideal casein-containing protein to aid recovery overnight. Additionally, the inclusion of tryptophan and magnesium should enhance sleep quality and quantity.

Introduction

Many studies have focused on the benefits of whey protein to stimulate muscle protein synthesis (MPS) as well as to attenuate muscle protein breakdown (MPB) with an overall positive Net Protein Balance (NPB). This is quite natural since whey protein has a higher amount of BCAAs and EAAs than other sources of protein (see Science Behind "Big Whey"). Does that mean that other sources of protein, such as casein, should be disregarded? The simple answer is No. In this article, the facts about casein are reviewed as well as its potential as a useful pre-bedtime protein source.

Additionally, the importance of quality and duration of sleep has been shown to impact on performance (Watson, 2017). The athlete repairs muscle during sleep and this process does not just require the appropriate 'building blocks' of amino acids but also the ability to sleep soundly. A number of nutritional products have been demonstrated to aid sleep, and these include tryptophan (an essential amino acid), and magnesium. A brief exploration of these components will be examined.

Casein

Casein is an important protein constituent of milk (Figure 1), in which it can be seen to make up 80% of milk protein as opposed to 20% being whey protein. The Science behind articles on "Milk Proteins" and "Whey vs Casein" should be examined for more detail.

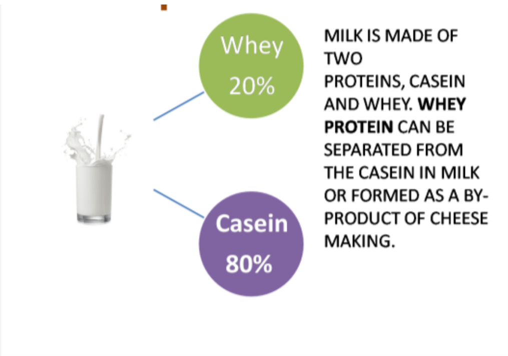

Figure 1. Components of Milk.

As previously described, the amino acid composition of casein is not as high in either the EAAs or the BCAAs (Table 1). This would suggest that casein is an 'inferior' source of protein than whey. To an extent this is true, although the fact that casein curdles in the stomach and is slower to digest and absorb may present some beneficial feature under certain circumstances. In effect, casein is a slow-release protein which could be useful as a protein source at bedtime.

Table 1. The amino acid compositions of Whey and Casein.

	Amount (in grams) per 100 g Protein	
Concentration	**Whey**	**Casein**
Alanine	4.66	2.84
Arginine	2.65	3.31
Asparagine	11.25	6.71
Cysteine	2.65	0.35
Glutamine	16.58	20.61
Glycine	1.78	1.73
Histidine	2.25	2.87
Isoleucine	5.72	5.03
Leucine	11.77	8.77
Lysine	9.68	7.44
Methionine	2.10	2.71
Phenylalanine	3.54	4.80
Proline	4.62	10.10
Serine	4.78	5.73
Threonine	5.05	3.97
Tryptophan	2.04	1.16
Tyrosine	3.59	5.38
Valine	5.29	6.46
Total	**100**	**100**
EAA	**50.09**	**46.53**

EAA, essential amino acids

Muscle protein

A single session of exercise stimulates the rate of MPS, and to a lesser extent, MPB (Phillips et al.,1997). As reported in the article on "Big Whey", if the exercise is undertaken in the fasted state there is a negative NPB. Protein ingestion stimulates MPS and inhibits MPB, resulting in a positive NPB and so leads to net muscle protein accretion during the acute stages of post-exercise recovery. Consequently, post-exercise protein ingestion is widely applied as a strategy to stimulate MPS and, as such, to facilitate the skeletal muscle adaptive response to exercise training. Various factors have been identified which can modify the post-exercise muscle protein synthetic response to exercise. These include the amount, type, timing, and distribution of protein ingestion.

A large variety of dietary protein sources have been shown to stimulate post-exercise muscle protein synthesis rates, including egg protein, whey and casein, milk and beef protein, and soy protein. However, dietary protein sources can differ in their capacity to stimulate MPS, which is largely dependent on differences in rates of protein digestion and absorption as well as their amino acid composition, with the leucine content being of particular relevance.

Besides the amount and type of ingested protein, the timing and distribution of protein ingestion throughout the day can modulate post-exercise muscle protein synthesis rates. An even distribution of total protein intake over the three main meals stimulates 24 h muscle protein synthesis rates more effectively than an unbalanced distribution in which the majority (>60%) of total daily protein intake is consumed at the evening meal. During 12 h of post-exercise recovery Areta et al., (2013) showed that an intermediate pattern of protein ingestion (20 g every 3 h) increases MPS to a greater extent than the same amount of protein provided in less frequent but larger amounts (40g every 6 h), or in more frequent, smaller amounts (10g every 6 h). Therefore, an effective pattern of daily protein intake distribution to support MPS is to provide at least 20g of protein with each main meal with no more than 4–5 h between meals.

Since overnight sleep is likely to be the longest post-absorptive period during the day, the concept of protein ingestion prior to sleep as a means to augment post-exercise overnight MPS needs to be considered.

Overnight Protein Metabolism

In general, most studies assess the effects of food intake on the MPS responsre to exercise performance when in an overnight fasted state. Such conditions differ from normal everyday practice in which training is often performaed in the morning and/or afternoin or in the evening after a full day of habitaual work and food intake.

Beelen et al., (2008) evaluated the impact of exercise performed in a fed state in the evening and the efficacy of protein ingestion immediately after exercise on muscle protein synthesis during overnight recovery. The ingestion of 20–25 g of protein during exercise increased MPS during the exercise bout, but no increase was observed during the prolonged overnight recovery period. In this investigation the MPS during overnight sleep was unexpectedly low, with values being lower than those observed in the in the morning following an overnight fast. Thus, a day of habitual food intake and the ingestion of 20–25g of protein during and/or immediately after an exercise bout performed in the evening does not appear to promote overnight muscle protein reconditioning.

Could these results be due to the gut not functioning optimally during sleep? In order to check that Groen et al., (2012) gave 40g of casein via a nasogastric tube while participants slept. They found that such a feeding strategy resulted in normal digestion and absorption, and they concluded that the gut does function normally at night when asleep if fed.

Protein feeding before sleep as a strategy to increase MPS

The first investigation to examine if protein feeding before sleep was of benefit after an evening training session was undertaken by Res et al., (2012). Recreational athletes were studied during overnight recovery from a single bout of resistance exercise performed in the evening after a full day of a standardised dietary intake. Immediately after exercise, all athletes ingested a recovery drink containing 20g of protein to maximize MPS during the acute stages of post-exercise recovery, and this was followed by either 40g casein or a placebo drink immediately prior to sleep. The protein ingested prior to sleep was observed to be properly digested and absorbed throughout overnight sleep. The greater plasma amino acid availability following pre-sleep protein ingestion improved the overnight whole-body protein balance, allowing the NPB to become positive. MPS was approximately 22% higher during overnight recovery when protein was ingested prior to sleep when compared to the placebo treatment. Figure 2 shows the positive effect of casein ingestion on overnight muscle protein synthesis compared with placebo. From these data the authors concluded that “pre-sleep protein ingestion represents an effective dietary strategy to further augment the skeletal muscle adaptive response to resistance-type exercise training”.

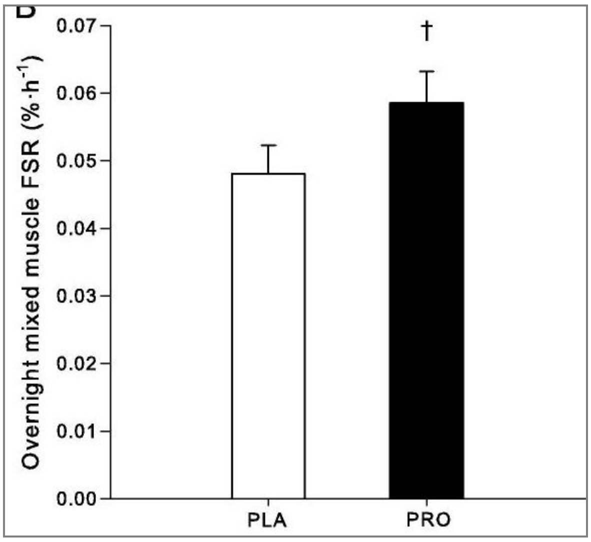

Figure 2. Efficacy of casein feeding (PRO) before sleep on overnight MPS compared with placebo (PLA).

As a consequence, Trommelen & van Loon (2016) produced a schematic (Figure 3) to highlight the variation in MPS as a consequence of feeding through the day as well as feeding casein (or not 'A') before sleep.

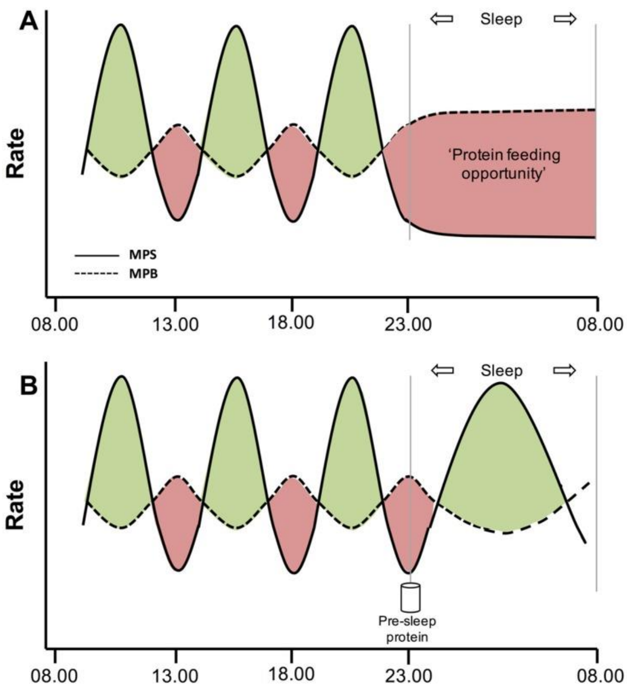

Figure 3. Schematic of MPS and MPB throughout the day.

Protein ingestion stimulates MPS rates and allows for net muscle protein accretion (green areas). During the post-absorptive phases MPB exceeds MPS resulting in net loss of protein (red areas).

Overnight sleep is the longest post-absorptive period (A). Protein ingested before sleep stimulates overnight MPS and so enhances muscle recovery during sleep (after Trommelen & van Loon, 2016)

More recently, Snijders et al., (2015) selected healthy young men in a 12-week resistance exercise training program (three exercise sessions per week) during which they ingested either 27.5g of casein prior to sleep, or a non-caloric placebo. Muscle mass and strength increased to a greater extent in the group that ingested protein prior to sleep (Figure 4). These results indicate that protein supplementation prior to sleep represents an effective dietary strategy to enhance the gains in muscle mass and strength during resistance training.

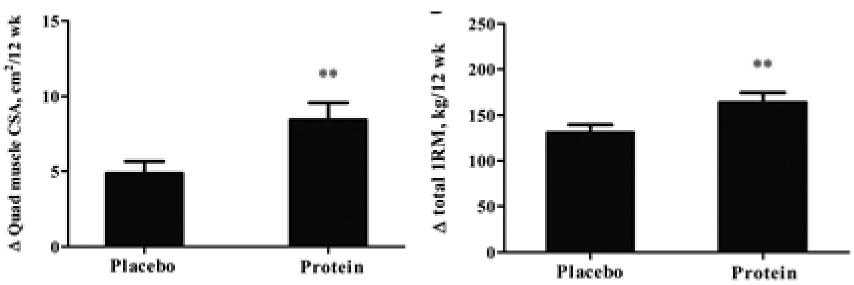

Figure 4. Change (Δ) in quads muscle cross section area (CSA) and also in 1 repetition maximum (1-RM) from casein or placebo taken before sleep during 12 weeks training programme (after Snijders et al., 2015).

It should be noted that the ingestion of the pre-sleep casein supplements in both the acute and long-term studies were compared with a non-protein placebo, and not compared with protein supplementation provided at other time points. Therefore, the benefits of pre-sleep protein provision have to be treated with some caution. As such, additional pre-sleep protein ingestion represents a practical strategy to increase the total daily protein intake, add another meal, and increase the overnight MPS. This effect is likely to be additive to MPS observed throughout the day.

While the overnight sleeping period can be seen as a new window of opportunity to augment post-exercise training adaptations, it remains to be established how much casein is required to maximize the impact of pre-sleep protein feeding on overnight MPS. The ingestion of 40g casein prior to sleep stimulates overnight MPS is considerably more than the 20 g of protein that is supposed to maximize MPS during the first few hours of post-exercise recovery in the day. Would a lower dose of casein be just as useful?

Trommelen & van Loon (2017) addressed this issue by performing a study in which they provided 30g casein prior to sleep, with or without an additional 2g of free leucine. In contrast to previous findings with 40g protein, the ingestion of 30g protein prior to sleep did not significantly increase overnight MPS. This suggests that a pre-sleep protein dose-response relationship exists, and that this differs from the immediate post-exercise recovery period during which the ingestion of 20g protein seems to maximize post-exercise muscle protein synthesis rates in young adults.

The authors also observed that the ingestion of casein was incorporated into new (*de novo*) muscle protein following overnight recovery. So, casein before sleep provides amino acids as precursors for *de novo* myofibrillar protein accretion during overnight sleep. This supports observations that the ingestion of 30g protein prior to sleep promotes muscle mass during 12 weeks of resistance-type exercise training (Snijders et al., 2015). However, data suggest that at least 40g of pre-sleep protein is required to induce a more substantial and detectable increase in MPS when assessed acutely over a 7.5-h overnight period.

The 30g of casein before sleep might not be sufficient to adequately increase overnight MPS, and that is why Trommelen et al., (2017) included a treatment in which 2 g crystalline leucine was added to the 30g of protein. The addition of supplemental free leucine to a suboptimal amount of protein has been shown to enhance post-exercise MPS in previous studies but failed to do so in this overnight feeding study. Given the extended duration of overnight sleep compared to a typical postprandial period (8 vs. 4–5 h), it is tempting to speculate that larger amounts of protein (≥40 g) are required to maximize muscle protein synthesis rates during overnight sleep.

Type of Pre-Sleep Protein

As protein sources differ in their capacity to stimulate MPS, the type of protein ingested prior to sleep may modulate the overnight muscle protein synthetic response. So far, all studies assessing the efficacy of pre-sleep protein ingestion on exercise reconditioning have used casein. Casein is a more slowly digestible protein source, allowing a more moderate but prolonged rise in plasma amino acid concentrations (see Figure 5). Given the extended nature of overnight sleep (~8-h), it could be speculated that a more sustained postprandial increase in available amino acids during overnight sleep is preferred as it will provide precursors to support MPS throughout the night. In contrast, whey protein is a more rapidly digestible protein, resulting in a pronounced but transient rise in plasma amino acid concentrations. Ingestion of a single bolus of whey protein has been shown to stimulate muscle protein synthesis rates to a greater degree than casein over periods up to

6 h. It remains to be established if whey is superior to casein when ingested prior to sleep and MPS are assessed over a more prolonged overnight period of 7.5 h.

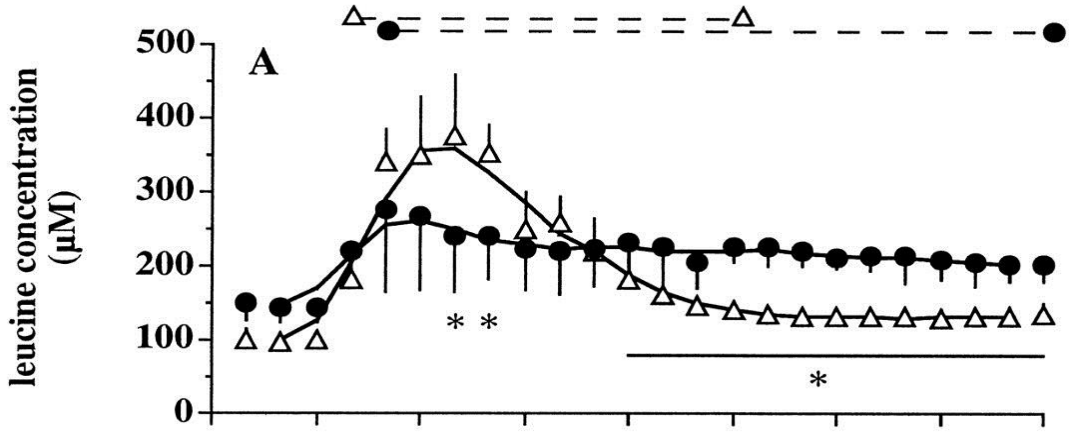

Figure 5. Plasma leucine concentrations after a labelled Whey and a Casein meal (from Boirie et al. 1997).

What happens during sleep?

There is a common perception that sleep is for rest, a period during which the mind and body can rejuvenate after a hard day's work. This assumption is not unfounded—during sleep, humans are less responsive and less mobile, not dissimilar to other states of unconsciousness such as coma (but unlike comas, sleep is rapidly reversible). However, sleep is a time of significant brain activity that can be observed using an electroencephalogram (EEG), which can measure the electrical activity that takes place in the brain during sleep. Sleep is also measured by observing eye movements, which closely correlate to the type of brain waves observed in the EEG.

The two main states of sleep have been defined as non-rapid eye movement (NREM) and rapid eye movement (REM). During NREM sleep, neuronal activity in many parts of the brain is decreased, and the waves that appear on the EEG are characteristically slower than waking states. In addition, this sleep stage is accompanied by noticeable physiological changes, including the increased secretion of growth and sex hormones and decreased motor activity, heart rate, metabolic rate, breathing rate, blood pressure and intestinal mobility. Conversely, scientists have found that during REM sleep, brain waves are similar to those observed when humans are awake. REM sleep is characterized by pupil constriction and rapid movement of the eyes. Accompanying physiological responses include irregular heart rate, breathing and blood pressure. In addition, REM sleep is also when dreams occur, which have been described as intense bursts of activity in certain populations of neurons.

Throughout the night, the brain will alternate between periods of REM and NREM sleep every 90 minutes, repeating this cycle five to six times every night. Although both REM and NREM periods will occur during this 90 minute time window, the proportion of REM to NREM sleep increases during the night. NREM sleep dominates just after falling asleep, while periods of REM sleep dominate in the later sleep cycles.

Sleep and circadian rhythm

The body responds differently depending on the time of the day. For example, it is easier to fall asleep at night than in the middle of the day. This is because darkness activates production of melatonin, a hormone that promotes sleep. Indeed, the human body runs on a 24-hour cycle of wakefulness and sleep. This so-called circadian rhythm (circa-, “approximately,” –diem, “day”) is driven by pacemaker cells in the hypothalamus, the part of the brain that controls a range of vital functions, including hunger, thirst, blood pressure and body temperature. Circadian rhythm not only controls when alertness and tiredness, but also coordinates countless chemical reactions. For example, during the day, when blood sugar levels are likely to be high from eating, the body activates the chemical reactions that break down sugar into stored energy. Conversely, during the night, when blood sugar is likely to be low, the specific processes that create sugar from stored glycogen are activated. The effects of the circadian pacemaker can also be seen at a whole-organism level. Human alertness and performance is highest during the day and lowest in the hours before daylight (3:00 – 6:00am), correlating with the time that humans are likely to be awake and asleep (see Figure 6). Additionally, immediately after waking, there is a period of time when you may feel groggy and not alert. This span of time is known as sleep inertia and can last for hours. In opposition to this sleep inertia, the circadian clock sends out wake-promoting signals throughout the day, which counteracts the propensity to go back to sleep. The opposite process happens at night, with sleep-inducing signals eventually overpowering the wake-promoting ones, resulting in sleep. This natural cycle of sleep/wake signals, driven by the circadian rhythm, explains why humans tend to sleep more efficiently at night and less efficiently during the day.

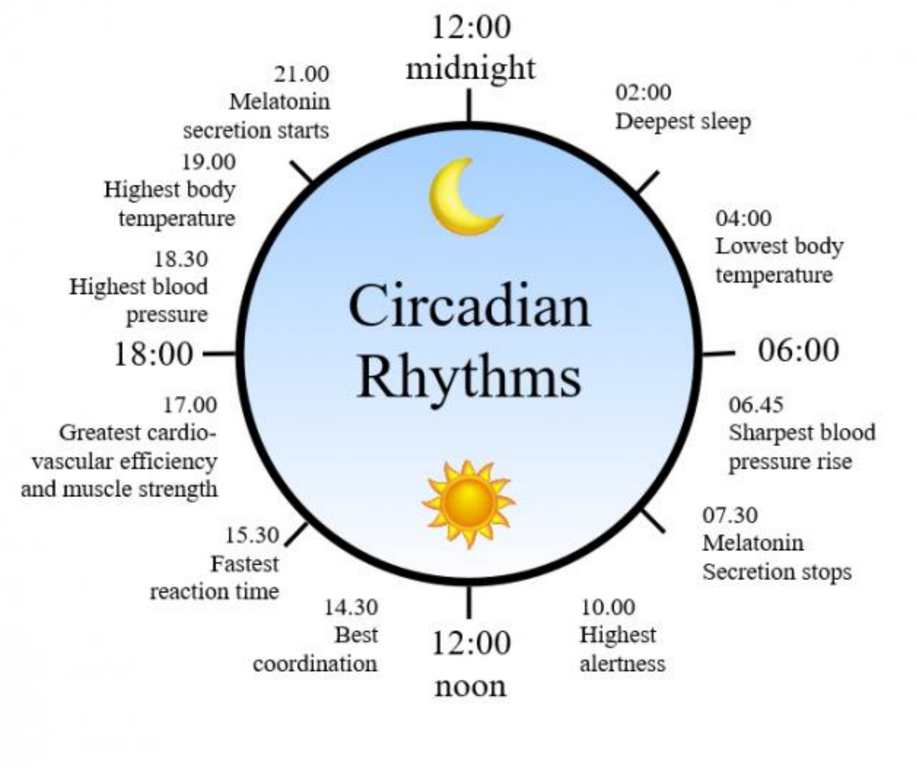

Figure 6. Schema illustrating likely changes during sleep.

The importance of sleep

The recommended amount of sleep to achieve optimal health and quality of life varies across the ages, with a gradual decrease from birth to adulthood and on to becoming elderly. According to the American Academy of Sleep Medicine, adults require between 7 and 9 h of sleep for optimal performance and health, while adolescents require additional sleep (ideally between 8 and 10 h). The necessary amounts of sleep varies between individuals and may even differ day to day for the same individual based on factors such as illness, sleep debt, and physiological or psychological stress. Duration is only one component of sleep, and the importance of sleep quality has been increasingly recognized as a vital element of overall health and well-being. Recent guidelines from the National Sleep Foundation suggest that sleep quality is improved at all ages by sleep continuity (decreased sleep latency, night time awakenings, and wake after sleep onset). It has been suggested that athletes may require more sleep than sedentary individuals to allow for adequate recovery and adaptation between bouts of exercise, perhaps requiring closer to 9 or 10 h of sleep instead of the 7- to 9-h general recommendation for adults. Despite this, there are currently no specific guidelines regarding sleep duration or quality for athletes.

The majority of the available evidence seems to suggest that athletes exhibit similar or perhaps slightly better sleep duration and quality than sedentary counterparts, and an active lifestyle that includes moderate exercise is consistently recommended as an effective means to improve sleep. Nonetheless, with respect to the current adult and adolescent sleep recommendations, athletes have been consistently shown to average less than 8 h of sleep per night across a variety of adult and youth sports without any clear difference between sexes (Gupta et al.,2017). In a study of more than 800 elite South African athletes, nearly three quarters reported sleeping less than 8 h, while 11% reported sleeping less than 6 h (Venter, 2012). A study of precompetitive sleep behaviour in 103 athletes found that the majority slept less than 8 h, and 70% reported worse sleep quality than usual, largely attributable to mood and anxiety disturbances before competition (Lastella et al.,2014). In addition, Olympic athletes demonstrated poorer sleep quality in terms of sleep efficiency and sleep fragmentation compared with age and sex-matched controls (Leeder et al.,2012). In a systematic review regarding elite athletes and sleep quality, poor sleep quality was reported in 38% to 57% of participants and may be more prevalent among female athletes and participants in aesthetic sports (Gupta et al.,2017).

With the above in mind, a number of nutritional products have been reported to aid sleep (both duration and likely quality), and a selection of these will now be considered.

Tryptophan

Tryptophan is an essential amino acid required for the in vivo synthesis of proteins. After consumption, it is metabolically transformed to bioactive metabolites, including serotonin and melatonin – both of which are involved in sleep and alertness.

Figure 7 provides a schematic which highlights the role of tryptophan in serotonin and melatonin production. Most of the investigations in relation to tryptophan intake and sleep have employed more elderly populations in which poor quality sleep is an issue. Lieberman et al. (2016)) used available data for 29 687 US adults to determine the effect of the average daily intake of 826 mg/d of tryptophan on liver and kidney function, depression, and sleep outcomes. The authors concluded that the high intake of tryptophan did not affect liver and kidney function or carbohydrate metabolism but was positively associated with sleep duration. In a related investigation, Bravo et al (2013) demonstrated that consumption of cereals enriched with tryptophan improved sleep as a consequence of elevating melatonin and serotonin levels in elderly participants. Likewise, Wenefrida et al, (2013) found that middle-aged/elderly individuals who consumed tryptophan added to cereal led to increased sleep efficiency and sleep time, and improved anxiety and depression symptoms. The latter authors suggest that high-tryptophan cereals might be useful as a 'chrononutrition' treatment that can alter the age-related sleep/wake cycle. Further studies have revealed that tryptophan has a direct effect on sleep, producing an increase in rated subjective sleepiness and a decrease in total wakefulness (Hartman, 1982; Silber & Schmitt, 2010), and that this improved quality of sleep is associated with an improvement in cognitive measures (Mohajeri et al., 2015).

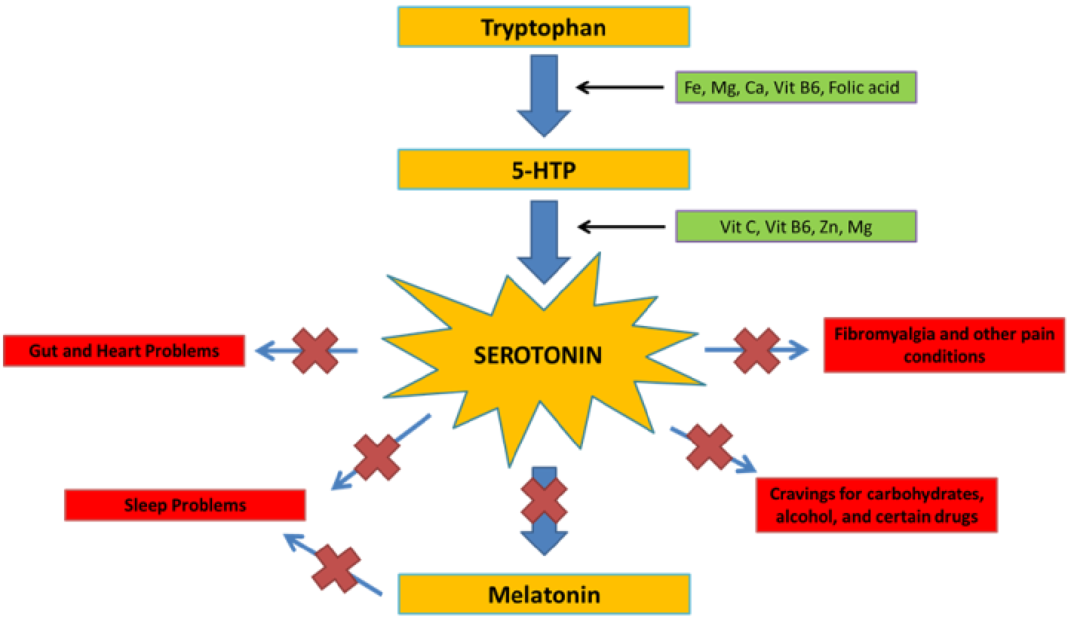

Figure 7. Production of serotonin and melatonin from tryptophan.

So all-in-all there is merit in using tryptophan as an aid for sleep quality and quantity. For further reading see Friedman (2018).

Magnesium

Magnesium is the fourth most abundant cation in the body and the second most abundant intracellular cation. It is involved in more than 300 biochemical reactions of the body, and is an essential cofactor for many enzymatic reactions, especially those that are involved in energy metabolism and neurotransmitter synthesis. Recently, magnesium has also been shown to regulate cellular timekeeping in both animal and plant cells (Feeney et al.,2016), and so it appears magnesium intake would prove beneficial to maintain the normal circadian rhythms and ensure a quality sleep in humans. Furthermore, magnesium supplementation has been shown to improve quality and duration of sleep among older people in a double-blind placebo-controlled clinical trial (Abbasi et al.,2012).

The magnesium intake of younger cohorts has also been shown to improve sleep quality. In a 5 year follow-up study between 2002-2007 employing 1487 adults over the age of 20 years old, Cao et al.(2018) concluded that those who had a higher intake of dietary magnesium slept better at night and were less likely to fall asleep during the day.

Wakefulness is driven by the release of neuromodulators by the ascending arousal system in the brain. Ding et al. (2016) found arousal was associated with a decrease in magnesium in interstitial spaces, whereas natural sleep and anaesthesia resulted in an increase in magnesium. They concluded that extracellular ions (such as magnesium) control the patterns of neural activity in the brain sleep-wakefulness cycle. Therefore by increasing magnesium levels in the blood, there is likely an elevated level of magnesium in the brain interstitial space and hence the promotion of sleep. So, magnesium induces sleep.

Conclusions

MPS is particularly low during sleep, even when 20g protein is ingested immediately after exercise performed in the evening. Protein ingested immediately prior to sleep is effectively digested and absorbed, and so increases amino acid availability during overnight sleep. Greater amino acid availability during sleep stimulates MPS and improves whole-body NPB during overnight recovery. At least 40g of dietary protein should be ingested prior to sleep to stimulation of MPS throughout the night. Resistance exercise performed during the day enhances the overnight MPS response to pre-sleep protein ingestion and allows more of the protein-derived amino acids to be used as precursors for *de novo* muscle protein synthesis. When applied during prolonged resistance-type exercise, pre-sleep protein supplementation can be used effectively to further increase gains in muscle mass and strength.

Since sleep can be problematic in athletes – particularly the night before competition – any nutrients which can promote sleep would be helpful. Tryptophan and magnesium have been used (independently) to improve sleep quality and duration. A combination of these nutrients may prove efficacious.

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