Original Article

Protein Blend and Casein Supplementations before Inactive Phase Similarly Activate Mechanistic Target of Rapamycin Signaling in Rat Skeletal Muscle

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Abstract

During overnight sleep, the longest postabsorptive and inactive phase of the day causes protein catabolism and loss. However, the daytime ingestion of dairy proteins has been shown to stimulate muscle protein synthesis and growth. This study compared the effects of pre-sleep supplementation of a protein blend (PB) composed of micellar casein (MCa) and whey protein (1:1) versus isolate MCa on the plasma levels of branched-chain amino acids (BCAAs) and the activation of the mechanistic target of rapamycin (mTOR) signaling, a critical intracellular pathway involved in the regulation of muscle protein synthesis. After 10 h of fasting during the active phase, rats were fed with a single dose of PB or MCa (5.6 g protein/kg of body mass) by gavage, and samples of blood and gastrocnemius muscle were collected at 30, 90, and 450 min. PB and MCa supplementations induced an increase (~3-fold, $P < 0.001$) of plasma BCAAs at 30 and 90 min. Most importantly, the stimulatory phosphorylation levels of mTOR and its downstream target p70 ribosomal protein S6 kinase (p70S6K) were similarly higher (~2.5-fold, $P < 0.001$) 30 and 90 min after MCa and PB. Plasma levels of leucine, isoleucine, valine, and overall BCAAs were correlated with the activation of mTOR ($P < 0.001$) and p70S6K ($P < 0.001$). MCa and PB supplementations before the inactive phase of rats resulted in an anabolic milieu in the skeletal muscle by inducing a transient increase in plasma BCAAs and a similar activation of the mTOR/p70S6K axis.

Keywords: Micellar casein, mTOR signaling pathway, protein supplementation, protein synthesis, skeletal muscle, whey protein

Introduction

Muscle mass is regulated by the balance between the processes of protein synthesis and protein breakdown.[1] During overnight sleep, the longest postabsorptive and inactive phase of the day causes protein catabolism and loss.[2] In contrast, micellar casein (MCa) protein ingestion before sleeping, with or without exercise,[3] is efficient in stimulating protein synthesis rates throughout the night.[4,5] However, to our knowledge, no study has compared the effects of presleep MCa ingestion with other protein sources on muscle protein anabolism.

The positive protein balance induced by dietary protein may preserve or even increase skeletal muscle mass,[6] which is of fundamental importance for the maintenance of functional and metabolic capacity throughout the life.[7,8] More importantly, the stimulation of protein synthesis by dietary protein intake could be beneficial for sarcopenic elderly,[8] diabetic,[9] and cancer[10] patients, who present a

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loss of muscle mass and function along with several adverse health outcomes.

Protein synthesis response during overnight to presleep protein intake may be determined by the type and amount of ingested proteins.[4,11] Among dairy proteins, MCa has been considered a "slow" protein since it precipitates in the stomach and is more slowly digested, which results in a modest but prolonged (up to 7 h) increase in plasma amino acid levels[12] conferring on MCa the ability to prolong the stimulation of muscle protein synthesis.[4,13] In contrast to MCa, whey protein has been referred to as a "fast" protein according to its digestion and absorption rates, resulting in a pronounced and transient increase in plasma amino acid levels that peaks at 40–60 min.[12,14,15] In addition, whey protein ingestion has been shown to stimulate muscle protein synthesis rates to a greater extent compared with MCa when evaluated for up to 4 h.[16,17] Therefore, the co-ingestion of MCa and whey proteins in the form of a protein blend (PB) could result in a synergistic effect on aminoacidemia and protein synthesis.

PB is composed of high-quality proteins such as MCa, and whey has all essential amino acids,[16] including the branched-chain amino acids (BCAAs; leucine/Leu, isoleucine/Ile, and valine/Val), which can stimulate muscle protein synthesis.[18] Leu has been considered the primary activator of the mammalian target of rapamycin (mTOR), a key intracellular regulator of muscle protein synthesis.[19] Activation of mTOR with subsequent phosphorylation of its downstream targets p70 ribosomal protein S6 kinase (p70S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) plays an essential role in the initiation of the mRNA translation and *de novo* muscle protein synthesis.[20] In according to Wolfe[21] for the synthesis of new muscle proteins, the synergic action of all essential amino acids, including the BCAAs and nonessential ones, is necessary.

The purpose of this study was to compare the effects of pre-sleep supplementation of a PB composed of MCa and whey protein (1:1) versus a concentrate MCa on muscle, anabolic protein signals, such as the plasma levels of BCAAs and the activation status of the mTOR signaling pathway. We hypothesized that PB would result in higher and prolonged BCAAs levels and mTOR signaling activation compared with MCa.

Materials and Methods

Experimental animals

Male Wistar rats (8 weeks old; ~250 g; $n = 40$) from São Paulo State University (Botucatu, Brazil) were used in the study. All animals were housed in polypropylene cages (three animals per cage) in a rack with the controlled temperature (22°C ± 2°C), with lighting on from 8 a. m. to 8 p. m. and off from 8 p. m. to 8 a. m. The animals had free access to water and food (Purina chow). All experimental procedures were performed according to the Brazilian College of Animal Experimentation and were approved by the Ethics Committee of the University of São Paulo (2018.5.14.90.3).

Experimental protocol

The night before the experiment, the animals fasted for 10 h.[22] Subsequently, at 8 a. m, the rats were supplemented with MCa (Grow Dietary Supplements, USA), PB (MCa plus concentrate whey, Grow Dietary Supplements, USA), or water (placebo [PLA]) by gavage, as summarized in Figure 1. PB was prepared by mixing the proteins in the ratio of 1:1, and the amount of protein was adjusted according to the percentage of purity of each one. The aminoacid composition of each supplement is shown in Table 1. The macronutrient composition of each supplement is shown in Table 2. Each rat was supplemented with an equal amount of protein (2.4 ml/100 g of body mass [BM], 5.6 g protein/kg of BM).[23]

The rats were anesthetized with an intraperitoneal injection of xylazine (10 mg/kg of BM) and ketamine (100 mg/kg of BM), and euthanized by exsanguination 30 min ($n = 15$), 90 min ($n = 10$) and 450 min ($n = 15$) after supplementation. In the present study, PLA 30 min was used as a control for PLA 90 min as well. MCa and whey protein are proteins with different rates of digestion and abortion, which gives each protein the ability to change the amioacidemia and the expression of proteins. Therefore, to detect these possible

Table 1: Amino acid composition of the supplements

	MCa (g/100 g)	Whey protein (g/100 g)	PB (g/100 g)
Alanine	2.55	3.69	3.12
Arginine	3.01	2.05	2.53
Aspartic acid	5.93	7.77	6.85
Cystine	0.48	1.55	1.02
Glutamic acid	15.93	13.15	14.54
Glycine	1.42	1.35	1.39
Histidine	3.62	1.31	2.47
Isoleucine	3.75	4.24	4.00
Leucine	7.43	7.99	7.71
Lysine	6.24	7.01	6.62
Methionine	2.16	1.65	1.90
Phenylalanine	3.40	2.32	2.86
Proline	7.45	4.28	5.87
Serine	4.15	3.75	3.95
Threonine	3.40	5.14	4.27
Tryptophan	1.31	1.23	1.27
Tyrosine	3.95	2.19	3.07
Valine	4.73	4.32	4.52
Overall BCAAs	15.91	16.56	16.23

MCa: Micellar casein, PB: Protein blend (MCa plus whey protein), BCAAs: Branched-chain amino acids (leucine, isoleucine, and valine)

Table 2: Macronutrient composition of the supplements

	Carbohydrate (g/100 g)	Protein (g/100 g)	Lipids (g/100 g)	Energy (kcal/100 g)
MCa	5.7	80.9	2.9	372.5
PB	7.6	77.9	3.85	376.6

MCa: Micellar casein, PB: Protein blend (MCa plus whey protein)

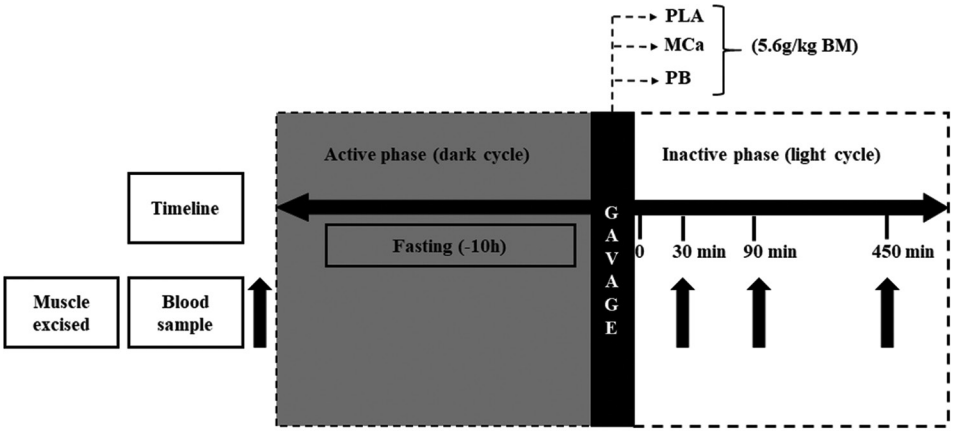

Figure 1: Experimental design. PLA: Placebo; MCa: Micellar casein; PB: Protein blend (MCa plus whey protein); BM: Body mass.

initial changes and to verify if this effect would last for a long period during the rest of the animals (i.e., light cycle), 30, 90, and 450 time points were used in the present study. Blood was taken by cardiac and plasma collection. Gastrocnemius muscles were collected and immediately frozen in the liquid nitrogen for future biochemical analysis and immunoblotting.

Amino acid measurements

Plasma Leu, Ile, and Val analyses were performed by Shimadzu® High-Performance Liquid Chromatography (HPLC), model LC 10 AD, using the Deyl method. Leu (99%), Ile (99%), and Val (99%) (Sigma-Aldrich®, St. Louis, MO, USA) were used as standard, and values were expressed in μM.

Immunoblotting technique

Gastrocnemius muscles were homogenized in extraction buffer (1% Triton X-100, 100 mM Tris, pH 7.4, containing 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, and 0.1 mg/ml aprotinin) at 4°C with a Polytron PTA 20S generator (model PT 10/35; Brinkmann Instruments, Westbury, NY, USA), operated at maximum speed for 30 s. The extracts were centrifuged (9,900 g) for 40 min at 4°C to remove the insoluble material, and the supernatants were used for protein quantification using the Bradford method as previously described.[24] Proteins were denatured by boiling in Laemmli sample buffer containing 100 mM DTT, run on an SDS-PAGE gel, and transferred onto nitrocellulose membranes (GE Healthcare, Hybond ECL, RPN303D). The transfer efficiency onto nitrocellulose membranes was confirmed by brief staining of the blots with de Ponceau red stain. These membranes were then blocked with Tris-buffered saline (TBS) containing 5% BSA and 0.1 Tween-20 for 50 min at room temperature.

Antibodies used for immunoblotting overnight at 4°C were phospho-4E-BP1 (Thr70; CELL 9455S), phospho-mTOR (Ser2448; CELL 2971S), mTOR (CELL 2972S) from Cell Signaling Technology (Beverly, MA, USA) at a dilution of 1:1000 as well as 4E-BP1 (SC6936), p70S6K (SC230), phospho-p70S6K (Thr389; SC11759) from Santa Cruz Biotechnology (Santa Cruz, CA, USA) at a dilution of 1:750. After the membranes were washed with TBS containing 0.1% Tween-20, they were incubated for 1 h at 4°C with secondary antibody conjugated with horseradish peroxidase. The specific immune reactive bands were detected using chemiluminescence (GE Healthcare, ECL Plus Western Blotting Detection System, RPN2132). Images were acquired by the C-DiGit™ Blot scanner (LI-COR^R, Lincoln, NE, USA) and quantified using the software Image Studio for C-DiGit Blot Scanner.

Statistical analysis

Data are expressed as means ± standard error (SE) of the mean. Multiple comparisons were made using a two-way analysis of variance followed by a Holm-Sidak *post hoc* test. The Western blot data were log-transformed before statistical analyses to avoid skewed data and are presented as means ± back-transformed SE. Pearson's correlation coefficients determined the correlation between data. The normality of the dependent variables was assessed using the Kolmogorov–Smirnov test. $P < 0.05$ was taken as the criterion for significance.

Results

Micellar casein and protein blend supplementations induce a transient and temporarily different increase in plasma aminoacidemia

In order to compare the effects of pre-sleep ingestion of different protein sources on aminoacidemia, rats were fasted for 10 h during overnight (active period) and after that, they were supplemented with MCa, PB, or water (PLA) by gavage. Both MCa and PB supplementations caused a significant increase in the plasma levels of Leu (~3.5-fold), Ile (~3-fold), Val (~2.5-fold), and overall BCAAs (~3-fold) from 30 to 90 min, when compared with PLA ($P < 0.001$), returning to basal values at 450 min (Leu, $P = 0.74$; Ile, $P = 0.54$; Val, $P = 0.08$ and BCAAs, $P = 0.44$) [Figure 2a-h]. These effects were similar between MCa and PB at 30 min (Leu, $P = 0.25$; Ile, $P = 0.71$; Val, $P = 0.96$ and BCAAs, $P = 0.49$). PB induced a further increase in Ile (60%, $P < 0.001$), Val (21%, $P < 0.001$), and

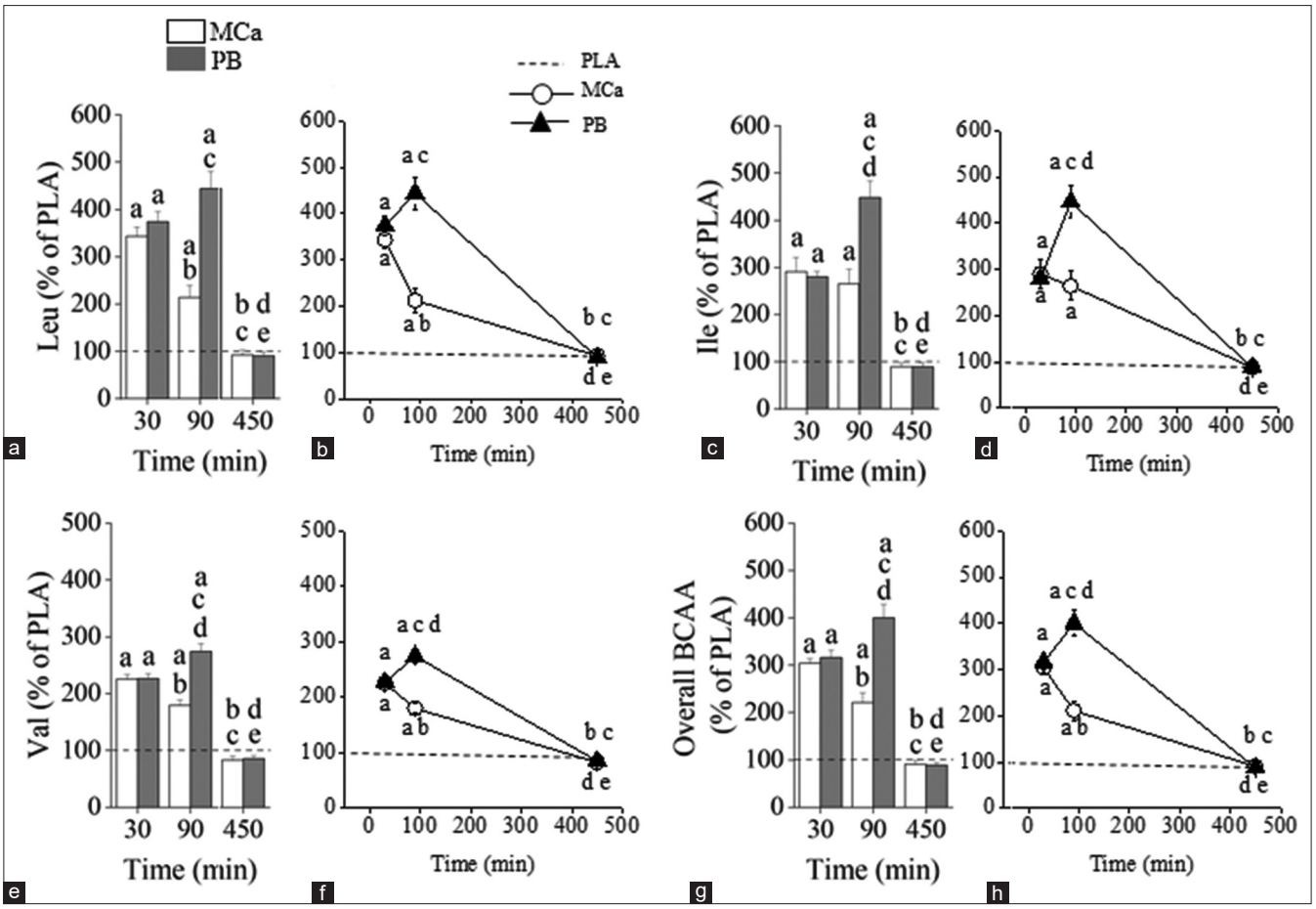

Figure 2: Effects of PLA, MCa, and PB supplementation on plasma Leu (a and b), Ile (c and d), Val (e and f), and overall BCAAs (Leu, Ile, and Val; g and h) levels of Wistar rats. Values are presented as means ± SE of 4–5 animals. Data from rats treated with protein supplementations at 30 and 90 min are expressed as % of values from rats treated with PLA at 30 min (considered as 100%; a and b, 232 ± 28.7 μM plasma of Leu; c and d, 100 ± 14 and μM plasma of Ile; e and f, 198 ± 34.6 μM plasma of Val; g and h, 505 ± 56.4 μM plasma of overall BCAAs), while data from rats treated with protein supplementations at 450 min are expressed as percentage of values from rats treated with PLA at 450 min (considered as 100%; a and b, 233 ± 18.2 μM plasma of Leu; c and d, 98 ± 5.1 μM plasma of Ile; e and f, 151 ± 2.7 μM plasma of Val; g and h, 481 ± 20.7 μM plasma of overall BCAAs). [a]$P < 0.05$ versus PLA 30 min; [b]$P < 0.05$ versus MCa 30 min; [c]$P < 0.05$ versus MCa 90 min; [d]$P < 0.05$ versus PB 30 min; [e]$P < 0.05$ versus PB 90 min (two-way analysis of variance, followed by a Holm-Sidak posttest). Leu: Leucine; Ile: Isoleucine; Val: Valine; BCAAs: Branched-chain amino acids; PLA: Placebo; MCa: Micellar casein; PB: Protein blend (MCa plus whey protein).

overall BCAAs (26%, $P < 0.001$) at 90 min when compared with 30 min [Figure 2c-h]. At the same time point, plasma levels of Leu tended to be higher in PB group [19%; $P = 0.07$; Figure 2a and b].

MCa caused an elevation of the plasma levels of Leu (2.1-fold; $P < 0.001$), Ile (2.6-fold; $P < 0.001$), Val (1.8-fold $P < 0.001$), and overall BCAAs (2.2-fold $P < 0.001$) at 90 min compared with PLA [Figure 2a-h]. Except for Ile [10%; $P = 0.48$; Figure 2c and d], the plasma levels of Leu (61% $P < 0.001$), Val (25% $P < 0.001$), and overall BCAAs (37% $P < 0.001$) in MCa-supplemented rats were significantly lower at 90 min than at 30 min [Figure 2a, b, e and h], indicating that PB supplementation induced more pronounced effects on Leu (108%, $P < 0.001$), Ile (69%, $P < 0.001$), Val (52%, $P < 0.001$) e overall BCAAs (81%, $P < 0.001$) than MCa ($P < 0.05$). Altogether, these findings suggest that MCa and PB supplementations are able of transiently increasing plasma BCAAs levels, which may stimulate protein anabolic intracellular pathways like mTOR signaling.

Micellar casein and protein blend supplementations promote a similar anabolic status by stimulating p70S6K branch of motor signaling in the skeletal muscle

The anabolic actions of BCCAs, particularly Leu, in the skeletal muscle, are mediated in great part by the activation of mTOR, which in turn can phosphorylate its downstream targets p70S6K and 4E-BP1.[25] In the present study, the phosphorylation levels of mTOR were similarly higher 30 and 90 min after MCa (~2.5-fold, $P < 0.001$) and PB (~3-fold, $P < 0.01$ and $P = 0.02$, respectively) supplementations compared with PLA [Figure 3a and b]. Curiously, at 450 min, phosphorylated mTOR was reduced (~2.2-fold) in MCa ($P < 0.001$) and PB ($P < 0.001$)-supplemented rats compared with PLA [Figure 3a and b]. There were no

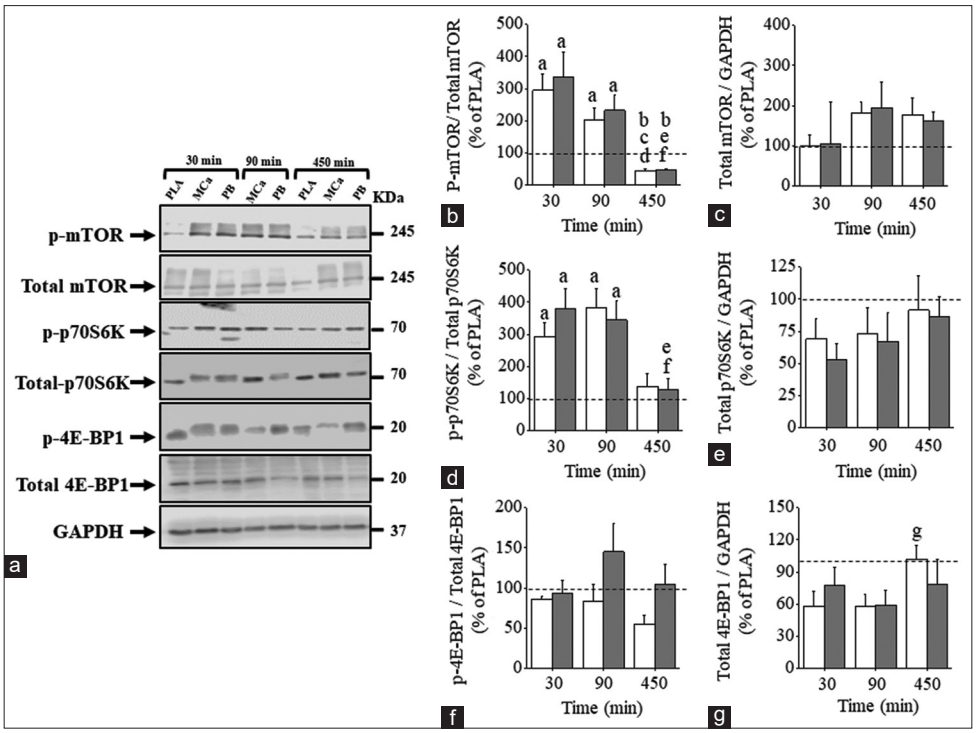

Figure 3: Representative immunoblots of p-mTOR, total mTOR, p-p70S6K, total p70S6K, p-4E-BP1, and total 4E-BP1 (a). Effects of PLA, MCa, and PB supplementations on phosphorylation levels of p-mTOR/total mTOR (b), total mTOR/GAPDH (c), p-p70S6K/total p70S6K (d), total p70S6K/GAPDH (e), p-4E-BP1/total 4E-BP1 (f), and total 4E-BP1/GAPDH (g) in gastrocnemius muscle from Wistar rats. Values are presented as means ± SE of 4-5 animals. $^{a}P < 0.05$ versus PLA 30 min; $^{b}P < 0.05$ versus PLA 450 min; $^{c}P < 0.05$ versus MCa 30 min; $^{d}P < 0.05$ versus MCa 90 min; $^{e}P < 0.05$ versus PB 30 min; $^{f}P < 0.05$ versus PB 90 min and $^{g}P < 0.05$ versus MCa 30 min (two-way analysis of variance, followed by a Holm-Sidak *post-test*). PLA: Placebo; MCa: Micellar casein; PB: Protein blend (MCa plus whey protein); mTOR: mechanistic target of rapamycin.

differences in total mTOR among groups at any studied period ($P = 0.74$) [Figure 3a and c].

Similar to mTOR, phosphorylated p70S6K was similarly higher (~3.5-fold) 30 and 90 min after MCa ($P < 0.001$) and PB ($P < 0.001$) compared with PLA [Figure 3a and d]. At 450 min, the levels of phosphorylated p70S6K returned to the basal values in MCa ($P = 0.57$) and PB ($P = 0.74$)-supplemented rats [Figure 3a and d]. Total p70S6K was unaffected in any group ($P = 0.53$) [Figure 3a and e]. Another branch of mTOR signaling is the 4E-BP1 protein; however, MCa and PB supplementations did not alter its phosphorylation levels at any period ($P = 0.28$) [Figure 3a and f]. Total 4E-BP1 was higher at 450 min compared with 90 min after MCa supplementation ($P = 0.02$) [Figure 3a and g].

Plasma levels of Leu, Ile, Val, and overall branched-chain amino acids positively correlate with mechanistic target of rapamycin and p70S6K

Next, we tested the correlation between BCAAs and mTOR signaling. Phosphorylated mTOR and p70S6K showed a positive correlation ($P < 0.001$) with the plasma levels of Leu [$r = 0.64$, $P < 0.001$; Figure 4a; $r = 0.46$, $P < 0.001$, respectively], Ile ($r = 0.57$, $P < 0.001$; $r = 0.49$, $P < 0.001$, respectively), Val ($r = 0.70$, $P < 0.001$; $r = 0.51$, $P < 0.001$, respectively), and overall BCAAs [$r = 0.65$, $P < 0.001$; Figure 4b; $r = 0.49$, $P < 0.001$; Figure 4c, respectively]. There was no correlation between phosphorylated 4E-BP1 and the plasma levels of Leu ($P = 0.69$), Ile ($P = 0.58$), Val ($P = 0.95$), and overall BCAAs ($P = 0.72$) [Figure 4d]. Thus, these findings suggest that BCAAs play an important role in activating mTOR pathway.

Discussion

The present study shows that, although PB supplementation caused a more robust elevation of most plasma BCAAs in the intermediary period (i.e., 90 min), both PB and MCa induced a similar activation of mTOR signaling in rat skeletal muscles. These findings partially confirm our hypothesis that PB would promote a more potent anabolic extracellular signal (i.e., BCAAs) than MCa. However, the anabolic intracellular signal (i.e., mTOR pathway) was similarly upregulated by both protein supplementations.

MCa and whey protein are considered high-quality proteins since they contain all the essential amino acids,[26] although in different proportions for each protein. Whey protein has a higher content of BCAAs, particularly Leu, besides being rapidly digested, which results in a rapid increase in plasma around 45–60 min.[14,27] However, this effect is transient. Thus, plasma Leu levels return to baseline after 2–3 h.[14,16] In contrast to whey, MCa is slowly digested, which causes smaller but more prolonged aminoacidemia.[17]

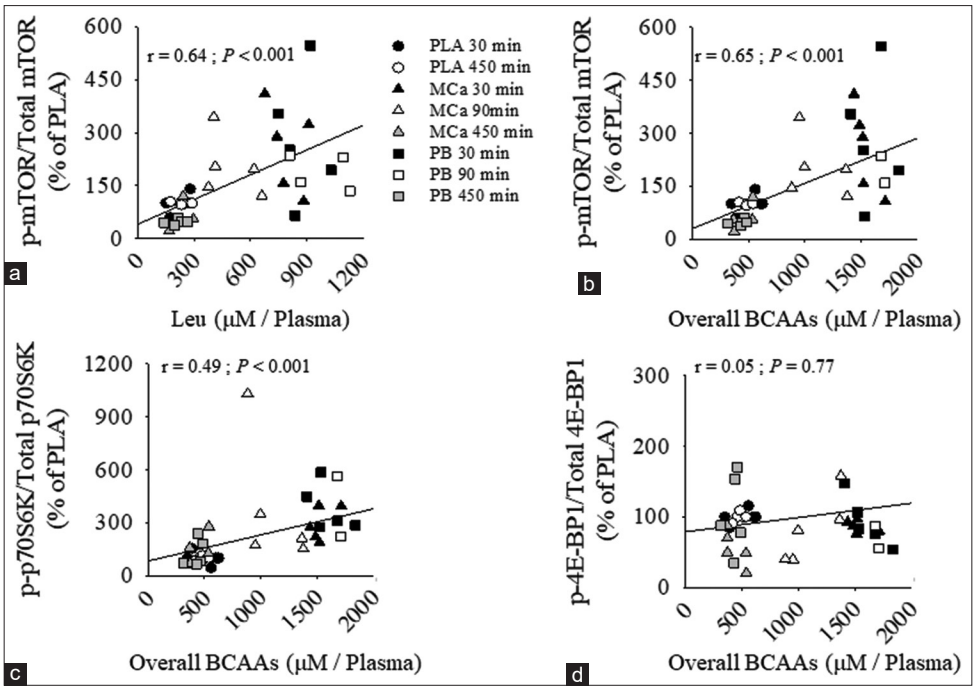

Figure 4: Correlations between phosphorylated mechanistic target of rapamycin and plasma Leu (a), and overall BCAAs levels (b). Phosphorylated p70S6K and total BCAAs levels (c). Phosphorylated 4E-BP1 and total BCAAs levels (d). Data were analyzed as two-tailed of significance using Pearson's correlation coefficients ($n = 40$) assumed $P < 0.05$. PLA: Placebo; BCAAs: Branched-chain amino acids; Leu: Leucine.

According to previous studies,[14,27] we demonstrated that PB and MCa supplementations caused rapid (i.e., 30 min) and similar increase in BCAAs and Leu plasma levels. Nevertheless, PB caused a further elevation in BCAAs plasma levels over time, an effect that was not observed in the MCa group at 90 min. It is also possible that PB has increased BCAAs between 90 and 450 min. Traylor *et al.*[27] reported that a PB composed of dairy whey protein and MCa in a proportion similar to that used in our study, but not MCa, maintained the high levels of plasma overall BCAAs up to 360 min in healthy young men. Therefore, our findings corroborate with the idea from a previous study[27] that whey protein and MCa co-ingestion in the form of a blend could confer a synergic effect increasing the amplitude and prolonging aminoacidemia.

It is known that the increase in plasma availability of essential amino acids,[14,27] followed by the ingestion of either MCa or a blend of proteins, is effective in activating and prolonging the stimulation of protein synthesis.[5,26,28] Although protein synthesis was not measured in the present study, an essential marker of muscle pro-synthetic activity was evaluated, mTOR. Activation of mTOR can phosphorylate and activate its downstream target p70S6K, which, in turn, phosphorylates the ribosomal protein S6. mTOR can also induce phosphorylation in its other branch, 4E-BP1, which leads to the dissociation of eIF4E with 4E-BP1 allowing eIF4E to bind with eIF4G forming active translation initiation complexes.[25] Both downstream targets of mTOR, p70S6K, and 4E-BP1, are crucial to promoting translation initiation and elongation.[25]

In the current study, both PB and MCa supplementations were able to similarly activate mTOR and its downstream target p70S6K at early periods. However, mTOR and p70S6K phosphorylation returned to baseline levels, as did aminoacidemia, 450 min after supplementations. Our results corroborate the study of Trommelen *et al.*,[11] who did not find an effect on mTOR and p70S6K activation 450 min after ingestion 30 g of MCa followed by resistance exercise pre-sleep in the healthy young men. According to our results, previous studies have shown that the peak of mTOR and p70S6K activation occur 1–2 h following protein ingestion, and this effect disappears between 3 and 5 h.[29,30] In contrast with our findings, Reidy *et al.*[26] have demonstrated that PB intake (50% sodium caseinate, 25% whey protein isolated, and 25% soy protein isolated) following exercise was more efficient in extending mTOR activation and muscle protein synthesis compared with isolated whey in healthy young men. Butteiger *et al.*[28] showed that rats fed 4 g meal consisting of ratios of 1:2:1 for whey:caseinate:soy, respectively, prolonged protein synthesis, which was superior when compared with whey protein.

Previous studies have shown the anabolic potency of BCAAs, particularly Leu, on mTOR signaling and skeletal muscle protein synthesis in both rodents[20,31] and humans.[11] Crozier *et al.*[31] demonstrated that following incremental amounts of Leu administration in rats, a dose of 0.14 g/kg BM produced a near-maximal increase in protein synthesis. Norton *et al.*[32] showed that a specific threshold of Leu intake is needed (47 mg) to boost protein synthesis in the skeletal muscle of rodents. In addition, Kanda *et al.*[14] observed that mTOR reached a plateau

after caseinate and whey protein co-ingestion containing ~43 mg of Leu (0.29 g/kg of BM) and that higher amounts of Leu ingested were not efficient in further-stimulating mTOR. Therefore, although the amount of Leu present in the groups supplemented with MCa (133 mg, 0.51 g/kg of BM) and PB (143 mg, 0.53 g/kg of BM) was not matched, both groups provided similar and sufficient amounts of circulating Leu to activate mTOR completely, phosphorylate p70S6K and thus, possibly have induced stimulation of protein synthesis. These results provide support for the role of Leu as a critical trigger for postprandial stimulation of protein synthesis after supplementation with PB and MCa.

In this line, the present study demonstrated a positive correlation between plasma Leu levels with mTOR and p70S6K activation. Although other amino acids from protein intake, such as glutamine and arginine, may also activate mTOR signaling,[33,34] the present study focused on evaluating the effects of BCAAs on the mTOR and its downstream targets. The importance of p70S6K in muscle physiology has been shown in knockout animals that present muscle atrophy.[35] Marabita *et al*.[36] showed that p70S6K can independently mediate rapamycin-sensitive muscle growth and is required to increase adult muscle strength in mice. Thus, the phosphorylation and activation of the p70S6K induced by protein supplementations, as demonstrated in the present study, may be essential to promote the well-known growth-promoting effects of protein supplementations on the skeletal muscle.

Human clinical trials are needed to confirm the observation made in the present study that supplementations of MCa and PB cause a similar activation of mTOR signaling and may be potent anabolic stimuli for protein synthesis in healthy young participants. In pathological conditions, PB has been shown to enhance muscle protein synthesis in cancer patients,[37] which could prevent the development of cachexia-induced muscle wasting and death. From an economic point of view, PB supplementation may be a less costly strategy when compared with MCa, since the costs of MCa production are higher.[38] In addition, MCa can be challenging to work with, as it is non-water soluble, making PB a more viable and more applicable supplement.[38] More research is needed to confirm that PBs, in general, can induce muscle growth in long treatment and whether the results can be replicated in the elderly or atrophic patients.

Conclusion

We demonstrated that although PB supplementation before the inactive phase (i.e., sleep) caused a more robust elevation of most plasma BCAAs in the intermediary period (i.e., 90 min), both PB and MCa induced a similar activation of mTOR/p70S6K signaling pathway in rat skeletal muscles. The findings of the present study provide new insights into the effects of protein supplementation as an efficient strategy to promote an anabolic milieu that can favor the maintenance of muscle mass during sleep.

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Conflicts of interest

There are no conflicts of interest.

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