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Effects of Postexercise Protein Intake on Muscle Mass and Strength During Resistance Training: Is There an Optimal Ratio Between Fast and Slow Proteins?

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While effects of the two classes of proteins found in milk (i.e., soluble proteins, including whey, and casein) on muscle protein synthesis have been well investigated after a single bout of resistance exercise (RE), the combined effects of these two proteins on the muscle responses to resistance training (RT) have not yet been investigated. Therefore, the aim of this study was to examine the effects of protein supplementation varying by the ratio between milk soluble proteins (fast-digested protein) and casein (slow-digested protein) on the muscle to a 9-week RT program. In a double-blind protocol, 31 resistance-trained men, were assigned to 3 groups receiving a drink containing 20g of protein comprising either 100% of fast protein (FP(100), *n* = 10), 50% of fast and 50% of slow proteins (FP(50), *n* = 11) or 20% of fast protein and 80% of casein (FP(20), *n* = 10) at the end of training bouts. Body composition (DXA), and maximal strength in dynamic and isometric were analyzed before and after RT. Moreover, blood plasma aminoacidemia kinetic after RE was measured. The results showed a higher leucine bioavailability after ingestion of FP(100) and FP(50) drinks, when compared with FP(20) ($p < .05$). However, the RT-induced changes in lean body mass ($p < .01$), dynamic ($p < .01$), and isometric muscle strength ($p < .05$) increased similarly in all experimental groups. To conclude, compared with the FP(20) group, the higher rise in plasma amino acids following the ingestion of FP(100) and FP(50) did not lead to higher muscle long-term adaptations.

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Changes in muscle mass result from imbalances between muscle protein synthesis (MPS) and breakdown (MPB). The acute effects of resistance exercise on MPS are largely determined by the mode of contraction, the exercise workload and intensity (Atherton & Smith, 2012), but also by the availability of essential amino acids (EAA) and leucine (Kimball and Jefferson, 2006). There is now a lot of experimental evidence showing that the acute period postexercise appears to be an optimal time to ingest protein, increase AA bioavailability, and enhance the magnitude and duration of the rise in MPS (Esmarck et al., 2001; Levenhagen et al., 2001; Pennings et al., 2011).

The AA bioavailability differs according to the rate of protein digestion, the type of protein and its digestion kinetics (Dangin et al., 2001 ; Wilkinson et al., 2007 ; Tang et al., 2009). Greater rates of MPS were observed after milk ingestion than after soy ingestion at the end of resistance exercise (Wilkinson et al., 2007). However, milk contains two protein fractions, milk soluble protein and casein, characterized as "fast" and "slow" proteins, respectively, based on their digestion rate and speed of AA absorption. Whey protein at the end of resistance exercise stimulates MPS to a greater extent than casein (Tang et al., 2009 ; Pennings et al., 2011), but a few studies suggested that casein ingestion inhibited whole body protein breakdown by approximately 30% (Boirie et al., 1997 ; Dangin et al., 2002). Taken together, these studies suggest that whey and casein have different metabolic fates and uses that could have complementary effects on MPS, MPB and protein deposition.

However, the practical applications of these results remain limited since successive sessions of resistance exercise are required to lead to measurable muscle hypertrophy. A meta-analysis examined the efficacy of protein supplementation on the muscle responses to RT (Cermak et al., 2012). In this review, data that were included from 22 randomized controlled trials (RCT) that included 680 subjects. It was shown that compared with either isocaloric or nonisocaloric placebo, protein supplementation significantly augmented the gain of lean mass and improved the gain in muscle strength in response to prolonged RT. Nine RCTs were included in a second meta-analysis that compared the effects of whey protein (alone or as part of a multi-ingredient) with those of other interventions such as carbohydrates or other protein sources, on body composition and muscle performances (Naclerio et al., 2016). It was shown that whey protein improved the responses of lean body mass, as well as muscle strength after RT, compared with subjects ingesting isoenergetic supplements containing carbohydrates or other sources of proteins. To our knowledge, only one study compared the effects of whey alone with those of casein alone after 10 weeks of RT (Cribb et al., 2006). In this study, the supplementation with whey protein was associated with a greater gain in lean mass and improvement in strength, than with casein. However, despite the potential respective roles of whey and casein proteins on skeletal muscle protein balance, to date no study has directly compared muscle responses to RT with the consumption of supplements with differing compositions in whey and casein proteins.

The aim of this randomized, controlled and double-blind trial was to examine the effects of different postexercise supplements varying by their milk soluble protein and casein ratio, on muscle responses to 9 weeks of RT. Given the expected complementary effects of whey and casein on protein deposition, we aimed to test the hypothesis of whether the muscle responses to RT were affected by the plasma availability of amino acids derived from dietary proteins in postworkout supplements. To test this hypothesis, subjects consumed isonitrogenous protein drinks immediately following every training bout, containing, either only fast dietary protein, a mix of equal amounts of fast and slow proteins, or the ratio of fast-to-slow proteins found in milk (i.e., 20/80).

Methods

Subjects

Thirty one healthy young (19–35 years) recreational resistance trained men participated in the study. The subjects were randomly assigned to supplements with 20 g of milk soluble proteins (FP(100), n = 10), 10 g of milk soluble and 10 g of casein proteins (FP(50), n = 11), or 4 g of milk soluble and 16 g of casein proteins (FP(20), n = 10). They were all nonsmokers and had no musculoskeletal disorders likely to affect their ability to respond to RT. Subjects who were allergic to milk protein, lactose intolerant or who had taken any protein supplementation during the last year were not included in the study. The protocol complied with the guidelines set by the Declaration of Helsinki of 1975, as revised in 1983, and was approved by the local Ethics Committee (Paris IDF VI, France).

Experimental Design

This was a randomized, and double-blind study in which each participant completed all treatments serving as his own control. The subjects were first familiarized with the RT during a three-week preparatory period. Subjects exercised three times per week without consuming the recovery beverage, to standardize their training status. After this preparatory period, subjects started the actual RT program, supplemented with the nutritional beverage assigned. They all completed 9 weeks of the supervised RT. Each subject received a recovery drink to be consumed within 15 min after the end of each RT session. The ingestion of the recovery beverages was supervised. The participants were asked not to eat or drink anything (except water) during training sessions and until 3 hr after having consumed the supplement. The three recovery drinks differed only by the ratio between fast (milk soluble proteins) and slow proteins

(casein). Body composition and strength changes to RT and recovery drinks were tested at inclusion (PRE), then every three weeks (Wk-3, Wk-6) and at the end of RT (POST). All tests were conducted 48 hr after the last training session.

Resistance Training

Whole-body RT started after the preparatory period. This familiarization period lasted 3 weeks with 3 training per week. The program consisted in the same exercises that were undertaken for the intensive 9 weeks program; subjects performed 2 sessions per week with lower and upper body resistance exercises and 1 session with upper body exercises only, which are described below. The only difference with sessions included within the training program was the workload of each exercise which was determined at 50–60% of 1RM. Thereafter, subjects started the 4 times a week RT. The participants trained on Monday, Tuesday, Thursday and Friday between 4pm and 7pm for a total of 9 weeks. During the training program, Monday and Thursday sessions mixed lower and upper body training consisting of back squats, leg extension and leg curl for the lower body, and bench press, lying triceps extension, inclined barbell press, dumbbell press, and dumbbell curls for the upper body. On Tuesdays and Fridays, participants completed an upper body session focused on biceps, back and shoulders exercises such as bent over row, lat pull down, cable row, biceps barbell curls, hammer dumbbell curls, and dumbbell press. It was divided into 3 blocks, each consisting of 3 different weeks with mainly sets of 10–12 repetitions with 70–75% of 1 RM the first week, 8–10 repetitions with 75–80% of 1 RM, the second week, and 6–8 repetitions with higher loads such as 80–85% of 1 RM the third week. The 1-RM values were determined every 3 weeks, and then the relative intensity of resistance sessions was adjusted accordingly. This RT program was elaborated to develop both hypertrophy (10–12 repetitions) and maximal strength (6–8 repetitions), which is in line with the American College of Sports Medicine position stand recommendations of progression models in RT (Kraemer et al., 2002).

Protein Supplementation

The protein supplements were presented in the form of chocolate flavored mixed powder, packaged in individual unidose sachets, and identified with the randomization number. These nutritional supplements were to be consumed in the form of drinks reconstituted in 250 ml of water. The supplements were manufactured by the company TRIBALLAT-Nutrisun (La Galmandière—35220 Châteaubourg—France).

The drinks were isoenergetic (167 kcal) and all composed of 20 g proteins, 20 g carbohydrate (50/50; Glucose/Maltodextrin), and 0.5 g fat. The protein composition varied according to the "fast /slow" proteins ratios: 100/0, 50/50, 20/80 respectively for the groups FP(100),

FP(50) and FP(20). The participants were asked to drink the protein beverage within the 15 min after the end of each training session.

Diet Control

Before the preparatory, participants were asked to complete a one-week food diary. Their nutritional intakes of proteins, carbohydrates and lipids were estimated at each meal and snack during the day. According to their habitual nutritional intake, they had a nutritional program in which protein intake varied between 1.5 and 2g/kg/day, including a standardized meal in the evening, which had to be taken at least 3 hr after the protein supplement. During the 9 weeks of RT, each participant had to complete a food diary 2 days-a-week on training days. Food diary was debriefed with a nutritionist. Energy, protein, carbohydrate and lipid intakes before and during the follow-up period were calculated for each meal using dietary-analysis based on information from the French database of food composition sources (CIQUAL, 2013).

Body Composition

Body weight, fat mass and lean body mass were measured using dual energy X-ray absorptiometry (DXA, dual-energy X-ray absorptiometry, General Electrics, Madison, USA). Body composition was measured at the end of the preparatory period (PRE) and every 3 weeks until the end of the RT (wk-3, wk-6 and POST). These measurements were always performed in standardized conditions, on the morning, the subjects in a fasted state.

Muscle Performances

Every 3 weeks, 48 hr after the last RT session, dynamic muscle strength was assessed using a 3 RM technique in back squat and 1 RM in bench-press. The first set of 10 repetitions with a light initial load was used as a warm up. Thereafter, the load was increased until maximal effort was achieved. To validate the maximal load in back squat the leg had to be at 90° from the ground. For the bench press, the bar had to touch the chest and the bottom had to touch the bench. Participants had 3 attempts, with 3–5 min rest between each try. The performance of the last test (3 weeks before) had to be confirmed at the first try. According to the difficulty perceived during this first try, the participants chose to increase the load by 2.5 or 5kg for the 2 following tries.

Moreover, isometric muscle strength was measured before and after RT. Maximal isometric strength was assessed for lower and upper body at a 90 °C position for knee and elbow flexors and extensors on an isokinetic dynamometer (Con-Trex Multi-Joint System, Dübendorf, Switzerland). Maximal voluntary contraction (MVC) was assessed three times at one minute-interval rests. If the last try was much better (more than 20N/m) than the two first tries, another MVC was conducted so as to make sure that the highest performance was achieved. Finally, after

10 min of rest, participants had to perform a leg resistance test to fatigue consisting in 30 isokinetic repetitions in flexion and extension at a maximal voluntary force with their dominant leg on the isokinetic dynamometer. Muscle fatigue was estimated as a difference between the average of the two first contractions and the average of the two last contractions.

Plasma Amino Acid

Plasma amino acid (AA) concentrations were measured at different time-points, immediately after RT session, and 30, 60 and 120 min after ingestion of the protein supplement. Blood samples were collected into 5-ml lithium heparin blood-collection tubes and preserved on ice until the last sample was collected. Within 10 min after the end of the blood sampling, the tubes were centrifuged at 4,500 rpm for 10 min at 4 °C. The plasma supernatant was separated, and samples stored in 2-ml Eppendorf tubes at -80 °C until further analysis. Plasma AA were measured by ion exchange chromatography with ninhydrin detection after dilution of the samples with a lithium citrate buffer containing D-glucosaminic acid and amino-ethylcysteine as internal standards, using an AA analyzer (AminoTac, JLC-500/V, Jeol, Tokyo, Japan). Areas under the curve (AUC) were measured.

Statistical Analysis

All data were analyzed using two-factor repeated-measures ANOVA to examine the global effects of time and supplement, and time x supplement interaction. If any main effects were observed, a Newman-Keuls post hoc procedure was used to locate where differences occurred. Paired Student's *t* tests using the Holm-Bonferroni adjustment, were used to ascertain between-group differences in AUC data. Values are presented as means ± *SD* and statistically significant differences were accepted when $p < .05$.

Results

Subjects and Dietary Intakes

All subjects' characteristics are shown in Table 1. There were no differences in any anthropometric characteristics at baseline. Protein intake increased in the 3 groups during the 9-weeks RT ($p < .01$), with no significant interaction (Table 2).

Plasma AA Concentrations

Changes in plasma AA concentrations after ingestion of protein-enriched beverages are shown in Figure 1. There was a significant time and time × supplement interaction ($p < .01$) for total (TAA), essential (EAA), branched-chain AA (BCAA) and Leucine. FP(100) and FP(50) ingestion resulted in a more pronounced plasma Leucine concentration than after FP(20) ingestion ($p < .05$). Both AUC_{0-120} for plasma BCAA and Leucine were higher after FP(100) ingestion than FP(20) ($p < .05$, $p < .01$, respectively). The AUC_{0-120} for plasma BCAA

Table 1 Descriptive Characteristics of Participants in the Three Experimental Groups: FP(100), FP(50) and FP(20)

Characteristics	FP(100) *n = 10*	FP(50) *n = 11*	FP(20) *n = 10*	*p*-value
Age (year)	27 ± 6	23 ± 3	26 ± 5	*ns*
Height (m)	1.78 ± 0.09	1.81 ± 0.06	1.81 ± 0.05	*ns*
Body mass (Kg)	74.3 ± 6.8	76.0 ± 6.8	77.5 ± 6.9	*ns*
BMI (Kg/m²)	23.5 ± 2.4	23.1 ± 1.1	23.7 ± 1.6	*ns*
Body fat (%)	17.72 ± 5.3	17.26 ± 5.88	16.96 ± 1.8	*ns*

Note. All values are expressed in mean ± *SD*. BMI = body mass index *ns* = nonsignificant

Table 2 Dietary Intakes

	Baseline			During the 9-week intervention			*p*-value	
Intakes	FP(100)	FP(50)	FP(20)	FP(100)	FP(50)	FP(20)	Time	Time x Supplement
Protein (g/kg/d)	1.5 ± 0.3	1.6 ± 0.4	1.5 ± 0.4	1.9 ± 0.4*	1.9 ±0.3*	1.8 ±0.3*	$p < .01$	*ns*
CHO (g/kg/d)	3.9 ± 1.2	4.3 ± 0.6	3.7 ± 0.9	3.7 ± 1.6	3.9 ±1.0	3.3 ±0.7	*ns*	*ns*
Lipid (g/kg/d)	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.4	1.0 ± 0.3*	0.9 ± 0.3*	0.9 ±0.3*	$p < .01$	*ns*

Note. Values are mean ± *SD*. *ns* = nonsignificant

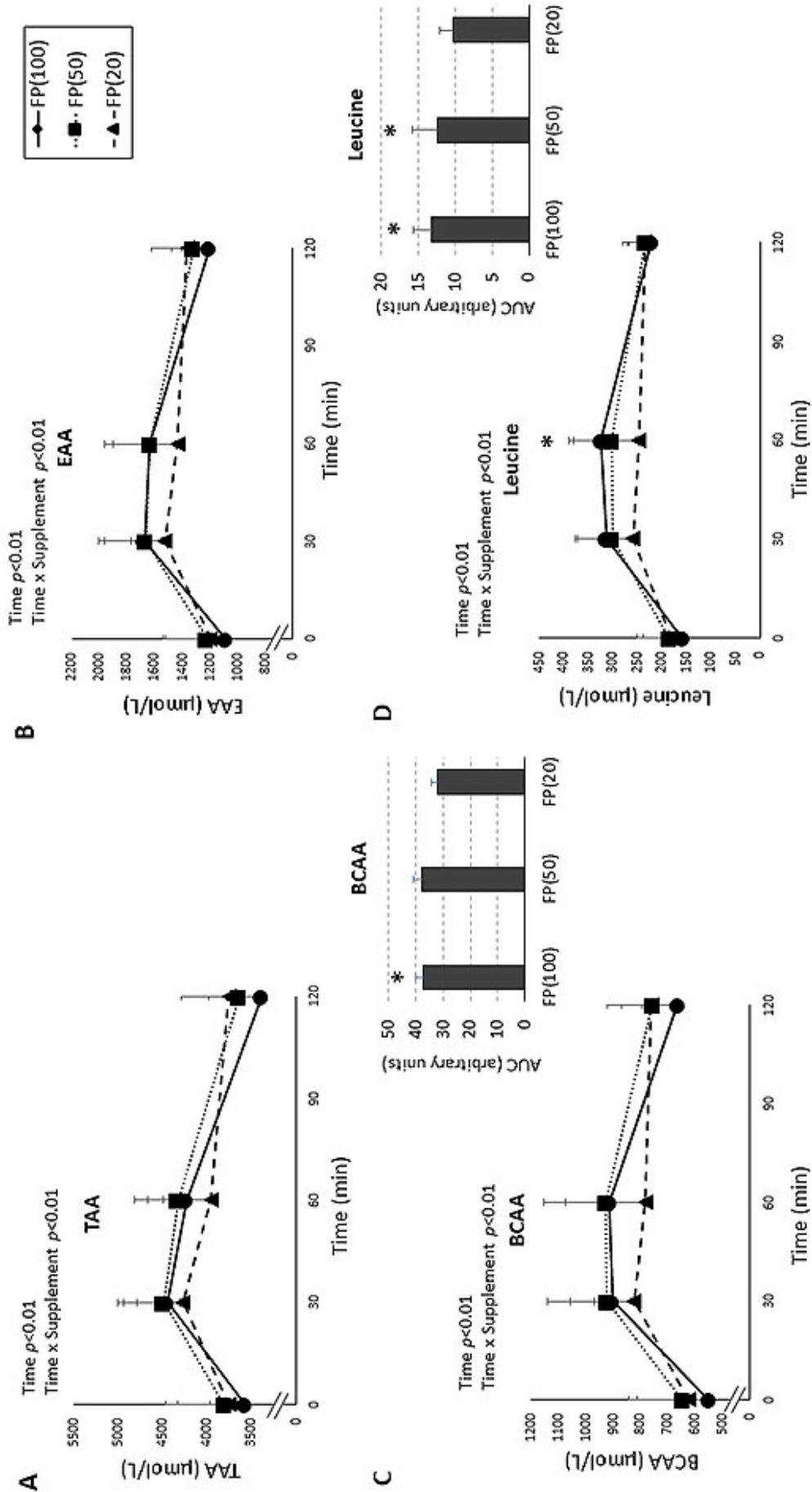*Greater change compared with Baseline ($p < .01$.)

Figure 1 — Plasma amino acid (AA) concentration (μmol/L) over 2 hr after resistance exercise and after the intake of FP(100), FP(50) or FP(20) drinks, with individual profiles for total AA (TAA, figure A), Essential AA (EAA, figure B), Branched-chain AA (BCAA, figure C) and Leucine (figure D). The insert shows the area under the curve (AUC) for 120 min following the ingestion of the protein beverage. *, different from FP(20), $p < .05$.

tended to differ between FP(50) and FP(20) ingestions (p = .07) and was significant higher for leucine with FP(50) compared with FP(20); (p < .05; Figure 1D).

Body Composition

Lean body mass.   Whole lean body mass (LBM) values are shown in Figure 2A. LBM increased significantly with time in FP(100) (from 58.9 ± 6.8kg to 60.4 ± 7Kg), FP(50) (from 60.2 ± 5.5kg to 61.9 ± 6Kg), and FP(20) (from 62 ± 5.4kg to 63.4 ± 5Kg), groups (p < .01). No time × supplement interaction was observed.

Significant increases with time were shown for arms lean mass (from 8 to 8.6kg ± 1, from 8.3 to 8.7kg ± 0.7, from 8.8 to 9.3kg ± 0.9, for FP(100), FP(50) and FP(20), respectively; p < .01).

Legs LM also significantly increased with time in FP(100) (from 23.6 to 23.9kg ± 3), FP(50); (from 23.6 to 23.9kg ± 3) and FP(20) groups (from 24.5 to 25.1kg ± 2; p < .01), with no detectable difference between groups.

Fat Mass (%)

Fat mass values are displayed in Figure 2B. After 9 weeks of training, fat mass did not change for FP(100); (-0.4 ± 1%), FP(50); (-1.13 ± 1.7%) and FP(20) groups (+0.4 ± 1.2%), (time effect p = .08).

Muscle Performance

Dynamic muscle strength.   There was a significant time effect (p < .01) for bench-press performance, which increased similarly in all groups, from 85 to 93.5kg ± 19.5 in FP(100), from 83 to 87.5kg ± 12 in FP(50), and from 97 to 104.5kg ± 21 in FP(20) groups. For back squat, there was a marked time effect (p < .01), such as performance increased in all experimental groups from 96.25 to 111kg ± 26, 91.3–103kg ± 17, and 97–111kg ± 17 in FP(100), FP(50) and FP(20) groups, respectively. No time × supplement interaction effects were observed for dynamic muscle performance (Figure 3).

Isometric muscle strength.   Maximal isometric torque significantly increased after the RT program (p < .05), for upper body (elbow extensors and flexors) and for knee extensors, but it only tended to be significant for knee flexors (p = .08; Figure 4). However this increase in isometric strength did not differ between groups (no time x supplement interaction effect).

Resistance to Fatigue

Neither RT nor protein supplement did affect the resistance to fatigue (data not shown, p = .7).

Discussion

To our knowledge, this is the first study to examine whether the ratio between "fast" and "slow" dietary proteins consumed immediately at the end of each resistance exercise could affect the muscle responses to a RT program. Given the well-known effects of whey protein alone (Naclerio & Larumbe-Zabala, 2016), we tested the hypothesis that a combination of milk soluble protein and casein would improve the muscle responses to RT, and we examined the specific effects of two fast-to-slow ratios, i.e., FP(50) and FP(20) supplements. Our data show that postexercise drinks varying by the fast-to-slow protein ratio did not affect the responses of fat mass, fat-free mass and muscle strength to 9 weeks of RT.

In the current study, our aim was not to verify the effects of protein supplementation on muscle mass and performance, but rather to examine whether the fast-to-slow dietary protein ratio impacts the muscle responses to RT. Protein supplements at the end of resistance exercises have additive effects beyond the muscle responses to exercises alone (Hartman et al., 2007; Willoughby et al., 2007). Previous studies have shown that supplements containing whey protein alone or combined with casein and other ingredients improve the lean body mass, upper and lower body strength gains observed after 6–12 weeks of RT, compared with the ingestion of carbohydrates or other protein sources (Naclerio & Larumbe-Zabala, 2016).

Increasing amino acid availability after exercise is effective in stimulating MPS (Pennings et al., 2011), as well as in lowering the rise in MPB observed at the end of resistance exercise (Biolo et al., 1997). Here we showed that the consumption of 20g of milk soluble proteins, FP(100), and the consumption of a mixture of 10g of milk soluble proteins and 10g of slow proteins, FP(50), induced similar rises in circulating TAA, EAA, BCAA and leucine. While EAA themselves play a major role in the rise in MPS, leucine in particular is a potent activator of MPS, in part through upregulation of the mammalian target of rapamycin complex 1 (mTORC1), and an inhibitor of MPB through downregulation of the expression of the key components of the ubiquitin/proteasome pathway (Dodd and Tee, 2012). Ingestion of FP(100) and FP(50) drinks led to higher leucine bioavailability, compared with FP(20) whose composition is close to that of the milk, containing 4g of fast protein and 16g of slow protein. Given the slight differences in leucine content of beverages, we estimated that 20 g of FP(100), FP(50) and FP(20) provided 2.2, 2 and 1.9 g of leucine, respectively. The slight lower plasma concentrations of leucine in the FP(20) group, compared with both FP(100) and FP(50) groups could be due to the lower amount of leucine provided by the dietary supplement, and/or the lower digestibility of casein which accounts for almost 80% of total proteins in FP(20) supplement.

The long-term muscle adaptations to specific protein supplementations during training interventions are mainly determined by both the amino acid composition of proteins and their digestibility. Previous studies clearly suggested that fast-digested and slow-digested proteins have synergistic effects on muscle anabolism (Dideriksen et al., 2013; Boirie et al., 1997). When measured for 0–6 hr of recovery from resistance exercise, whey and casein
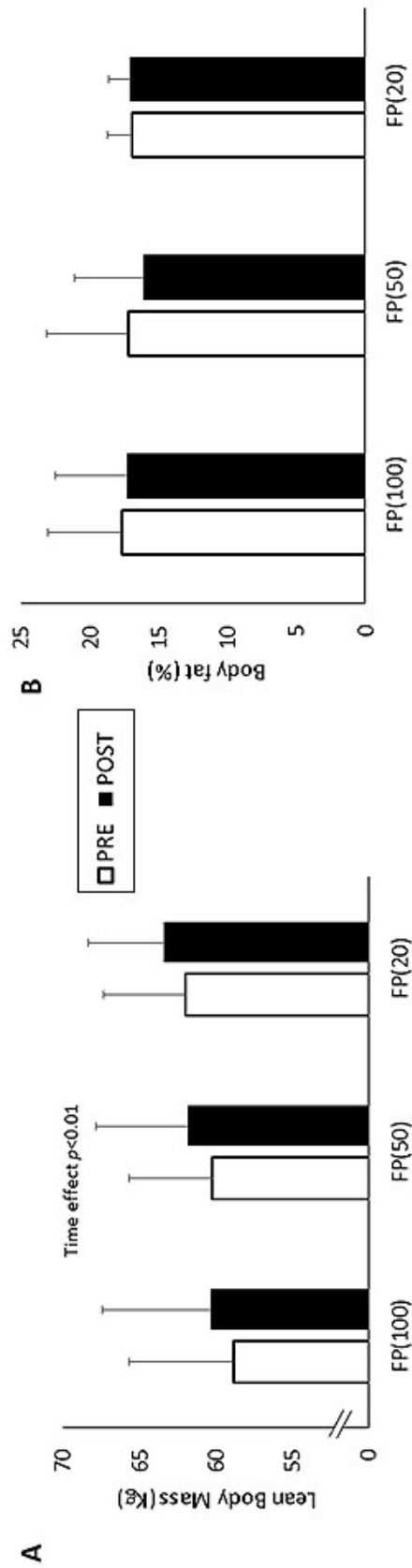
Figure 2 — Total body composition for lean body mass (LBM, figure A) and body fat (figure B) before (PRE) and after 9 weeks of RT and protein supplementation (POST).
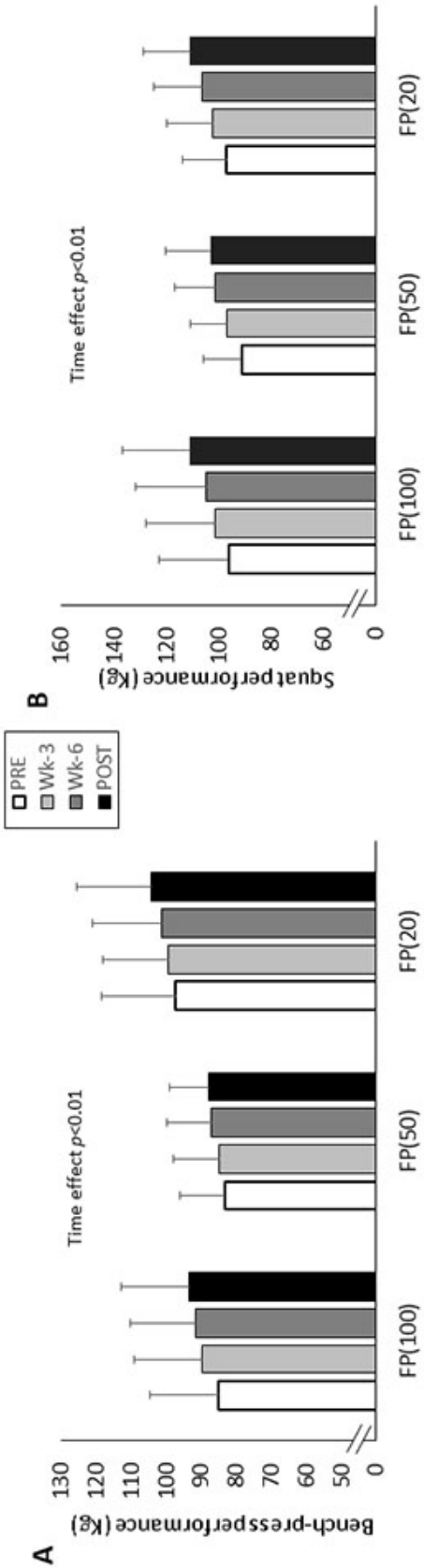
Figure 3 — 1 RM bench-press (figure A) and 3 RM squat performance (figure B) before (PRE), after 3 weeks (Wk-3), 6 weeks (Wk-6) and 9 weeks (POST) of resistance training with supplementation. RM = maximal repetition.
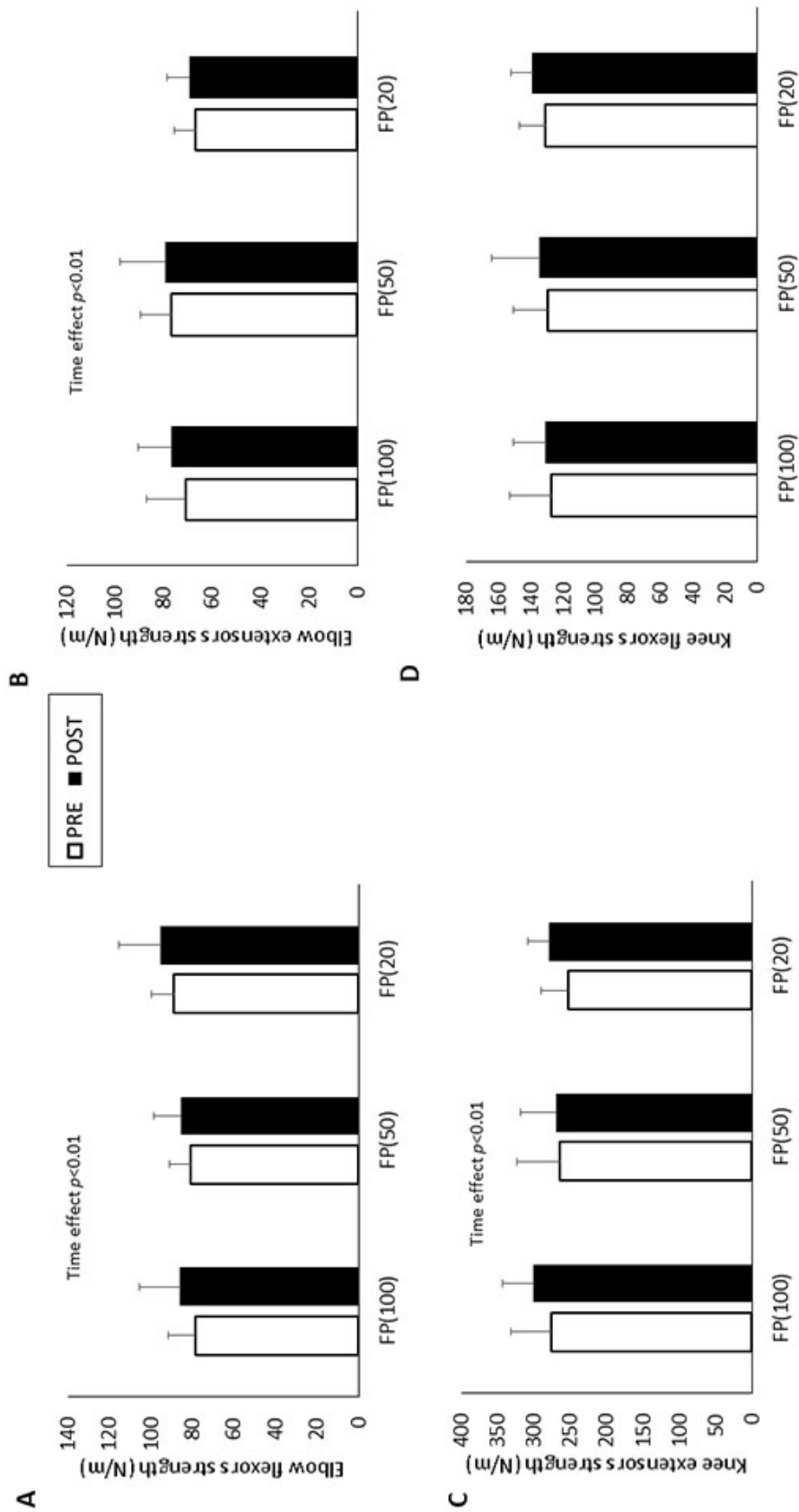
Figure 4 — Evolution in maximal isometric torque for elbow flexors (figure A), elbow extensors (figure B), knee extensors (figure C) and knee flexors (figure D) before (PRE) and after 9 weeks of supplement and resistance training (POST).

proteins have similar effects on myofibrillar MPS (Tipton et al., 2004; Reitelseder et al., 2011). Moreover, whole body protein breakdown was also inhibited after casein ingestion in resting subjects (Boirie et al., 1997). Whether these effects observed at rest could be extended to recovery from resistance exercise needs to be examined.

However, despite expected synergistic effects of casein and soluble protein, the protein supplements tested in the current study led to similar changes in lean body mass, fat mass, and strength performances, especially with no difference between the supplement comprising milk soluble protein alone, and supplements comprising a mix of fast and slow proteins. The higher circulating leucine and BCAA concentrations following ingestion of the FP(100) supplement, compared with the FP(20), did not have a measurable and positive impact on the long term adaptations to RT. Because the consumption of whey protein at the end of resistance exercise stimulates MPS to a greater extent than casein, the present results highlight the lack of consistency between acute studies and longitudinal training studies. Several factors may explain this apparent discrepancy. Healthy volunteers enrolled in the current study were recreationally active, and regularly involved in resistance exercises. However, it cannot be excluded that experimental groups may include responders and nonresponders to RT and/or protein supplements. The individual's inherited genetic predisposition, epigenetic changes and transcriptional plasticity may influence the variability in response to RT and/or nutrition (Mitchell et al., 2015).

Moreover, it has been recently suggested that spread and changes in protein intakes may be important factors predicting benefits from increased dietary protein during RT (Bosse and Dixon, 2012). The protein spread theory postulated that there must be sufficient difference in daily protein intake, expressed as g/kg/day, between groups to detect changes in muscle mass and performance. No significant differences in total protein intake were observed between experimental groups during the period of RT and protein supplementation. However, since the control of nutrient intake was achieved through a food diary 2 days-a-week during the 9 weeks of RT, it cannot be excluded that further changes in protein intake may be missed. Moreover, the protein change theory postulated that there must be a sufficient change from baseline protein intake to observe lean mass gain and strength gain in response to protein supplementation (Bosse and Dixon, 2012). As reported in Table 2, the total daily protein intake during the 9 weeks of RT was significantly higher than habitual protein intake before training. Therefore, the average change in habitual protein intake was high, and was likely effective to improve the responses of lean mass and muscle performances to RT. However, the lack of detectable changes in the muscle responses to RT depending on the type of protein supplement is consistent with the protein spread theory, and in accordance with a previous study comparing whey and soy proteins (Candow et al., 2006). The higher plasmatic availability of BCAA and leucine after FP(100) or FP(50) ingestions did not seem to significantly affect the responses to RT. Interestingly, the current study failed to demonstrate that higher AA availability during the anabolic window that follows the end of exercise, enhances the magnitude of muscle responses to RT.

From a practical standpoint, the present results suggest that although the nutritional supplement comprising a mix of proteins similar to milk (i.e., FP(20)) resulted in lower aminoacidemia when compared with both FP(50) and FP(100), additional dietary proteins taken at the end of each exercise bout, independent of source, contribute similarly to optimize the long-term muscle adaptations to RT. What really matters to maximize the responses to RT is to provide a sufficient amount of high-quality proteins, at the end of exercise, and the ratio of fast-to-slow digested proteins makes little difference.

In conclusion, recovery drinks enriched with milk soluble proteins, provide a higher bioavailability in BCAA and leucine than a recovery drink comprising mostly slow proteins. However, this does not result in higher responses of lean tissue mass and strength to resistance training, at least under our experimental conditions.

Acknowledgments

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