


## Proceeding

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# Effects of protein supplementation in fitness world: A 12-week cross-over studio

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### ABSTRACT

The aim of this project was to evaluate the effect of isolated protein supplementation in young amateur athletes. Sixteen subjects aged between 20 and 30 were recruited for this study. Before to start sports performance was assessed at T0, in all subjects, using physical performance test and evaluated body composition. Therefore, the subjects were randomly assigned in two groups (group A and B) of 8 subjects each. The group A start to intake 30 g of protein powder diluted in water after each training session (3 times a week) for six weeks (T0) whilst the group B was the placebo. After 6 weeks (T1), the measurement were repeated for all subjects and the group A became placebo and the group B started with the supplementation. At the end of 6 weeks (T2) we carried out all the tests were performed again. Results showed a significant improvement in almost all tests between T0 and T2 within the same group ( $p < .05$ ), but not significant difference was found between the start and the end of protein's intake period in both groups. In conclusion, supplementation did not have affect the performance and body composition significantly. Instead, training seems to influence the performance more the supplementation.

**Keywords:** Training; Supplementation; Body composition; Neuromuscular strength; Hypertrophy.

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## INTRODUCTION

Many studies have shown different strategies to support training and to obtain for example a good performance; several studies on genetics have recently emerged aimed to adapt training to the subject (Amato et al., 2018) but certainly nutrition and supplementation with specific nutrients play a key role. Indeed, to maintain muscle mass through a positive muscle protein balance, daily protein intake should be in the range of 1.4-2.0 g of protein for kg of body weight (Jager et al., 2017). However, only with daily nutrition it is difficult to maintain an adequate amount of protein intake. As known, it has an anabolic effect on the lean body mass (Jager et al., 2017), they favour a fast repair and construction of muscle tissue leading to a trophic effect especially after training (Churchward-Venne, Murphy, Longland, & Phillips, 2013; Jager et al., 2017; Moore et al., 2009). An important distinction must be made between endurance training and resistance training. In fact, some studies show that the post workout supplementation with isolated proteins does not improve endurance or aerobic/anaerobic performance more than carbs supplementation (Amato et al., 2017; D'Lugos et al., 2016). But at the same time, it can be useful to reduce muscle damage markers such as creatine kinase and muscle pain 12-24 hours after exercise (Breen, Tipton, & Jeukendrup, 2010; Romano-Ely, Todd, Saunders, & Laurent, 2006; Saunders, Luden, & Herrick, 2007; Valentine, Saunders, Todd, & St Laurent, 2008). As regards the effects of protein supplementation with resistance training, no studies report improvements in strength performance, often with supplements of three weeks or less (D'Lugos et al., 2016; Willoughby, Stout, & Wilborn, 2007). On the other hand, studies reported improvements in maximum strength with the ingestion of about 30 / 40g of post workout protein for at least 6/12 weeks (Hoffman, Ratamess, Kang, Falvo, & Faigenbaum, 2007; Josse, Tang, Tarnopolsky, & Phillips, 2010; Taylor, Wilborn, Roberts, White, & Dugan, 2016). However, these studies have several variables that influence the results such as intensity (Proia et al., 2016) and volume of the workout, the duration, the fitness of the subject and diet (Jager et al., 2017). The proteins, in addition to contribute to the increase in maximum strength, contribute to the change in body composition. Especially, by changing the area of the muscle section in both type I and type II muscle fibres (Cermak, Res, de Groot, Saris, & van Loon, 2012) more when the completion of a resistance training program is also associated (Pasiakos, McLellan, & Lieberman, 2015; Phillips, 2011; Tipton & Phillips, 2013). The anabolic function of proteins is carried out in different ways such as increasing the IGF1 plasma concentration (Campbell et al., 2007). However, the key role is played by dose, timing and amount of intake as highlighted by recent International Society of Sports Nutrition Position Stand about protein and exercise (Jager et al., 2017). A protein dose of 20-40 g of protein (10-12 g of EAAs, 1-3 g of leucine) stimulates the synthesis and helps to have a positive nitrogen balance, also decreasing muscle damage marker (Areta et al., 2013; Tinsley et al., 2017) even more if the protein supplementation occurs with a maximum of three hours away from each (Areta et al., 2013). Indeed, the skeletal muscle is sensible to the effects of proteins and amino acids for a maximum of 24 hours after the resistance training (Paddon-Jones, Sheffield-Moore, Aarsland, Wolfe, & Ferrando, 2005). Particularly, for our study we have considered animal proteins because at the same concentration, are more efficient than vegetable proteins in muscle synthesis increase (Breen et al., 2010; D'Lugos et al., 2016; Witard, Jackman, Kies, Jeukendrup, & Tipton, 2011). We considered whey proteins because they are soluble in water, easy to swallow, are quickly digested and are more effective in stimulating increases in anabolism and maximum strength compared to other protein classes such as casein especially post workout (Taylor et al., 2016; Wilson & Wilson, 2006). However, it is difficult to establish how much this lean body mass increase is attributable to the supplementation alone, to training with weights, or both and also there is no clarity as to whether the beneficial effect on muscle mass is in terms of hypertrophy or maximum strength, in fact studies show how protein supplementation can give benefits both in neuromuscular strength and in hypertrophy (Hartman et al., 2007). Therefore, the objective of this study was to verify if supplementation with isolated proteins assumed after a 12-week of resistance training (three times a week) in a crossover study can increase the hypertrophy and improve the neuromuscular strength.

## METHODS

### Subject

Sixteen of Caucasian male subjects (age  $26.25 \pm 2.81$ ) was enrolled in the study. Inclusion criteria were: age between 20 and 30 years and performed physical activity at least three times a week for at least 6/8 months, in order to exclude the physical conditioning period needed. None of them performed the resistance training with a load over 60% of 1 RPM in the period before to start the study or suffer of some pathologies. At the beginning of the project everyone signed the informed consent. All the subjects followed a healthy diet with the right and balanced ratio of nutrients (55% carbs, 25% protein, 20% fat of daily calorie intake) distributed in 5 daily meals.

### Physical performance evaluations and anthropometric measures

At the beginning of the study (T0), personal data, anthropometric measurements (weight, height and arm, chest, waist, thigh circumferences measurement) were collected (Table1).

Table 1. Anthropometric measurements.

	Age	Weight (Kg)	Height (m)	BMI(kg/m <sup>2</sup> )
Group A (n = 8)	$26.37 \pm 0.26$	$76.02 \pm 4.54$	$1.77 \pm 0.06$	$24.17 \pm 0.62$
Group B (n = 8)	$26.12 \pm 0.29$	$76.56 \pm 8.98$	$1.77 \pm 0.04$	$24.12 \pm 1.53$

In addition, the strength and endurance of all subjects was assessed with a battery of tests that included:

- Squat,
- Deadlifts,
- Bench press,
- Plank.

For the first three exercises, 5 sets of 5 repetitions with a recovery of 1.30 minutes were performed to evaluate physical condition. For squat, deadlift and bench press test the maximum load (in kg) with which the subject was able to complete 5 repetitions was considered as test results. However, the plank test was performed holding the plank position as long as possible (in minutes). The study lasted 12 weeks during which all subjects performed the same training three times a week, aimed to increasing muscle mass. We considered a period of 12-week because seem to be effective to stimulate muscle adaptation to training as already demonstrated in pathological subjects too (Proia et al, 2019).

On the first day of the week they trained chest, upper limbs and abdominal; the second day of the week subject trained leg, shoulders and abdominal and the third day week they trained legs, back and abdominal.

At T0, after the evaluations, the subjects were randomly assigned into two groups, A and B, of 8 people each.

Group A for the first 6 weeks, at the end of the workout, took 30 grams of protein powder diluted in 200 ml of water. Instead, for the first 6 weeks, Group B performed the training session without supplementation. At the end of the sixth week (T1) all the assessments were repeated for both groups. Therefore, Group A interrupted the supplementation until the end of the study, carried out only the training sessions. Group B instead from T1 began the supplementation with 30 grams of isolated protein powder diluted in 200 ml of water after each training session (3 times a week). At the twelfth week (T2), all the evaluations were repeated.

### Statistics analysis

Data were expressed as the mean and standard deviation and were analysed by two-way repeated measures analysis of variance (ANOVA, within and between subjects) with post-hoc Bonferroni correction, performed with IBM SPSS statistics software 23 version. Data were also analysed in terms of the absolute values. The significance level was set at  $p < .05$ . Supplementation time was completed as the independent variable, and the different circumferences measurement and test performance as the dependent variable.

## RESULTS

Table 2. Anthropometrics measurements and physical performance in the three evaluations time: at the beginning of the study (T0), after 6 workout weeks (T1), after 12 workout weeks (T2) in the two group. Variables in absolute values, are reported as mean and SD.

	T0 (mean $\pm$ SD)	T1 (mean $\pm$ SD)	T2 (mean $\pm$ SD)
<b>Chest (cm)</b>			
Group A	94.13 $\pm$ 2.90	94.56 $\pm$ 2.57	95 $\pm$ 2.5
Group B	95.36 $\pm$ 6.39	95.51 $\pm$ 6.04	96.08 $\pm$ 6.22
<b>Arm (cm)</b>			
Group A	32.38 $\pm$ 2.83	33.43 $\pm$ 2.75	34.14 $\pm$ 2.79
Group B	32.99 $\pm$ 2.94	33.69 $\pm$ 2.85	34.65 $\pm$ 2.77
<b>Waist (cm)</b>			
Group A	83.44 $\pm$ 4.91	83 $\pm$ 4.64	82.67 $\pm$ 4.51
Group B	85.25 $\pm$ 7.25	84.86 $\pm$ 7.07	84.43 $\pm$ 7.05
<b>Thigh (cm)</b>			
Group A	54 $\pm$ 3.70	54.60 $\pm$ 3.64	55.29 $\pm$ 3.44
Group B	52.20 $\pm$ 2.09	52.76 $\pm$ 2.02	53.85 $\pm$ 1.89
<b>Plank Test (minutes)</b>			
Group A	2.27 $\pm$ 0.32	2.34 $\pm$ 0.19	2.33 $\pm$ 0.22
Group B	2.29 $\pm$ 0.25	2.28 $\pm$ 0.39	2.45 $\pm$ 0.28
<b>Squat Test (kg)</b>			
Group A	30.25 $\pm$ 5.30	33.88 $\pm$ 4.94	35.25 $\pm$ 5.06
Group B	28 $\pm$ 5.66	30.13 $\pm$ 4.94	33.13 $\pm$ 3.72
<b>Bench Test (kg)</b>			
Group A	26.81 $\pm$ 3.46	30.75 $\pm$ 3.36	31.50 $\pm$ 3.40
Group B	24.94 $\pm$ 6.10	28.69 $\pm$ 5.99	31.56 $\pm$ 5.33
<b>Deadlift Test (kg)</b>			
Group A	59.75 $\pm$ 17.74	62.31 $\pm$ 15.85	61.50 $\pm$ 14.68
Group B	39.94 $\pm$ 18.73	41.88 $\pm$ 17.61	44.20 $\pm$ 16.40

Results of the repeated measures ANOVA showed a significant difference of circumferences measurement (cm) between the three-evaluation time (T0-T2) in group A in: chest circumference ( $F_{2,6} = 13.36$ ;  $p < .05$ ), thigh circumference ( $F_{2,6} = 25.37$ ,  $p < .05$ ) and arm measurement ( $F_{2,6} = 108.106$ ,  $p < .05$ ). No significant difference was found in waist measurements between T0-T2 in group A ( $F_{2,6} = 2.47$ ,  $p > .05$ ). As regard circumferences measurement (cm) between T0-T2 in group B, repeated measures ANOVA showed a significant difference in: chest circumference ( $F_{2,6} = 13.60$ ,  $p < .05$ ), waist circumference ( $F_{2,6} = 7.59$ ,  $p < .05$ ), thigh circumference ( $F_{2,6} = 42.93$ ,  $p < .05$ ) and arm measurement ( $F_{2,6} = 21.40$ ,  $p < .05$ ). Data of

physical performance evaluation, with repeated measures ANOVA test, showed in group A statistic significant results: in bench-press test ( $F_{2,6} = 81.34$ ,  $p < .05$ ) and in squat test ( $F_{2,6} = 53.70$ ,  $p < .05$ ).

No statistic difference was found in plank test ( $F_{2,6} = 0.85$ ,  $p > .05$ ) and deadlift test in group A ( $F_{2,6} = 2.75$ ,  $p > .05$ ). While physical performance evaluation in group B, with repeated measures ANOVA test, showed statistic significant in bench-press test ( $F_{2,6} = 42.02$ ,  $p < .05$ ), in squat test ( $F_{2,6} = 17.79$ ,  $p < .05$ ), in deadlift test ( $F_{2,6} = 22.92$ ,  $p < .05$ ) and in plank test ( $F_{2,6} = 4.73$ ,  $p < .05$ ). Table 2 shows the results of all anthropometric and physical test in absolute values for the different variables analysed in groups A and group B. A one-way between-subject ANOVA was run between the two group for each test and not significant result was found in all tests ( $p > .05$ ).

## DISCUSSION

As shown in the results section, between T0-T2 there were change in physical performance and body composition: most of the evaluations performed showed a significant change (apart from deadlift test, plank test and waist measurement but only for group A). Definitely in this case training seems to have a greater influence than supplementation. Indeed, group A has the greatest significance over time between T0 and T2 in chest ( $p = .02$ ), thigh ( $p = .002$ ), arm ( $p = .007$ ), and not between the T0 e il T1 ( $p > .05$ ) (supplementation time for this group). As well group B has the greatest significance in all measurements of the circumferences between T0 and T2 with chest ( $p = .003$ ), waist ( $p = .047$ ), arm ( $p = .003$ ); instead between T1 and T2 was not significant ( $p > .05$ ) (supplementation time for group B). This means that training had a greater influence than protein supplementation, on body composition, in both groups. However, as regards physical performance, protein supplementation seems to have a positive effect; this statement would show how it is possible to discriminate neuromuscular strength from hypertrophy of skeletal muscle (Hartman et al., 2007).

Indeed, in group A both in the bench-press test and in the squat test the significance between T0 and T1 (for both tests  $p = .000$ ) is greater than T1-T2 ( $p > .05$ ) and T0-T2 ( $p > .05$ ); the stronger statistical significance value was found between T0 and T1 both in plank-test ( $p = .72$ ) and deadlift test ( $p = .082$ ). Differently it is evident from the physical performance evaluation results in group B, in which the stronger statistical significance value was found in all tests between T0 and T2 in bench-press test ( $p = .000$ ), in squat test ( $p = .008$ ), in deadlift test ( $p = .005$ ) and in plank test ( $p = .005$ ). Therefore, these results shown a greater effect of training than supplementing with isolated proteins on performance.

## CONCLUSIONS

The supplementation of 30g of protein, taken only three times a week, after a resistance training aimed to increase muscle mass is insufficient to cause a significant change in body composition and physical performance. Instead, the resistance training performed for 12 weeks is effective at changing both.

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