



### Effects of Protein Supplement Timing during 4-Week Resistance Training on Muscle Hypertrophy in Males

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**Objective** Nutrient timing is a new system of exercise nutrition that can help improve strength and lean body mass in a short time and does not require a change in exercise plan and energy intake. The concept of nutrient timing began to be used to solve problems such as the stagnation of muscle strength growth in high-protein diets, high quality proteins to gain weight and strength, but to obtain and utilize nutrient supplements at the right time to maximize muscle growth. Therefore, it is necessary to supplement the appropriate nutrients at different times.

At present, some studies have found that the type and protein supplementation timing have some influence on the resistance training of high-level athletes. Protein supplementation before or after exercise plays a positive role in improving sports performance, recovery after exercise, muscle hypertrophy and muscle strength improvement. Due to the different types, dosage and time of protein supplementation, there are still some differences.

It is still necessary to study the effect of nutrient timing of whey protein and the CEUS on the morphological indices of the rectus femoris. Therefore, by observing the thickness, circumference and area of the rectus femoris, we observed the effect of different nutrient timing and lower limb strength training on the shape of rectus femoris in healthy male youth. It is suggested that the different timing and dosage produce different effects and provide scientific suggestions for the later strength training and whey protein supplementation.

**Methods** A total of 32 healthy male students participated in a randomized, double-blind, placebo-controlled study. Subjects were randomly divided into four groups, the control group (Group A), the pre-exercise supplementary group (Group B), the after-exercise supplementary group (Group C) and both pre-exercise and after-exercise supplementary group (Group D), which were supplemented by: two bottles of placebo before and after the training in Group A. two bottles of whey protein water before training and two bottles of placebo after training in Group B. two bottles of placebo before training and two bottles of whey protein water after training in Group C. two bottles of whey protein water before and after training in Group D. the bottles of supplements before training should be supplemented half an hour before the start of training, and the bottles of supplements after training should be supplemented within half an hour after training. Each bottle of whey protein water was 350ml which contained 15g whey protein. The training method was 70% 1RM for barbell squat, 4 sets of 10 times, each set rest for 60s. Three training sessions were conducted each week, and the training lasted for four weeks. During this period, in order to deal with the training effect, at the end of the second week, we performed a maximum squat strength test to adjust the training intensity for the following two weeks. The body composition test used GE lunar IDXA, a dual-energy X-ray scanning method and the indexes were body weight, muscle mass and FFM. The CEUS used GE Vivid 7 holographic Color Doppler Ultrasound Diagnostic system. The test sites of CEUS were in the anterior superior iliac crest and the upper margin of the patella 1/4, and the indexes were the thickness of the femoral rectus femoris, the circumference of the rectus femoris, and the cross-sectional area of the femoral rectus femoris. Tests were performed before and after 4-week resistance training. After the intervention, the variance of paired sample t test and One Way ANOVA were used to test the significance of each group.

**Results** According to the change of the body composition, compared with the control group (Group A), the FFM of Group B, Group C and Group D were decreased, but there were no significant differences ( $p>0.05$ ). The muscle mass of Group C increased from  $54.53\pm 4.64$  kg to  $58.54\pm 5.82$  kg, and muscle mass of Group D increased from  $55.74\pm 4.09$  kg to  $58.75\pm 4.74$  kg, compared to group A, Group C and Group D had significant increase in muscle mass ( $p<0.05$ ). Body composition is mainly composed of adipose tissue and lean tissue, the body composition is influenced by the acquired factors, resistance training will also decrease the FFM and the increase the lean weight. Combined with 4-week resistance training with protein supplementation, we can see that the FFM in Group B, Group C and Group D declined a lot, which may be related to protein supplementation. The muscle mass of Group C and Group D were significantly improved, it was indicated that after resistance training, protein supplementation can improve muscle mass, but there was no differences between Group C and Group D, which indicates that the increase of protein supplementation has little effect on the increase of muscle mass, which may be due to the increase of protein supplement, the amount of muscle synthesis will increase, but the amount of decomposition will increase, too. In addition, the changes in the synthesis and decomposition, resulting in little change in muscle mass.

About the morphological changes of rectus femoris in dominant leg, compared with the control group (Group A), the thickness of the rectus femoris (changed from  $12.55\pm 3.94$  mm to  $16.71\pm 3.04$  mm), the circumference of the rectus femoris (changed from  $8.38\pm 1.98$  cm to  $10.08\pm 1.79$  cm), the cross-sectional area of the rectus femoris (changed from  $3.64\pm 1.91$  cm<sup>2</sup> to  $5.43\pm 1.61$  cm<sup>2</sup>) in Group C and the thickness of the rectus femoris (changed from  $14.12\pm 2.33$  mm to  $15.91\pm 2.10$  mm) in Group D were significantly increased ( $P<0.05$ ). The thickness of the rectus femoris, the circumference of the rectus femoris, the cross-sectional area of the rectus femoris in Group B and the circumference of the rectus femoris and the cross-sectional area of the rectus femoris in Group D were also increased, but there were no significant changes ( $p>0.05$ ). About the morphological changes of rectus femoris in non-dominant leg, compared with the control group (Group A), the thickness of the rectus femoris (changed from  $13.54\pm 3.82$  mm to  $16.77\pm 3.37$  mm), the cross-sectional area of the rectus femoris (changed from  $4.07\pm 2.11$  cm<sup>2</sup> to  $5.42\pm 1.86$  cm<sup>2</sup>) in Group C and the thickness of the rectus femoris (changed from  $13.46\pm 2.91$  mm to  $16.39\pm 1.24$  mm) in Group D were significantly increased ( $P<0.05$ ). The thickness of the rectus femoris, the circumference of the rectus femoris, the cross-sectional area of the rectus femoris in Group B, the circumference of the rectus femoris in Group C and the circumference of the rectus femoris and the cross-sectional area of the rectus femoris in Group D were also increased, but there were no significant changes ( $p>0.05$ ).

It could be seen from the results that the thickness, circumference and cross-sectional area of rectus femoris in Group C and Group D had a significant increase, and the change in Group B was not significant, indicating that after resistance training, protein supplementation will have better effects, more conducive to promoting the synthesis of muscle protein, so that the morphology of muscle was more obvious. From the comparison between Group C and Group D, we can see that the morphology of the rectus femoris in Group C has a significant change, although there were significant changes in the thickness of the rectus femoris in Group D, there was no significant increase in the circumference and cross-sectional area of the rectus femoris, which showed that the increase of protein supplementation had little effect on the morphology of the rectus femoris. This may be related to the synthesis and decomposition rate of the muscle, the increase in protein intake, to some extent, accelerated the rate of muscle synthesis, but the rate of decomposition of the muscle will also be accelerated, the synthesis rate and the rate of decomposition to achieve a positive balance. The rectus femoris of Group D will have a significant increase, but compared to group C, the effects were not obvious.

**Conclusions** For the nutrient timing of the whey protein, taking whey protein after exercise is the better timing to improve the thickness, circumference, cross-sectional area of rectus femoris and the muscle mass by the lower limb resistance training for males. There is a certain effect on the reduction of body FFM, but no significant improvement is found. The increase of whey protein supplementation may have little effect on body composition and muscle hypertrophy.