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Research Article
Impact of Resisted Exercises and Whey Protein on Growth Hormones and Testosterone in Normal Subjects

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Abstract

Background and Objective: Protein timing is a popular dietary strategy designed to optimize the adaptive response to exercise. The strategy involves consuming protein in and around a training session in an effort to facilitate muscular repair, remodelling and thereby, enhance post-exercise strength and muscle anabolism. The purpose of the current study was to investigate the effect of whey protein (WP) on muscle performance through the evaluation of the growth and the testosterone hormones. **Materials and Methods:** Sixty normal subjects were selected and randomly divided into two groups. A study group took whey protein orally for 6 weeks (1.2 g kg^{-1}/day) following exercise and control group did only resistance exercise. Resistance exercise was done for both upper limbs (biceps and triceps) using dumbbell and the initial weight was 75% of the one repetition maximum (RM). Three sets were done at 10 repetitions for each set. The training protocol consisted of three sessions/week for 6 weeks for both groups. Subjects were tested for performance before, after 3 and 6 weeks of training and blood hormone concentrations before and after 6 weeks and the comparison between the training programs were investigated. Samples were tested by t-test and two-way ANOVA. **Results:** Growth hormone showed significant difference between both groups (p=0.0001), while testosterone showed no significant difference between both groups (p = 0.166) at the end of the 6th week. One RM also showed significant difference in study group than control group in biceps muscle where p-value was 0.0001. **Conclusion:** Whey protein combined with resistance exercise has a significant difference on anabolic hormones and muscle strength at the end of the study period. These findings determined that whey protein might enhance muscle strength and anabolism.

Key words: Whey protein, resistance exercise, anabolic hormones, testosterone, growth hormone, one repetition maximum, muscle strength

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Whey protein is considered one of the sources of amino acids that can help to decrease the risks of heart disease, cancer and diabetes, it was clear in the ongoing research work since 2007 that focus on how to decrease risk factors affecting humans[1]. Whey is an abundant source of branched-chain amino acids (BCAAs)[2] and stimulation of protein synthesis may accelerate healing rate of muscle injuries if leucine is added to whey protein and ingested in high amounts[3].

Whey has approximately 3 g of leucine per serving, where the threshold for optimal protein synthesis is 3 g[4]. As with other forms of protein, consumption of whey protein shortly after vigorous exercise can boost muscle hypertrophy[5].

Several studies reported that protein timing play an important role in muscle synthesis especially after resistance training exercise[6]. Protein is the critical nutrient required to improve protein synthesis after exercise. It was believed that essential amino acids are responsible to enhance this effect than nonessential amino acids[7].

To maximize the use of anabolic hormones and increase protein synthesis in the muscle, whey protein should be taken before and/or immediately after exercise training[8].

Several findings suggested that whey promotes a rapid and large increase in muscle protein synthesis. Therefore, adding casein to a whey shake may keep muscle-building state for a longer period of time than just taking whey standalone[9].

Resistance exercise has been shown to elicit a significant acute hormonal response. It appears that this acute response is more critical for tissue growth and remodelling than chronic changes in resting hormonal concentrations. Anabolic hormones, (testosterone (T) and growth hormone (GH)), have been shown to be elevated post exercise. Different protocols (as high intensity volume and moderate to high intensity, using short rest intervals and stress on a large muscle mass), tend to produce the greatest acute hormonal changes (e.g. testosterone, GH and the catabolic hormone cortisol) compared with low-volume, high-intensity protocols using long rest 20 intervals. Other anabolic hormones such as insulin and Insulin-Like Growth Factor-1 (IGF-1) are critical to skeletal muscle growth[10].

The interplay and constant fluctuation of the body hormonal balance may work to our muscle building advantage or create a litany of problems, including a sluggish metabolic rate, failure to properly recover between workouts, low energy levels and hampered protein synthesis[11].

There have been a few studies that have examined the effect of prolonged protein supplementation (e.g. length of a typical off-season resistance training program) on changes in hormonal concentrations in resistance trained men. Studies examining the effect of protein supplementation on strength enhancement are limited and results have been inconclusive. However, other studies did not find any significant changes in the profiles of hormonal responses to resistance exercise due to long-term strength training in adult men[12-15]. Thus, the purpose of the study was to examine the effect of whey protein supplementation on strength and hormonal response during a 6 weeks resistance training program in normal subjects.

MATERIALS AND METHODS

This study was conducted in the Faculty of Physical Therapy, Cairo University from April, 2016-January, 2017, the duration of this study was 10 months and was approved by the Ethical Committee No.: P.T.REC/012/001182.

Demographic data: There were no differences among groups in any of the baseline measurements.

Subjects: Sixty normal male subjects participated in this experiment with mean age ranged from (18.82±0.72) years, mean weight was (68.08±5.36) kg, mean height was (176.63±4.61) cm and mean body mass index (BMI) was (21.83±1.58) kg m^{-2}.

Study group: It represented the mean age (18.53±0.68) years, weight (68.13±5.12) kg, height (177.27±4.02) cm and BMI (21.71±1.87) kg m^{-2}.

Control group: It represented the mean age (19.1±0.66) years, weight (68.03±5.68) kg, height (176±5.12) cm and BMI (21.94±1.26) kg m^{-2}.

Exclusive criteria items: Exclusive criteria items were diabetic subjects, any history of abnormal hormonal function, any history of muscle disease and any abnormal nutritional diseases.

Procedures:

- Whey protein (4 Dimension Nutrition Whey Phase, USA) was calculated based on the subject's weight (1.2 g kg^{-1}/day for study group[16])

- One scoop (35 g) containing 24 g of protein from powder whey was mixed with 180 mL of water using a spoon in a plastic glass

Resistance exercise: Resistance exercise was used for both groups. It was done as (biceps and triceps resistance exercise using dumbbell for both upper limb). Three sets were done each set at 10 repetitions[17]. The training protocol consisted of three sessions/week for 6 weeks[18].

One RM was calculated for biceps muscles according to a formula for estimating one's maximum strength, based on the amount of weight lifted and the number of repetitions achieved during a particular set.

All subjects started with light warm-up (one set of 10-12 repetitions at a light weight selection), then went with moderate warm-up (one set of 8-10 repetitions at a moderate weight selection) according to Baechle *et al.*[19] Eq.:

$$\text{Weight} \times (1+0.033 \times \text{Number of repetitions})$$

The intensity of exercise was 75-80% of one-repetition maximum (1RM) with a 1 min rest between sets to avoid muscle injuries[20].

Procedure of analyzing hormones: Plasma was taken to analyze growth hormone and testosterone through cobas E411 analyser using kits: Growth hormone: 05390125-190 (Roche diagnostics, Sandhofer Strasse 116-D-68305 Mannheim-Germany) and testosterone: 05200067-190 (Roche diagnostics, Sandhofer Strasse 116-D-68305 Mannheim-Germany).

Results had been compared with reference range before training and at the end of 6th week within and between groups as shown in Table 1.

The key findings of this research showed how protein supplementation can promote a large increase in muscle protein synthesis and muscle strength. GH and muscle strength results showed significant increase in study group more than control group while testosterone showed no difference between study and control groups.

Statistical analysis: Descriptive statistics and two-way ANOVA were conducted to compare 75% of one RM of biceps within and between groups (study and control). Inferential statistics and two-sample t-test were conducted to compare anabolic hormones (testosterone and growth hormone) differences within and between groups (study and control).The level of significance for all statistical tests was set at $p<0.05$.

RESULTS

Mean values of 75% of 1RM for biceps: A significant increase was shown within same group when comparing 75% of 1RM of biceps before, after 3 and 6 weeks as the results were: (SS =77.561, mean square =2.675, F value =278.56 and p-value = 0.0001). In addition, it also showed significant increase between study and control groups as the results were: (SS = 1191.8, mean square = 238.35, F value = 278.56 and p-value = 0.0001).

Multiple pair wise comparison tests (*post hoc* tests) within and between groups for 75% of 1RM of biceps

Study group: One RM showed a statistical significant increase (p-value<0.05), before starting versus both after 3 and 6 weeks. Also, there was a statistical significant difference after 3 weeks versus after 6 weeks of training.

Furthermore, 1RM in the control group, before starting versus both after 3 and 6 weeks and after 3 weeks versus 6 weeks, all showed a significant increase.

However, when comparing mean values of study and control groups, there was no statistical significant difference (p-value>0.05) before starting and after 3 weeks, while there was statistical significant increase after 6 weeks of training as shown in Table 2.

Mean values of GH and T hormones within same group: A significant increase in GH and testosterone pre and post exercise training program in both study and control groups as shows in Table 3.

Mean values of GH and T between study and control groups: Comparing results of the mean values of GH in study and control groups showed significantly increase in the study

Table 1: Reference range of the analyzed hormones

Tests	Patient type	Lower limit	Upper limit	Unit
Testosterone (total), serum	Male<50 years	2.5	8.4	ng mL^{-1}
	Male>50 years	1.9	7.4	ng mL^{-1}
	Female	0.1	0.5	ng mL^{-1}
Serum growth hormone (fasting)		0.0	5.0	ng mL^{-1}

Coded according to the manufacturer analyzer

Table 2: Mean values of 75% of one-RM of biceps within and between groups

Comparison	MD	p-value
Study group		
Before training-after 3 weeks	-2.367	0.001*
Before training-after 6 weeks	-6.833	0.001*
After 3 weeks-after 6 weeks	-4.467	0.001*
Control group		
Before training-after 3 weeks	-2.600	0.001*
Before training-after 6 weeks	-5.467	0.001*
After 3 weeks-after 6 weeks	-2.867	0.001*
Between groups		
Before training study group-before training control group	0.3000	0.050^{NS}
After 3 weeks study group-after 3 weeks (control group)	0.06667	0.050^{NS}
After 6 weeks study group-after 6 weeks (control group)	1.667	0.001*

*Significant, NS: Non-significant, p-value: Probability value, MD: Mean difference

Table 3: Mean values of GH and T within same group

	Before starting		After 6 weeks			
Groups	Mean	SD	Mean	SD	95% CI for mean difference	p-value
GH						
Study group	0.447	0.299	2.111	1.289	1.194, 2.132	0.0001*
Control group	0.4256	0.2611	1.0492	0.4246	0.4302, 0.8171	0.0001*
T						
Study group	7.710	1.528	8.100	1.355	0.1875, 0.5916	0.0001*
Control group	7.780	1.475	8.000	1.266	0.0771, 0.3629	0.004*

* Significant, SD: Standard deviation, CI: Confidence interval, p-value: Probability value, GH: Growth hormone, T: Testosterone

Table 4: Mean values of GH and T between groups

Groups	Mean	SD	95% CI for mean difference	p-value
GH				
Study	1.660	1.260	0.537, 1.542	0.0001*
Control	0.624	0.518		
T				
Study	0.390	0.541	-0.073, 0.412	0.166^{NS}
Control	0.220	0.383		

*Significant and NS: Non-significant, SD: Standard Deviation, CI: Confidence Interval, p-value: Probability value, GH: Growth hormone and T: Testosterone

group more than the control group. However testosterone comparisons showed no significant difference between groups as shown in Table 4.

DISCUSSION

This study was designed to investigate the effect of resistance exercise and whey protein on growth hormone and testosterone objectively through blood analysis and subjectively through 1RM for biceps muscle.

The results of this study showed that whey protein combined with resistance exercise had an effect on growth hormone as it showed a significant increase in the study group than in the control group, while testosterone was affected by resistance exercise only. Biceps strength also showed a significant difference pre and post training program at the end of the 6th week but the study group had a greater improvement in 1RM compared to the control group. It is thought that protein supplementation can stimulate muscle protein synthesis to counteract the deleterious effects of muscle degradation seen after bouts of resistance exercise[6].

If protein degradation is reduced with a concomitant increase in protein accretion, the resulting effect would generate a greater stimulus for muscle growth and enhanced recovery and potentially resulting in greater strength gains[21], while other investigators have shown augmented strength gains from protein supplementation.

WP supplementation slightly increased endurance time and significantly increased grip strength and levels of albumin and total protein. WP supplementation improved exercise performance, body composition and biochemical assessments in mice and may be an effective ergogenic aid in aerobic exercise training[18].

The findings of the muscle strength through 1RM in the present study come in agreement with the findings of Coburn *et al.*[22], who randomly assigned adult male subjects to a supplement (20 g whey protein, 6.2 g leucine), carbohydrate placebo (26.2 g maltodextrin), or control group for 8 weeks of unilateral (non-dominant limb) leg extension resistance training. The protein supplemented subjects demonstrated a 30% increase in strength in the trained limb which was significantly higher than the strength increase (22%) achieved by the carbohydrate placebo group. Strength changes were observed in the control group, but more in the study group.

Similarly, Willoughby *et al.*[23], compared the effects of a 10 weeks resistance training program combined with 20 g protein (14 g whey and casein protein, 6 g free (essential and non-essential) amino acids) or 20 g dextrose placebo ingested 1 h before and after exercise on muscular strength in untrained males. Results showed significant difference in muscle strength in the study group more than the control group.

In addition, the efficacy of whey protein as a protein supplement was examined by Burke *et al.*[16], with respect to resistance training. The study demonstrated that males who received whey protein supplementation, while undergoing resistance training showed a slight but significant increase (3.8%) in lean muscle mass compared to males who did the same training but received a placebo (comprised of maltodextrin). The study acknowledged that the relatively small rather than large enhancement in lean muscle mass might be due to sufficient protein intake from the high protein diets of most strength-trained individuals.

Furthermore, Rankin *et al.*[24], demonstrated the effect of post-exercise supplement consumption on adaptations to resistance training through19 untrained men (18-25 years) consumed either a milk or a carbohydrate-electrolyte (CHO) drink immediately following each work-out during a 10 weeks resistance training program. Muscle strength (1RM for seven exercises), body composition, resting concentrations of serum total and free testosterone, cortisol, IGF-1 and resting energy expenditure (REE) were measured prior to and at the end of training. The results showed that resistance training caused an increase (44 +/- 4%, $p<0.001$) in muscular strength for all subjects. The training program reduced body fat percentage (8%, $p<0.05$, -0.9 +/- 0.5 kg) and increased fat-free soft tissue (FFST) mass (2%, 1.2 +/- 0.3 kg, $p<0.01$). Milk tended to increase body weight and FFST mass ($p = 0.10$ and $p = 0.13$, respectively) compared to CHO.

In addition, Esmarck *et al.*[25] studied the effect of the timing of protein intake on muscle hypertrophy in elderly humans. The study found that over a 12 weeks resistance training period, muscle hypertrophy increased significantly when protein was ingested immediately after exercise, while no significant changes were observed when protein was supplemented 2 h after exercise. These findings indicate that early consumption of protein after resistance training leads to higher net protein synthesis which is similar to present study results as study group showed more improvement in muscle strength than control group.

Furthermore, the results seem to contradict with the fact that the ingestion of amino acids through protein supplements augments muscle hypertrophy and causes retention of lean muscle mass, promoting positive nitrogen balance after resistance training. Whey protein has also been shown to provide these effects, although it may not contain the antioxidant benefits of other forms of protein.

However, it is important to note that all of these studies have demonstrated that post exercise protein intake in any form is required to promote muscle anabolism.

Furthermore, protein supplementation increases the availability of amino acids, which may result in an increased uptake of amino acids by the muscle. Increased uptake of amino acids by the muscle enhances net muscle protein balance and improves the anabolic environment[26].

In the present study, testosterone values showed significant difference post exercise program compared with pre measurement values and this was similar with Ahtiainen *et al.*[27], who reported significantly higher free and total testosterone concentrations during a 7 weeks high-volume training phase compared with pre-training values.

Similar study of Kalman *et al.*[28], demonstrated 20 subjects were supplemented with 50 g/day of one of four different protein sources (soy concentrate; soy isolate; soy isolate and whey blend and whey blend only) in combination with a resistance-training program for 12 weeks. Results showed that estradiol was significantly lower in the whey blend group (-9.1 ± 8.7 pg mL^{-1}, $p = 0.033$). This investigation shows that 12 weeks supplementation with soy and whey protein does not decrease serum testosterone or inhibit lean body mass changes in subjects engaged in a resistance exercise program. In this study, testosterone also did not affected by protein supplementation.

Resistance exercise has no noteworthy effect on circadian secretion of salivary testosterone throughout the 16 waking hours. These results indicated that athletes can undertake resistance exercise in either the morning or afternoon with the knowledge that a similar testosterone response can be expected regardless of the time of day[29].

In contrast with this study regarding increased growth hormone and no differences on testosterone between groups, Kraemer *et al.*[14] examined the effects of amino acid supplementation on muscular performance and resting hormone concentrations during resistance training overreaching. Seventeen resistance-trained men were randomly assigned to either an amino acid (AA) or a placebo (P) group and underwent 4 weeks of total-body resistance training designed to induce a state of overreaching. Significant elevations in serum sex hormone-binding globulin were observed during overreaching in the P group from T2-T5. This response was abolished in the AA group. Significant reductions in total testosterone were observed in

the P group at T4 compared with T1 and the total testosterone values were higher for the AA group than for the P group from T2-T4. Serum 22-kd growth hormone concentrations were elevated at T2-T5 in P group only. No differences were observed in resting cortisol and insulin-like growth factor 1. Haemoglobin concentrations were significantly reduced at T2-T5 in the P group.

In contrast also, Rankin *et al.*[24] demonstrated the effect of post-exercise supplement (milk) consumption on adaptations to resistance training and the results were: Resting total and free testosterone concentrations decreased from baseline values in all subjects (16.7, 11%, respectively, $p<0.05$). Significant changes in fasting IGF-1, cortisol and REE across training were not observed for either group.

Also, McCall *et al.*[15] demonstrated acute and chronic hormonal responses to resistance training that were evaluated in 11 college men who completed 12 weeks (33 sessions) of high volume resistance training. No differences in resting concentrations of growth hormone (GH), insulin-like growth factor-I, testosterone or sex hormone-binding globulin occurred from pre- and post-training in the trained vs. non trained control group. Findings showed that resistance training had no effect on resting serum hormone concentrations, whereas similar acute exercise responses occurred between the 10th and 20th training sessions which was in contradiction with the results of the current study as GH and testosterone showed significant difference pre and post exercise training program.

Another contrasted study, Arazi *et al.*[30] the results of this study demonstrated a significant increase in blood testosterone from PRE in both WP and PL groups. However, blood testosterone comparisons showed that subjects in WP had a significantly greater increase in blood testosterone compared to the PL group.

Kraemer *et al.*[21] also reported no differences in the training volume or intensity in experienced resistance-trained men during several days of protein supplementation. However, they suggested that supplementation for a longer period of time may have resulted in more favourable outcomes.

Exercise paradigms and whey protein are designed based on the assumption (not necessarily evidence-based mechanisms) that GH facilitates anabolic processes that lead to skeletal muscle protein accretion and hypertrophy, while testosterone was not affected by whey protein.

CONCLUSION

It is concluded that whey protein supplementation combined with resistance exercise in a period of 6 weeks can have a positive impact on muscular power, muscle strength and muscle protein anabolism in normal subjects. Additionally, the strategic consumption of a daily whey protein supplementation (1.2 g kg^{-1}/day) represents a simple but effective strategy that enhances performance during resistance training.

The results of the current study may encourage another research to determine the effect of whey protein on muscle cross sectional area using ultrasonography or any other modality.

SIGNIFICANCE STATEMENT

This study discovers the importance of resistance exercise and proteins' workouts which are ideal for building explosive speed that is beneficial for muscle performance. These types of programs train the muscles in a very particular way in order to create quick and powerful movements that take little reaction time to conjure. Workouts that use these types of exercises and proteins build strong and fast athletes because of the way they affect anabolic hormones, stretch and then contract the muscles. This study will help the researchers to discover the critical areas of the use of whey protein in sport injuries who aim through treatment, to protect muscle damage and earlier recovery to improve the quality of athletic performance requirements that many researchers were not able to explore. Thus, a new theory on whey protein combined with resistance exercises may be reached.

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