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Endocrine responses following exhaustive strength exercise with and without the use of protein and protein-carbohydrate supplements

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ABSTRACT: The aim of this study was to determine the effect of carbohydrate-protein supplementation with whey protein (CHO-PROw) after resistance training, and casein protein (PROc) before bedtime on the concentration of growth hormone (GH), insulin (I) and insulin-like growth factor (IGF-1), as well as serum creatine kinase (CK) activity. Twelve strength trained male subjects (age: 25.8 ± 4.7 years; training experience 6.1 ± 0.79 years; body mass 75.9 ± 2.7 kg; body height 171.8 ± 13.3 cm) were recruited for the study. They were randomly divided into an experimental group (group E, n = 6) and a control group (group C, n = 6). All study participants completed full barbell squats with a constant external load of 90% one-repetition maximum (1RM) and a volume of 12 sets. In each set three repetitions were performed with 3 min rest periods after each set. Immediately after the exercise protocol, the subjects from the experimental group received a carbohydrate-protein complex (CHO-PROw) with a dose of 0.5 g/kg of body mass, while before bedtime they ingested a protein supplement (PROc) consisting of 90% casein protein with a dose of 0.3 g/kg of body weight The results indicate that a ignificant increase in GH concentration occurred in the experimental group between the pre-exercise level and after 24 h of recovery ($p<0.01$), as well as between 1 h and 24 hours of recovery ($p<0.01$). Significantly higher levels of GH were also found between the control group and the experimental group 24 hours after exercise ($p<0.01$). The results showed significantly higher levels of IGF-1 in the experimental than in the control group after 24 hours of recovery ($p<0.05$). In the case of insulin, no significant differences were observed when comparing levels before exercise, after exercise, after 1 hour of recovery and after 24 hours of recovery. The CHO-PROw and the PROc supplements did not reduce post-exercise muscle membrane damage as evidenced by serum CK activity. The intake of these supplements after high-intensity resistance exercise caused an increase in GH and IGF-1 concentration, which could stimulate muscle hypertrophy and inhibit proteolysis.

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INTRODUCTION

Strength training is one of the strongest stress factors for the athlete's body and it significantly impacts changes in the concentration of various metabolites circulating in the blood, which is confirmed by numerous studies [1, 2]. Scientific reports indicate that among the many elements of strength training, high volume of work and overall training load are the main factors influencing post-exercise increase in the secretion of anabolic hormones such as growth hormone (GH) or insulin (I), which stimulate muscle protein synthesis [3, 4]. GH has a significant effect on the rate of protein synthesis and the type of substrates metabolized during and immediately after exercise [1]. This hormone also stimulates the activation and proliferation of satellite cells which facilitate myofibrillar hypertrophy [5]. Growth hormone has an anabolic effect on skeletal muscles, stimulates the synthesis of proteins, and facilitates the transport of amino acids into skeletal muscles, thus affecting hypertrophy of both type I and type II muscle fibres [6, 7]. Insulin is one of the most potent anabolic hormones [8]. It exerts an anabolic effect by the increase in the collection of amino acids in muscle cells, and stimulation of protein synthesis, indirectly inhibiting catabolic reactions occurring in the body. In addition to resistance training, other factors, such as diet and supplementation also have a significant impact on post-exercise concentration of selected hormones [9, 10, 11]. The energetic state of the organism and adequate supply of protein in the diet also affect the concentration of insulin-like growth factor (IGF-1) [12, 13]. The anabolic effect of IGF-1 is based on the stimulation of amino acid uptake by muscles, and this factor has a significant effect on the process of transcription and translation of mRNA, protein and chondromucoprotein synthesis, inhibition of proteolysis and increase in the number of cell nuclei. IGF-1 also stimulates repair processes in the body, activates glycolysis, inhibits lipolysis, stimulates the immune system, and inhibits cell apoptosis. Serum IGF-1 elevations are induced by strength training [14], but some studies suggest that this occurs only when the resting concentrations are low [15]. Maintaining the momentum of muscle protein synthesis is related to the turnover and metabolism of protein resources in the body, which greatly affects the anabolic processes [16, 17]. The recommended protein intake for athletes is 1.5-2 g/kg body mass (BM) and in specific cases even more than 3.0 g/kg BM [18, 19, 20, 21]. Adequate intake of protein, as well as the time and type of dietary supplements used, may have a direct impact on endocrine responses to resistance training and muscle hypertrophy [10, 11, 20, 21, 22, 23]. Some researchers indicate the significant role of carbohydrate-protein (CHO-PRO) supplementation and the intake of various fractions of proteins or free amino acids before and after exercise in the process of muscle hypertrophy [20, 24, 25]. Other researchers suggest a significant effect of the above-mentioned supplements ingested after exercise and before bedtime [11]. In light of the latest scientific research whey protein and casein are the most significant and most interacting protein fractions contained in supplements [10, 17, 25]. They exhibit varying effects on muscle recovery, particularly as a consequence of strength training [26]. Whey protein is most efficient in rapid delivery of amino acids into the blood stream. It is characterized by an optimal ratio of essential amino acids (EAA) with an exceptionally high share of branched chain amino acids (BCAAs) and glutamine [20, 27]. In addition, whey protein isolates are characterized by the highest biological value, but at the same time, due to the rapid release kinetics, their activity is brief when compared to all the proteins supplied with food [25, 27]. In contrast to whey protein, supplements containing a predominance of casein protein, due to their slower rate of digestion and release kinetics allow for prolonged supply of amino acids for skeletal muscles [20, 22, 25]. Numerous studies have confirmed that the use of protein dietary supplements before and after exercise increases sports performance [23, 28], affects muscle hypertrophy [19, 21] and muscular power [18, 19, 28], and increases lean body mass [18, 19, 29]. Important supplements used immediately after and during the first 2 hours of recovery include carbohydrate-protein products that also affect the concentration of GH and I [8]. In a study by Baty et al. [30] subjects who took CHO-PRO supplements 30 minutes before resistance training showed a reduction in creatine kinase activity (CK), a marker of muscle damage during the 24 h of recovery [31]. On the other hand, the study by Coombes and McNaughton [32] indicated that the use of BCAA supplementation affects the reduction of post-exercise plasma CK activity, and inhibits muscle protein degradation.

The most dynamic anabolic processes in skeletal muscle occur during elevated GH concentrations, which occur daily during the first 1-2 hours of sleep (deep sleep phase). During this period there is a significant recovery and growth of tissues, including muscle tissues [7, 13, 14]. Substrates consumed in both the circadian cycle and the period before and up to 90 minutes after the completion of resistance training have a significant impact on this process [7, 13, 17, 20, 21, 23]. The anabolic-catabolic balance is related to the concentration of specific hormones and growth factors (IGF-1), and to the availability of an adequate set of amino acids essential for various types of protein synthesis in order to rebuild damaged muscle and tendon tissues [11]. To use, and even enhance, the effect of GH night interaction it is necessary to maintain the optimum concentration of particularly important essential amino acids (EAA) in the blood during sleep, which in practice results in the need for supplementation in the evening or just before bedtime. Supplementation of casein protein fraction (PROc) allows for stable, long-running increase in the concentration of EAA in the blood, lasting up to 6 hours after its intake [25]. It seems that casein protein is of particular significance in the long-term process of reconstruction of damaged muscle cells, which is important during the night. Unfortunately, in the current literature there are no publications that confirm this pattern. To our knowledge, there are limited data available regarding the influence of casein protein supplementation on post-exercise endocrine responses.

Objective of the study

The primary aim of this study was to determine the effect of carbohydrate-protein supplementation with whey protein (CHO-PROw) used immediately after resistance exercise and casein protein (PROc) taken at bedtime on post-exercise and recovery concentration of growth hormone (GH), insulin (I) and insulin-like growth factor (IGF-1) in blood serum, in strength trained male subjects. Additionally the effects of CHO-PROw and PROc supplementation on creatine kinase (CK) activity during post-exercise recovery were evaluated.

MATERIALS AND METHODS

Subjects

This study involved a total of 12 subjects with 6.1 ± 0.79 years of experience in strength training. The age of the subjects was 25.8 ± 4.7 years, body mass 75.9 ± 2.7 kg and body height 171.8 ± 13.3 cm. The subjects were randomly divided into an experimental group (group E, n = 6) and a control group (group C, n = 6).

Testing protocol

The exercise protocol included full barbell squats with a constant external load of 90% 1RM and a volume of 12 sets. In each set 3 repetitions were performed. The duration of rest periods between sets was 3 min. The training loads were the same for both groups, yet 10 min after the cessation of the exercise protocol, subjects from the experimental group received CHO-PROw consisting of 0.5 g of nutrient per kilogram of body mass dissolved in 500 ml of water. The carbohydrate-protein drink with a high glycaemic index included short-chain and medium-chain peptides and consisted of 70% carbohydrate and 30% whey protein. Before bedtime, subjects from the experimental group ingested a protein supplement (PROc) dissolved in 500 ml of water, comprising of 90% casein protein with a dose of 0.3 g/kg body weight. The control group received still water at the same time as the experimental group ingested the provided supplements. None of the subjects took medications, additional supplements or other ergogenic substances during and six weeks prior to the experiment. During the week preceding the experiment, all subjects were placed on a mixed isocaloric diet (2934 ± 256 kcal/d) which included 53% carbohydrates, 22% protein and 25% fat. In addition, during the week before the experiment, all participants refrained from alcohol, coffee and tea, and performed 1 light resistance training session 3 days prior to testing. The study protocol was approved by the Bioethics Committee for Scientific Research, at the Academy of Physical Education in Katowice, Poland.

Evaluations

Before the investigation maximal strength of the participants in the barbell squat was determined on the basis of a 1-repetition maximal test conducted six days before the beginning of the experiment. The first 3 sets were used as a specific warm-up, and these were preceded by 15 min of general warm-up. Knee wraps were allowed and 3 spotters were present at all times during the testing protocol and warm-up sets.

Procedure of squat strength test

30% 1RM – 6 reps.
50% 1RM – 5 reps.
70% 1RM – 3 reps.
80% 1RM – 3 reps.
90% 1RM – 1 rep.
100% 1RM

Biochemical analysis

During the experiment venous blood samples were collected from the antecubital vein, in each case 10 ml of venous blood to determine pre- and post-exercise biochemical values of the analysed variables (GH, IGF-1, insulin) at rest, 3 minutes after the cessation of the last set of squats, and after 60 min and 24 h of recovery. The following biochemical assays were performed for the evaluation of hormone concentrations and IGF-1.

GH evaluation – DSL-1900 radioimmunoassay test of serum
IGF-1 evaluation – DSL-2800 test of serum
Insulin evaluation – DSL-2100 radioimmunoassay test of serum
CK activity evaluation – Analco GBG using a spectrophotometer.

Statistical analysis

Means and standard deviations as descriptive statistics were calculated for all measured variables. All variables were normally distributed as suggested by the Shapiro–Wilk test results ($p > 0.05$). So, to identify a significant number of tests by time interactions, (number of tests by time) analyses of variance with repeated measures were used for each dependent variable. When a significant interaction was found, Tukey's post hoc analysis was performed for pairwise comparisons [33]. The statistical significance was set at $p < 0.05$.

RESULTS

Table 1 contains the mean values, standard deviations and statistically significant differences in GH and IGF-1 of subjects in both groups participating in the four series of examinations (before exercise, after exercise, after 1 h of recovery and after 24 hours of recovery). Table 2 shows insulin and CK values recorded before exercise, after exercise, after 1 h of recovery and after 24 hours of recovery in the control and experimental groups and the statistical differences between them. Analysis of variance with repeated measures shows significant differences in the case of growth hormone, IGF-1 and CK. In the next stage of data analysis, the post-hoc Tukey's test was applied.

The results indicate that a significant increase in GH concentration (Table 1) occurred in the experimental group between the pre-exercise level and after 24 h of recovery (451.7%; $p<0.01$), as well as between 1 h and 24 hours of recovery (342.7%; $p<0.01$). Significantly higher levels of GH were also found between the control group and the experimental group 24 hours after exercise (257.6%;

TABLE 1. Changes in the concentration of growth hormone and IGF-1 in the experimental (group E, n=6) and the control group (group C, n=6) recorded during the experiment.

	GH [ng/ml]		IGF-1 [ng/ml]	
	group C	group E	group C	group E
Before exercise	0.199 ± 171	0.199 ± 0.177**	649.04 ± 125.02	657.96 ± 125.71
After exercise	1.141 ± 1.132	0.807 ± 0.591	633.75 ± 116.21	680.29 ± 289.58
After 1 h of recovery	0.293 ± 0.237	0.248 ± 0.167^ ^	557.76 ± 179.74	552.37 ± 219.89
After 24 h of recovery	0.307 ± 0.218##	1.098 ± 0.512^ ^ ** ##	586.65 ± 127.35#	670.64 ± 225.31#

* # ^ $p<0.05$, ** ## ^ ^ $p<0.01$ – statistically significant differences.

TABLE 2. Changes in insulin concentration and CK activity in the experimental (group E, n=6) and control group (group C, n=6) recorded during experiment.

	Insulin [mIU/l]		CK [U/l]	
	group C	**group E**	**group C**	**group E**
Before exercise	18.71 ± 7.241	19.82 ± 17.646	305.37 ± 117.06##	228.93 ± 78.49**
After exercise	14.53 ± 3.483	14.42 ± 4.7	369.57 ± 144.56	314.08 ± 118.85
After 1 h of recovery	11.33 ± 1.653	14.37 ± 6.21	370.06 ± 120.82	366.07 ± 91.53
After 24 h of recovery	10.01 ± 4.17	11.34 ± 0.65	560.48 ± 235.17##	562.66 ± 229.25**

* # ^ $p<0.05$, ** ## ^ ^ $p<0.01$ – statistically significant differences.

$p<0.01$). The post-hoc Tukey's test showed significantly higher levels of IGF-1 in the experimental than in the control group after 24 hours of recovery (14%; $p<0.05$).

In the case of insulin, no significant differences were observed when comparing levels before exercise, after exercise, after 1 hour of recovery and after 24 hours of recovery.

DISCUSSION

There are numerous interactions between the type and intensity of exercise, diet applied, supplement intake, and endocrine responses among athletes. Post-training hormone secretion is particularly intensified as a result of strength training with maximum external loads in exercises involving large muscle groups [14], as in our study. Additionally, the increase in daily energy supply in a diet significantly affects the changes in the concentration of GH, I, and IGF-1 [11, 21, 34]. Although many studies have demonstrated a relationship between supplementation and post-exercise changes in the concentrations of various hormones, our results showed no significant changes in the concentrations of GH, I and IGF-1 after one hour of recovery in group E which received the CHO-PROw supplement immediately after the exercise protocol, compared to group C. Variability in metabolic reactions under conditions of similar availability of energy substrates may be the result of secretion changes and tissue sensitivity to the action of hormones released. Our results are contrary to reports which clearly show that the use of CHO-PRO supplements before, during and after training significantly affects post-exercise serum hormone concentrations [9, 30, 35]. Dietary supplements used by athletes are supposed to reduce exercise-induced degradation of muscle cells and support protein synthesis; however, the obtained results showed no effect of CHO-PROw intake on serum CK after 1 hour of recovery. Negative correlations between the applied supplementation and the concentration of CK were found by Samadi et al. [36]. In our study we analysed not only the influence of CHO-PROw, but also PROc supplementation consumed on the training day, just before bedtime. Despite the use of supplementation, after 24 hours of recovery, there was no statistically significant decrease in CK activity, which confirms previous reports in which there was no effect of CHO-PRO supplementation on changes in serum CK after a day of recovery from resistance exercise [30, 35, 37]. The increase in CK activity after 24 hours of recovery indicates severe damage to muscle cell membranes, and leakage of CK into the bloodstream during exercise and after its completion. Most likely, this condition was caused by both mechanical damage of muscle cell membranes resulting from a substantial force of muscle contraction and oxidative damage to cell membranes [38]. Our results showed no exercise-induced increase in the concentration of insulin (directly after exercise and after 1 h of recovery), despite the consumption of a CHO-PROw supplement in the amount of 0.5 g/kg of body mass immediately after exercise. Despite the lack of accurate data on the direct effect of insulin on the regulation of carbohydrate and protein changes, most researchers indicate that insulin promotes the synthesis of muscle proteins, inhibits their degradation, and accelerates the transport of amino acids into muscle cells [39], thereby significantly impacting the process of muscle hypertrophy. In addition, high CK levels after an hour of recovery may indicate significant oxidative damage to the membranes of muscle cells caused by an increase in the production of free radicals [38]. The influence of CHO-PRO intake on insulin concentration changes after resistance exercise is not the same as after its consumption at rest. After the completion of a resistance training session, it appears that insulin has a negligible effect on the support of protein synthesis [40, 41], despite the increase in anabolic signalling in muscle [42]. In order to confirm this phenomenon, we analysed the results of experiments which showed no increase in insulin concentration immediately after and one hour after the end of exercise and intake of a CHO-PRO supplement. Other researchers suggest that only CHO-PRO supplementation before, during and after exercise affects changes in insulin concentration [30].

Intense exercise stimulates the pituitary to increase secretion of GH. GH plays an important role in the anabolic reactions of athletes' bodies through facilitating the transport of amino acids into skeletal muscle, induction of transcription and translation of mRNA. Increases in the concentration of GH are of particular importance in the process of reconstruction of damaged muscle cells, whereas additional supplementation with protein or amino acids accelerates intracellular protein synthesis, causing, among other things, an increase in the amount of muscle proteins, which in turn affects adaptation to resistance training [20, 25]. Our study showed no significant increase in exercise-induced GH concentration after 12 sets of squats with a barbell loaded at 90% 1RM. Analysis of changes in GH show that post-exercise consumption of CHO-PROw in group E did not significantly influence the changes in the concentration of this hormone during the first hour of recovery. The studies also showed an increase in resting GH concentration after a period of 24 hours of recovery. This was observed after the intake of CHO-PROw directly after exercise and PROc before bedtime. The observed increase in GH was statistically significant, both in relation to rest values and after 1 h of recovery. The GH concentration in group E after 24 hours of recovery was also significantly higher compared to group C. The increase in GH concentration after 24 h probably occurred as a result of PROc supplementation before bedtime. Supplementation with casein protein has a significant impact on muscular strength, by stimulation of recovery and growth of muscle fibres [26] as well as by an increase in resting GH values, which has been confirmed in our study. This may be related to the fact that the supply of amino acids and proteins affects the growth of GH. Nevertheless, it is possible that the impact on change in resting GH values resulted from a combination of post-workout supplementation with CHO-PROw and PROc before bedtime. Pre-exercise supplementation with CHO-PRO used by Kraemer et al. [24], as well as consuming a traditional meal containing 61% carbohydrate, 33% protein and 6% fats after exercise, in the studies by Bloomer et al. [43], together with post-exercise supplementation with an isocaloric drink in Baty's et al. work [30], had an impact on changes in the concentrations of numerous hormones, not only during training but also during daily activities, which was confirmed in our study. In addition, the low morning levels of glucose and low-carbohydrate diet stimulate the secretion of GH [10, 13, 35, 44], which highly justifies the use of PROc supplementation before bedtime. The time effect of supplements on changes in the concentration of selected hormones varies, and published scientific papers show conflicting results. Chandler et al. [35] reported that the use of CHO-PRO supplements two hours before and immediately after strength training causes elevated levels of GH during 8-hour post-exercise recovery. However, in our study, during 1 h of recovery no significant changes in the concentrations of GH or any other analysed variables versus rest were observed. In our study the supplementation with CHO-PROw after exercise and PROc before bedtime shows significant changes in resting concentration of IGF-1. The results presented in the literature confirm the occurrence of adaptive changes depending on the value of exercise-induced IGF-1 concentration [44, 45]. The increase in rest IGF-1 concentration significantly influences the adaptive processes, enhances the uptake of amino acids, accelerates the processes of transcription and translation of mRNA, affects the inhibition of proteolysis, increases the number of nuclei in muscle cells and stimulates muscle hypertrophy [46]. Our study showed no changes in IGF-1 concentration after the intake of CHO-PROw and within one hour of recovery, which is consistent with the results of Chandler et al. [35]. The concentration of IGF-1 drops to a quiescent level for a period of typically 60 to 90 minutes after the end of exercise [47]. This drop may be related to the use of the growth factor in the cell protection processes and to the influence on satellite cell stimulation [48] and also to proteolysis of IGF-BP3 complex and separation of IGF-1, which is bound to the cellular receptor in muscle. It seems that very intense exercise, where the energy demand far exceeds the ability to replenish cellular sources, leads to an energy deficit, which may result in lower plasma IGF-1, but at the same time protects against an increase in post-exercise hypoglycaemia [49]. Most researchers indicate that pre- and post-exercise supplementation does not cause significant changes in IGF-1 concentration [50]. Our study showed an increase in resting IGF-1 value after 24 hours due to the use of dietary CHO-PROw immediately after exercise and PROc before bedtime. It can therefore be speculated that the protein casein fraction reduces the need for the use of IGF-1 in order to secure the energy in muscle cells. Changes in resting concentrations of IGF-1 may also occur after a period of recovery longer than the circadian period [24]. The study indicates that self-consumption of CHO-PROw after exercise and PROc before bedtime, despite its proven impact on the process of protein resynthesis, may not be a sufficient stimulus to reduce the catabolism of muscle fibres confirmed by changes in CK activity. Therefore, it seems that the post-training rebuilding process of fibres and muscle cell membranes (damaged by exercise and oxidative stress) is dependent not only on supplements but also on a properly balanced diet [10, 20, 25, 29].

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