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Effects of Carbohydrate-Protein Intake During Exercise on Hormonal Changes and Muscular Strength after 12-Week Resistance Training

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ABSTRACT

The purpose of the current study was to investigate the effects of carbohydrate, protein and combination intake during exercise on hormonal responses, adaptations and muscle strength following 12 weeks resistance training. Subjects of this study included 40 untrained young men (age: 22.21± 1.77, body mass: 76.03±7.22) who performed 12 weeks resistance training twice a week with ~ 75% 1 repetition maximum (1RM) intensity while consuming 750 ml 6% carbohydrate beverage (CHO), 0.2 whey protein g/kg (PRO), combined 750 ml 6% carbohydrate beverage and 0.2 whey protein g/kg (CHO+PRO) and placebo (PLA). The Results indicate that exercise-induced cortisol responses inhibit resistance in CHO and CHO+PRO ($p<0.05$), while stimulating more insulin than in PRO and PLA ($p<0.05$). Cortisol responses were repeated in CHO and CHO+PRO at week 12 but reduced in PLA and PRO. All groups displayed reduced pre-exercise cortisol at week 12 compared to week 0 ($P<0.05$). Despite the increase of insulin responses in CHO and CHO+PRO at week 12, it remained constant in PLA and PRO. Post-exercise GH increased in all groups, No significant differences were observed among the groups. The data indicated that ingestion of carbohydrate and/or protein during resistance exercise improve hormonal milieu after exercise, but these improvements could not enhance muscular strength in 12 weeks resistance training.
KEYWORDS: Carbohydrate, Protein, Hormonal change, Muscular strength, Resistance training.

1. INTRODUCTION

A bout of resistance exercise stimulates acute changes in the hormonal response that mediate results of post exercise adaptations (Kraemer et al, 1998b; Kraemer et al, 2003). After a bout of resistance exercise, anabolic hormones such as testosterone, growth hormone and IGF-1 increase and improve net protein balance. In contrast insulin (INS) probably remains unchanged and CORT concentration that is a catabolic hormone increases (Kraemer et al, 2003). In support of these data, previous studies indicate that resistance exercise protocols designed to maximally stimulate the major muscle groups (i.e., whole-body; moderate volume, high intensity) often produce hypersecretion of cortisol (CORT) >500 nmol l^{-1} (Bird et al, 2005; Kraemer et al, 2005), while insulin (INS) levels remain unchanged or decrease (Roy et al, 1997). Thus attenuating exercise-induced CORT release may be an essential component for decreasing protein degradation and enhancing skeletal muscle hypertrophic response to resistance training (Goldberg, 1969).

One way that may influence hormonal response induced resistance exercise is nutritional intervention (Chandler et al, 1994; Kraemer et al, 1998b; Volek et al, 2004). Several investigations demonstrate that ingestion of carbohydrate (CHO), protein (PRO) or essential amino acids (EAA) around time of resistance exercise induce change in acute hormonal response and eventually affect result of resistance training (Bird et al, 2006; Chandler et al, 1994; Tarpenning et al, 2001; Volek et al, 1997). For example ingestion of a 6% CHO beverage during resistance exercise increase plasma INS concentration and attenuate CORT secretion (Tarpenning et al, 2001). Ingestion of protein or essential amino acids, also increase availability of external amino acids and enhanced uptake of amino acids by skeletal muscle and improve net protein balance (Biolo et al, 1997). Thus combined nutritive intervention increases insulin release and amino acids availability, as well as suppressing exercise-induced CORT secretion, can effective and aimed efficiency of resistance training. However a few studies compared ingestion of these supplements and investigated their effects according to the time of ingestion.

Recently, Bird et al demonstrated that ingestion of 6g EAA combined with %6 CHO solution during single bout of resistance exercise suppresses the exercise-induced CORT response and increases INS release (Bird et al, 2005). They concluded that the hormonal response to exercise can be influenced by ingestion of CHO, EAA and a combination of them during exercise toward more favorable for anabolism. At the same time, addition of 35 g of CHO to the 6 g of the mixture of EAAs and NEAAs had minimal effect and the response to a mixture of 6 g EAAs + 35 g CHO was actually less than the

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anabolic effect of 6 g EAA alone (Miller et al, 2003). In another study researchers compared ingestion of %6 CHO and 6g EAA in 12 weeks of resistance training and indicated that CHO+EAA ingestion enhanced muscle anabolism following resistance training to a greater extent than either CHO or EAA consumed independently (Bird et al, 2006). They explain that the synergistic effect of CHO + EAA ingestion maximizes the anabolic response presumably by attenuating the post-exercise rise in protein degradation. However, some studies show that amino acid uptake is different with either pre or post-exercise ingestion of amino acid (Tipton et al, 2001) versus whey protein (Tipton et al, 2007). Therefore, it can be concluded that results from studies of ingestion of crystalline amino acid may not be readily translatable to intact dietary proteins (Tang et al, 2007). Moreover, studies using whole proteins would likely be of greater relevance to athletes, many of whom regularly consume proteins supplement such as whey.

In this study, we chose whey protein since it is a high quality protein that is available for more athletes and is also rapidly digested, and thus should support muscle protein synthesis (MPS) and protein accretion and finally muscular strength with training (Burke et al, 2001; Chromiak et al, 2004; Kerksick et al, 2006). Our hypothesis was that CHO + PRO ingestion during an acute bout of resistance exercise will provide a best hormone milieu after resistance exercise, and when repeated, will result in greater gains in muscle strength following 12 weeks of resistance training.

2. METHODS AND MATERIALS

Subject: The subjects of this study were 40 young volunteered men with no regular exercise or resistance training prior to the start of the study. The subjects' physical characteristics are listed in table 1. Subjects completed a health history questionnaire and par-Q before the start of the study and gave written consent after being informed of the risk associated with the study. Subject with contraindications to exercise as outlined by the American college of sport medicine (ACSM, 2000) and/ or who had consumed any nutritional supplements anabolic steroids 6 months prior to the study were excluded from participation. All experimentations were approved by the Ethics in Research Committee of the Azad University (Iran). Subjects were randomly assigned to one of four groups: CHO group (n = 10), EAA group (n =10), combined CHO - EAA group (n =10), or placebo (PLA) group (n =10).

Anthropometrics and body composition testing: During the initial laboratory session (day 1), descriptive data including height, weight, and body composition were obtained during the initial session (session for resistance training equipment familiarization), five days prior to the first exercise session at wk 1 and 24 h after the final exercise session at the end of wk 12. Height was measured to the nearest 0.1 cm by using a stadiometer (seca, Italy) and weight was measured to the nearest 0.1 kg by using an electronic precision balance scale (Seca, Italy). Body composition was determined using a Body Space BF-350E bioelectrical impedance analyzer (BodySpace Corporation, Arlington Heights, South Korea). All sessions were performed between 4:00 and 6:00 PM to minimize the influence of diurnal variations on exercise performance and hormonal response [Reilly], Subjects were required to refrain from all strenuous activity, alcohol use, caffeine, and sexual activity and were notified to maintain normal nocturnal sleep habits (i.e., approximately 8 h/night) throughout the experimental timeline.

Muscular strength testing: Muscular strength was assessed Four days before the start of training in week 1 and 48 hours after finishing training in 12th week, using selected resistance exercises including leg press, leg curl, leg extension, calf raise, lat pull down, bench press, barbell bicep curl, and supine triceps extension by 1-RM test. The process of 1-RM test is the same as that executed by Bird et al. (Bird et al, 2005), including Warm-up consisting of one set of 5–10 repetitions at 40–60% of perceived maximum. Then Subjects rested for 1 min, performing light stretching. This was followed by 3–5 repetitions performed at 60–80% of perceived maximum. Thereafter, 3–4 subsequent attempts were made to determine the 1-RM, with the weight increased progressively until the subject failed at the given load. Three minutes of rest was allocated between the lifts. By definition, 1-RM is the maximum amount of weight that could be lifted one time through a full range of motion, using good form at a tempo of 2:0:2 (2 s eccentric; 2 s concentric).

Blood sampling: Blood sampling were obtained prior to exercise and immediately post-exercise period for analysis of glucose (GLU), insulin (INS), cortisol (CORT), Growth Hormone (GH), total testosterone (TT) and INS like Growth Factor -I (IGF-I) at weeks 1 and 12. After a 4-h fast, subjects sat quietly for a further 15-min period prior to blood collection to minimize hormonal fluctuations related to anticipatory responses Venous blood samples were obtained from the antecubital vein into 10 ml collection tubes. Blood samples were allowed to stand at room temperature for 20 min and then centrifuged for 10 min at 3000 rpm. The serum was then removed and frozen at _20 _C for later analysis.

Glucose was determined by an enzymatic spectrophotometric method (Dimension Xpand, Biomer Inc., Germany). INS, CORT, GH, total testosterone and IGF-1 concentrations were determined in duplicate and the average concentrations reported using commercially available ELISA kits (Diagnostics Systems Laboratories, Biomer Inc., Germany).

Resistance training protocol: A progressive resistance training principle was employed, with two times per week for 12 weeks and the load increased as necessary to maintain a training intensity at approximately 75% of each individual 1-RM (i.e., 8–10 repetitions per set), with subjects performing each set to failure. The resistance training protocol used for this investigation was that previously used by Bird et al. (Bird et al, 2006), which has been shown to influence hormonal concentrations. All sessions were supervised by the principal investigators and/or trained university students. The resistance training protocol consisted of a complete body workout, with a combination of machine equipment and free

weight exercises, including 4 exercises for lower body and 4 exercises for upper body in order: leg press, leg curl, and leg extension, and calf raise, lat pull down, bench press, barbell bicep curl, and supine triceps extension. The subject's should complete three sets of 8–10 repetitions at approximately 75% of their 1-RM. 1 minute of rest between each set and 2 minute between each exercise was allowed for recovery. These times of rest between sets and exercise induced influence plasma hormonal concentrations. The resistance exercise session lasted approximately 60 min (by a 6 min warm-up and 6 min warm-down period). All training sessions were performed between 16:00 and 18:00 h to minimize the influence of diurnal variations on hormone release. Subjects were informed that missing three training sessions or two consecutive training sessions resulted in disqualification from the study. Additionally, all aerobic exercises were conducted for only 1 h per week during the experimental period.

Nutritive intervention: In each of bout of resistance exercise, subjects consumed either a CHO (solution of 6% glucose, Quaker Oats, Inc., Chicago, IL, USA), a whey protein (0.2 g/kg of whey protein, Interactive Nutrition International Inc, Canada), a combined CHO + PRO supplement, or a PLA (aspartame, Quaker Oats, Inc.) dissolved in water at a fluid volume of 10 ml kg body mass^{-1} (an average of 750 ml of solution). The Total beverages ingested between each exercise were divided by 9 servings depending on body size. The whey protein composition observed in table 1 which had been shown to enhance muscle anabolism following resistance exercise (Chromiak et al, 2004; Kerksick et al, 2006).

Dietary records: For dietary control, used food record for 3 days pre, mid and post resistance training protocol to assess whether there were differences in energy intake and macronutrient composition between groups. Before beginning the protocol, subjects were instructed to consume their normal diet and record as accurately as possible everything they consumed during 3 days. Then each subject met with an expert Dietitian (National Nutrition Food Technology & Research Institute, Tehran, Iran) or maintained their normal diet throughout training period. All dietary records were analyzed by the same individual who had several years of experience of entering dietary records using the ESHA food processor software (version 2.5). Any unavailable information regarding the nutritional contents were entered into the database from manufacturer labels.

Statistical analysis: Data was analyzed utilizing standard descriptive statistics, paired *t* tests to compare within group differences between pre- and post exercise, one-way ANOVA to examine changes occurring with training for each group, and two-way ANOVA (4group × 2 time) with repeated measures to compare differences between the groups in dietary intakes, hormonal responses and strength changes. The source of significant differences was located using Tukey's HSD post hoc procedure. Regression analysis determined associations between selected variables. Analysis was performed using the Statistical Package for Social Sciences (SPSS v11.5). Significance was accepted when $P<0.05$. Values are expressed as mean (±SD).

3. RESULTS

Macronutrient intake: All nutritional data are presented relative to body weight in kilograms in table 2. There were no significant main effects for Group (p= 0.567) or Test (p= 0.423) or the Group × Test interaction (p=0.783) located for the average daily intake of total calories, carbohydrates, protein or fat during the course of 12 weeks resistance training and supplementation period (Table 2).

Body composition and anthropometric variables: There were no significant differences between groups at baseline (in week 0) for body mass, fat mass and fat free mass. All groups had significant increases in body mass and FFM in week 12, but no significant differences observed between groups. Although FM slightly decreased in all groups, but the differences were not significant.

Glucose and Hormonal responses: Hormonal responses at first and last bout of resistance exercise (in week 1 and 12) are presented in figure 1 to 5. No significant differences were observed between groups in pre exercise concentrations of GLU in wk 1 and 12. There were no significant Group × Test interaction for glucose concentrations (p=0.59); However, significant main effects for group (p=0.0005) and for Test were observed (p=0.037). The data indicate that GLU concentrations in CHO and CHO+PRO groups significantly increase after exercise at weeks 1 and 12 than PLA and PRO groups. But no significant changes were observed in this response after 12 weeks.

Pre exercise INS concentrations were similarly in all groups but, were higher for treatment groups after exercise at wks 1 and 12. There were no significant Group × Test interaction for INS concentrations (p=0.17); However, significant main effects for group (p=0.0005) and for Test were observed (p=0.33). The data indicate that INS responses in CHO and CHO+PRO groups significantly increase after 12 weeks resistance training than two other groups.

CORT significantly increases after exercise for PRO and PLA in wks1 and 12, but in CHO and CHO+PRO no changes were observed. Pre exercise CORT concentrations in all groups significantly reduced in week 12 compared to week 1. There were no significant Group × Test interaction for CORT concentrations (p=0.39); However, significant main effects for group (p=0.0005) and Test (p=0.02) were located. The data indicate that CORT concentrations in PRO and PLA groups significantly increase after exercise at weeks 1 and 12. While no significant increases were observed in CHO and CHO+PRO groups after exercise.

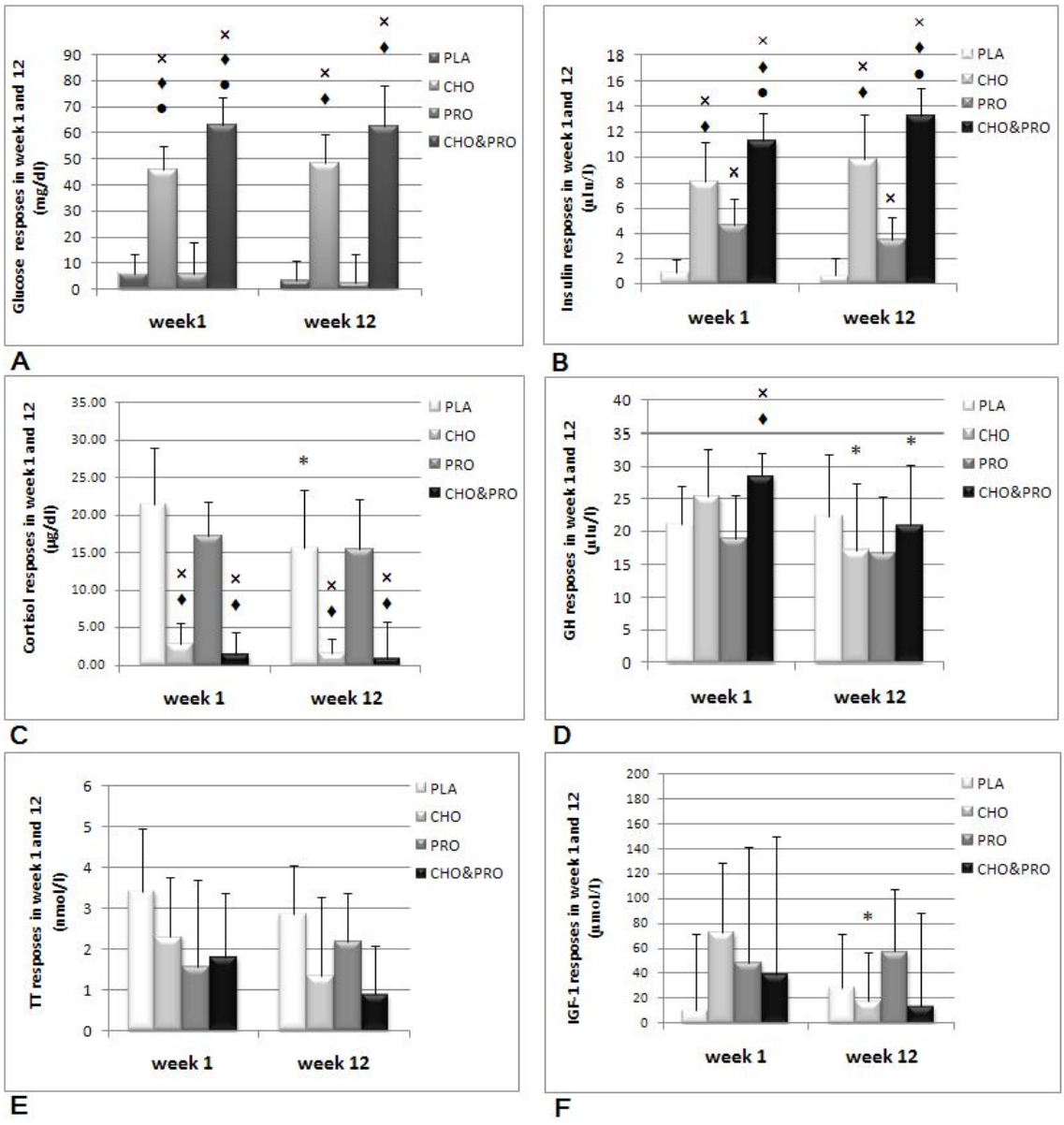

Figure1. A representation of the means (±SD) for **A**. Glucose responses at week 1 and 12 **B**. Insulin responses at week 1 and 12 **C**. Cortisol responses at week 1 and 12 **D**. GH responses at week 1 and 12 **E**. TT responses at week 1 and 12 **F**. IGF-1 responses at week 1 and 12. × Significantly different (P<0.05) from PLA; ♦ significantly different (P<0.05) from PRO; ●significantly different (P<0.05) from CHO; * significantly different (P<0.05) from pre training.

GH increased post exercise in all groups either in week 1 or 12. Also, rest concentrations of GH were significantly higher for all groups in week 12 compared to week 1. There were no significant Group × Test interaction for GH concentrations (p=0.31), In addition, no significant main effects for group or Test were located (p=0.099 and p=0.125, respectively). The data indicate that although GH responses in CHO and CHO+PRO groups decrease after exercise in week 12, but not significantly.

Rest concentrations of testosterone in all groups increased at week12 compared to 1. There were no significant Group × Test interaction (p=0.69) and main effect for group and Test for testosterone concentrations (p=0.19 and p=0.40, respectively). These data indicate that although testosterone concentrations increase in all groups significantly after exercise at weeks 1 and 12, no significant differences were observed between the groups.

There were no significant Group × Test interaction, Group and Test for IGF-1 concentrations (p=0.21, p=0.30 and p=0.20 respectively);

Muscular strength: Figure2 presents strength changes in leg press and bench press exercises for all four groups in weeks 1 and 12. There were no significant differences at baseline (at week 0) for 1-RM strength between groups (P<0.05),

pre- to post-training. After progressive resistance training 1-RM strength in bench press and leg press exercise significantly increases (in 6 and 12 weeks), but there were no significant differences between the groups. Although the treatment groups displayed slight increases in 1-RM strength in bench press and leg press, the differences were not significant.

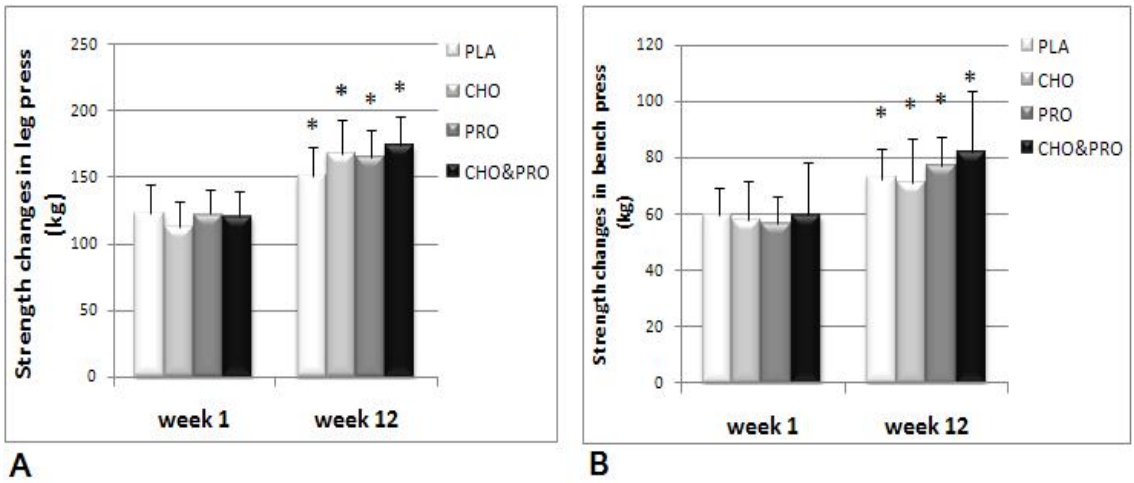

Figure2. A representation of the means (±SD) for **A**. maximal muscular strength (1RM) in leg press at week 1 and 12 **B**. maximal muscular strength (1RM) in bench press at week 1 and 12. *: significantly different (P<0.05) from pre training.

4. DISCUSSION

The results of this study indicate that consumption of CHO or PRO during a bout of resistance exercise affects the post exercise hormonal milieu. In addition these responses were repeated after all the resistance exercise bouts in 12 weeks of resistance training period. The hormonal milieu displayed by the treatment groups affects the results of a bout of resistance exercise and lowers the body muscular strength after 12 week of resistance training. Furthermore, such responses are augmented when the treatments are combined, with the CHO + PRO group exhibiting the greatest increases in INS, and the greatest relative gains in lowering muscular strength. This is while, PLA group shown significantly higher exercise-induced CORT concentration that is in contrast to CHO and CHO+PRO groups.

The experimental protocol and time of intake supplementations more closely resemble the study by Bird et al. who demonstrated that the ingestion of a 6g essential amino acid combined with ~675 ml of carbohydrate 6% during exercise induced greater stimulate INS after exercise (Bird et al, 2006). Findings of this study are in agreement with recent reports and indicating that the effect of ingestion of intact protein during exercise is similar to crystallizing essential amino acids (Bird et al, 2006). Furthermore, consumption of a liquid CHO or PRO beverage resulted in a substantial increase in the acute INS response to resistance exercise. However, responses are augmented when the supplement are combined (CHO + PRO). This is in contrast to the PLA group that displayed non-significant changes. These findings are in agreement with the recent reports that indicate addition of EAA or protein with CHO enhances the acute INS response (Bird et al, 2005; Koopman et al, 2005; Williams et al, 2001). In the current study although CHO ingestion induced increased post-exercise INS concentrations, probably these were not associated with an increase in muscle fractional synthetic rate (Roy et al, 1997), because the previous investigations showed that the increase rate of amino acid uptake by muscles is necessary for hypertrophic response of skeletal muscle (Tipton et al, 2001), although plasma amino acid concentration and rate of uptake of them by muscles were not determined in the current investigation. However, the results of this investigation showed that after 12 weeks of resistance training, the same responses in PRO and PLA groups were repeated, while CHO and CHO + PRO groups experienced significant increase in INS response in comparison with the response in week 1. This finding appears that the adaptive response of INS following resistance training may be influenced by CHO consumption (Tarpenning et al, 2001). However, the significance of such adaptations is yet to be elucidated.

In current investigation consumption of carbohydrate in CHO and CHO + PRO groups attenuated elevation of CORT concentration induced resistance exercise, in contrast to PRO and PLA groups that CORT concentrations significantly increased after exercise in week 1. This response was repeated after 12 weeks of resistance training in CHO and CHO + PRO, while responses in PRO and PLA groups were decreased. These adaptations that are happened in PRO and PLA groups are in agreement with our previous information that indicates CORT response attenuated after resistance training (Kraemer et al, 2005). Furthermore, the results of the current study has shown that all groups displayed a significant reduction in Pre exercise CORT concentrations at week 12 compared to week 0, that is an adaptation to resistance training and consistent with previous reports (Kraemer, 2003; Staron et al, 1994; , Tarpenning et al, 2001). Kraemer suggests that an accumulated reduction in CORT concentrations results in reduced total tissue exposure to CORT, thereby influencing the

subsequent phase of recovery by modulating anabolic and catabolic processes (Kraemer et al, 1998b). Recent investigations demonstrate that protein degradation 48-h post-exercise significantly correlates with CORT area under the curve concentrations and changes in muscle fiber cross-sectional area were significantly correlated with the changes in CORT response (Bird et al, 2005). Therefore, chronic reductions in the exercise-induced CORT response associated with CHO and PRO ingestion can positively impact the skeletal muscle hypertrophic adaptation to resistance training via reductions in hormone-mediated protein degradation (Borsheim et al, 2004; Tarpenning et al, 2001; Thyfault et al, 2004).
In the current study, post-exercise growth hormone concentrations were significantly greater than those of pre-exercise in all groups. Furthermore resting (pre-exercise) GH concentrations were significantly higher in week 12 than week 1, an adaptation to resistance training that is consistent with previous reports. These findings indicate that ingestion of carbohydrate or/and protein during exercise dose not influence the responses of GH to resistance exercise. This response happens despite the dramatic increase in the levels of glucose after exercise in CHO and CHO + PRO. This is in disagreement with previous reports indicating that high levels of glucose inhibit the exercise-induced increase in growth hormone (Goldberg, 1969). Addition of glucose levels, several circulating substrates and hormones can affect the growth hormone-secretory pulses, including lactate and H^+, branched chain amino acids (BCAA), fatty acids, catecholamines. For example, lactate and H^+ have been shown to play a role in exercise induced stimulation of growth hormone (Kraemer et al, 1998b). In the current study, although levels of these substrates were not monitored, it is likely that GH response affects with other regulators such as lactate or BCAA concentrations. The results of this investigation has shown that GH responses in CHO and CHO + PRO groups significantly decrease in week 12 compared to week 1, while PLA and PRO showed the same responses after 12 weeks of resistance training. This reduction in GH concentration is accompanied by a decrease in glucose responses after 12 weeks of resistance exercise in CHO and CHO + PRO groups. Thus at least a part of this reduction in GH concentration is attributed to fewer levels of glucose after exercise in week 12 (Goldberg, 1969).
The progressive resistance training protocol used in the current investigation resulted in significant increase in 1-RM strength for the leg press and bench press in all groups in week 12. This is in consistent with previous reports of strength gains following resistance training of similar durations (Bird et al, 2006; Chromiak et al, 2004; Kraemer & Ratamess 2005). This increase is greatest in CHO + PRO group and is minimal in PLA group, but differences between groups in leg press and bench press 1-RM strength were not significant. This finding indicates that although consumption of carbohydrate, protein or combined CHO + PRO during resistance exercise could improve muscular strength gain, but this improvement is not remarkable in 12 weeks of resistance training. Pervious works displayed that a training program with frequency of 2 day $week^{-1}$ in untrained individuals, following the progressive overload principle, is effective in promoting muscular adaptations in novice trainers. Exercise-induced changes in strength expression appear to be mediated by the complex interplay between neural-induced adaptations and the hypertrophic response of skeletal muscle, with early phase (first 6–8 weeks) strength gains primarily due to neural adaptations, while hypertrophic responses begin to occur at the later phases (12–26 weeks) of training (Volek, 2004). Therefore, training adaptation in early stage of training program is the same for all groups. This increases the possibility that short period of resistance training is most important reason of similarity of strength gain in PLA and treatment groups. Thus, it appears that use of longer period of time (i.e., 20 week) could display difference between the groups (Bird et al, 2006).

At least a part of disparity observed in strength gain between PLA and treatment groups is attributed to differences in the degree of hypertrophy over the 12-week training period. Although muscular hypertrophy in the current study is not assessed directly, but differences in fat free mass gain between groups indicate that the synergistic effect of combined CHO + PRO ingestion slightly is greater than individual effect of them and placebo. Therefore consumption of CHO + PRO in longer period of time may result in greater hypertrophy and eventually in increased strength gain.

In summary, the major findings of the present study indicate that ingestion of carbohydrate and/or protein during resistance exercise improve hormonal milieu after exercise, but these improvements could not enhanced muscular strength in period of 12 week resistance training.

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