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The Effect of Strength Training and Consumption of Glutamine Supplement on GH/IGF1 Axis

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***Abstract*—**Physical activity and diet are factors that influence the body's structure. The purpose of this study was to compare the effects of four weeks of resistance training, and glutamine supplement consumption on growth hormone (GH), and Insulin-like growth factor 1 (IGF-1) Axis. 40 amateur male bodybuilders, participated in this study. They were randomly divided into four equal groups, Resistance (R), Glutamine (G), Resistance with Glutamine (RG), and Control (C). The R group was assigned to a four week resistance training program, three times/week, three sets of 10 exercises with 6-10 repetitions, at the 80-95% 1RM (One Repetition Maximum), with 120 seconds rest between sets), G group is consuming l-glutamine (0.1 $g/kg^{-1}/day^{-1}$), RG group resistance training with consuming L-glutamine, and C group continued their normal lifestyle without exercise training. GH, IGF1, IGFBP-III plasma levels were measured before and after the protocol. One-way ANOVA indicated significant change in GH, IGF, and IGFBP-III between the four groups, and the Tukey test demonstrated significant increase in GH, IGF1, IGFBP-III plasma levels in R, and RG group. Based upon these findings, we concluded that resistance training at 80-95% 1RM intensity, and resistance training along with oral glutamine shows significantly increase secretion of GH, IGF-1, and IGFBP-III in amateur males, but the addition of oral glutamine to the exercise program did not show significant difference in GH, IGF-1, and IGFBP-III.

***Keywords*—**Strength, glutamine, growth hormone, insulin-like growth factor 1.

I. Introduction

REGULAR resistance training could cause hypertrophy in skeletal muscle [1]. Exercise as a potent physiological stimulus elevates GH and IGF-1 secretion [2]. IGF-1 is the major mediator of the anabolic and growth-promoting effects of GH [3]. The competing processes of cellular proliferation, cellular differentiation, and the increased protein synthesis required for muscle repair or hypertrophic adaptation is regulated by distinct roles of IGF-1 isoforms [4]. The Insulin-like growth factor-binding protein III (IGFBP-III) as a carrier protein for IGF-1, and had a role in the growth modulators [5].

Glutamine is a key polypeptide in a wide variety of synthetic and metabolic processes, and has been suggested to become conditionally essential during metabolic stress [6]. L-glutamine consumption had a positive effect on fatigue at the recovery stage after maximal intensity exercise [7]. Skeletal muscle is the major site of glutamine production and consequently, the activity of skeletal muscle may directly influence those tissues that utilize it [8].There is evidence from studies in vitro that intramuscular glutamine has a role in the regulation of muscle protein synthesis and breakdown [8]. Strenuous physical exercise as well as exhaustive training programs, and other physical stress conditions leading to glutamine depletion generally occur in conditions of metabolic acidosis, and could be considered as an over training marker [9]. Also, it has been shown muscle glutamine formation was suppressed in severely hyper catabolic, and during prolonged severe stress [10]. Soeters et al. noted in the lack of intracellular glutamine, consuming glutamine supplementation could be useful [10]. Nova glutamine synthesis with pharmacological or nutritional interventions could potentially counteract glutamine depletion [11]. Welbourne demonstrated that a small oral glutamine load is capable of elevating plasma GH [12], while, Biolo et al. mentined rhGH (releasing hormone GH) administration in the glutamine release from the muscle into circulation [13].

By regarding strength training as a stimulus for the production of GH, glutamine metabolism in muscle mass, and also declines glutamine concentration in plasma as a result of muscular activity, we have hypothesized to maintain glutamine muscle, and plasma level by consuming glutamine as a supplement may have an effect on GH/IGF1 axis, and IGFBP-III as a marker to elevate serum IGF-1 and GH production.

II. Methods

This study was conducted from April 2013 to June 2013 at the Islamic Azad University, Science and Research Ayatollah Amoli Branch. The study was approved by the Research Ethics Committee of Isfahan University of Medical Science and Health Services (IUMS). The participation in the study was voluntary. All subjects were informed of the objectives of this study. Each subject signed a written informed consent.

All subjects presented no symptoms of cardiovascular diseases, pulmonary disease, diabetes, high blood pressure, and their historically demonstrated that they had not taken any dietary supplements, and pharmaceutical drugs, or were not on any specific diet. Forty amateur male body builders (aged= 22.93±3.50 yrs., weight= 70.24 ±11.04 Kg, height= 174.00± 0.05 cm, and BMI= 24.20±4.00 kg/m^2) were selected randomly from 40 eligible volunteers. They were randomly divided into four equal groups, Resistance training (R), Glutamine (G), Resistance training and Glutamine (RG), and Control (C) group.

The resistance group took part in the Resistance training program for four weeks (three times/week). The duration of each session was 60-90 minutes. Each session was divided into three sections consisting of a 10-minute warm up, 70-

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minute resistance training at the 80 -95% 1RM, and 10-minute cool down. The warm-up section consisted of stretching movement, rhythmic movement with low intensity. The Resistance training section consisted of nine exercises (Staggered-Stance Squat, Barbell Bench Press, Barbell Behind Neck Press, Dumbbell Side Bend, Dumbbell Biceps, Seated Barbell Shoulder Press, Dead Lift, Half Squats, Standing Calve Raise) each exercise was performed in three sets. The cool down section consisted of rhythmic movement with low intensity, and stretching movement with deep breathing. The Glutamine group is consuming l-glutamine (0.1 $g/kg^{-1}/day^{-1}$). The Resistance training and Glutamine group took part in resistance training with consuming l-glutamine (0.1 $g/kg^{-1}/day^{-1}$). The Control group continued their normal lifestyle without exercise training or consumption of l-glutamine.

Body weight was measured on a digital scale (Tefal - WZ5100C0 with a precision to 100 grams, USA). To measure height, a stadiometer (Seca, Modell 214, Hamburg, Germany). Quetelet index (kg/m^2) was used to calculate BMI [14]. The body fat percentage based on weight, age, and thickness measurements of three skin folds (pectoral, abdomen, thigh) based on the Jackson-Pollock 3-point formula by using a caliper (Lafayette Skinfold Caliper II, Model 01128, USA) [14].

Venous blood samples were obtained from the antecubital vein of the subjects while in a seated position. The collecting of blood samples was done at the same time of day to reduce any diurnal variation of the hormonal response. Following overnight fasting 9-12 hours, the subjects came to the laboratory at 7:00 to 8:00 am, and took a rest for 30 min prior to the first blood collection. GH (µg/ml) was assessed by Enzyme-linked immunosorbent assay (ELISA) utilizing Diaplas kits (USA), IGF-1 and IGFBP-III assessed by ELISA kit utilizing Mediagnost IGF-1 and Mediagnost IGFBP- III (Germany), respectively.

Relevant statistical analyses were performed using SPSS version 16 on a personal computer ($P<0.05$ was considered to be statistically significant). Descriptive analysis was adopted for the demographic, and the clinical characteristics were reported as mean±SD (Standard Deviation). Before the statistical analysis, the Kolmogorov-Smirnov test showed a normal distribution of all variables, and the Levene's test was used to show differing variances between two groups before the start of the protocol ($P>0.05$). T-dependence Test was used to show a significant difference between the pre-test and post-test of variables in each group ($P<0.05$). An analysis of the covariance was used to compare the post-test of variables between the four groups. The power of the tests was 0.80 ($P<0.05$).

III. Results

Forty amateur male body builders (aged= 22.93 ±3.50 yrs., weight= 70.23±11.04 kg, height= 174.00±0.05 cm, and BMI= 24.03±3.57 kg/m^2, FP= 25.38±6.89%) completely took part in our study. The demographic and clinical characteristics of all 40 subjects that completed the study period are given in Table I, which shows the pre-tests and post-tests as mean±SD of the variables (BMI, FP, GH, IGF-1, and IGFBP-III) for R, G, RG, and C groups.

The KS test demonstrated distribution of the GH serum level in R ($Z= 0.51$, $p= 0.89$), G ($Z= 0.82$, $p= 0.51$), RG ($Z= 0.32$, $p= 0.94$), and C ($Z= 0.85$, $p= 0.54$), IGF-1 serum level in R ($Z= 0.62$, $p= 0.73$), G ($Z= 0.53$, $p= 0.87$), RG ($Z= 0.10$, $p= 0.97$), and C ($Z= 0.12$, $p= 0.92$), and the IGFBP-III serum level in R ($Z= 0.63$, $p= 0.82$), G ($Z= 0.43$, $p= 0.91$), RG ($Z= 0.24$, $p= 0.85$), and C ($Z= 0.22$, $p= 0.88$) in pre- test was normal distribution ($p> 0.05$). Levene's test showed there was no significant difference in the variance of the variables between the two groups prior to the start of the protocol ($P>0.05$).

The characteristics of the T-dependence test are shown in Table II ($p< 0.05$). One way analysis has showed GH ($F= 6.05$, $p= 0.01$*), IGF-1 ($F= 4.19$, $p= 0.01$*), and IGFBP-III ($F= 5.59$, $p= 0.01$*) ($P<0.05$).

TABLE I
Characteristics of Variables as a Mean, and Standard Deviation in Studying Subjects

Variables		Mean ±SD R	Mean ±SD G	Mean ±SD RG	Mean ±SD C
Weight (Kg)	Pre test	68.75 ±10.50	71.50 ±12.34	69.75 ±10.10	70.92 ±11.23
	Post test	69.80 ±10.80	71.83 ±14.50	70.23 ±10.80	70.80 ±12.52
BMI (kg/m2)	Pre test	23.65 ±3.50	24.72 ±3.50	23.85 ±3.60	23.92 ±3.70
	Post test	23.92 ±4.00	24.84 ±3.90	24.20 ±4.10	23.87 ±4.20
FP (%)	Pre test	24.21 ±6.00	26.36 ±4.50	25.80 ±4.54	25.16 ±4.54
	Post test	22.71 ±5.22	26.55 ±4.35	23. 51 ±6.41	25.21 ±4.93
GH (ng/ml)	Pre test	6.51 ±1.27	6.83 ±0.30	6.44 ±1.14	6.40 ±1.10
	Post test	14.36 ±0.83	6.30 ±0.80	12.50 ±2.40	6.43 ±0.70
IGF-1 (ng/ml)	Pre test	508.7 ±50.13	531.10 ±67.60	536.50 ±75.50	506.10 ±65.60
	Post test	576.2 ±56.02	516.20 ±35.60	620.90 ±138.78	514.0 ±40.90
IGFBP-III (ng/ml)	Pre test	5.27 ±1.10	5.96 ±0.70	5.74 ±0.90	5.50 ±1.08
	Posttest	7.30 ±0.96	5.19 ±0.80	6.39 ±0.62	4.92 ±0.80

BMI (Body Mass Index). Values expressed as Mean ±SD (Standard Deviation). Resistance Training (R) (n = 10), Glutamine (G) (n = 10), Resistance with Glutamine (RG) (n = 10), Control group (C) (n = 10). $P< 0.05$.

TABLE II
CHARACTERISTICS OF T-DEPENDENCE TEST IN STUDYING SUBJECTS

Group Variable	R		G		RG		C	
	t	p	T	P	t	p	t	p
GH (ng/ml)	22.95	0.01*	0.98	0.23	29.93	0.01*	0.07	0.94
IGF-1 (ng/ml)	3.84	0.01*	1.32	0.10	6.70	0.01*	0.56	0.58
IGFBP-III (ng/ml)	1.86	0.09	1.72	0.10	3.77	0.01*	1.48	0.17

Resistance Training (R) (n = 10), glutamine (G) (n = 10), resistance with glutamine (RG) (n = 10), Control group (C) (n = 10). P< 0.05.

Tukey test as a post hoc test of one way analysis in GH showed that there was a significant difference between R-RG (m= 1.22±0.68, p= 0.09), RG-G (m= 6.25±0.68, p= 0.01*), R-G (m= 15.20±0.68, p= 0.01*), RG-C (m= 14.15±0.68, p= 0.01*), R-C (m= 7.93±0.68, p= 0.01*), G-C (m= 0.87±0.68, p= 0.88) (P<0.05).

Tukey test as a post has the one way analysis in IGF-1showed there was a significant difference R-RG (m= 1.46± 40.28, p= 0.01*), RG-G (m= 324.25±40.28, p= 0.01*), R-G (m= 164.20±40.28, p= 0.01*), RG-C (m= 324.15±40.28, p= 0.01*), R-C (m= 166.93±40.28, p= 0.01*), G-C (m= 2.20 ±40.28, p= 0.96) (P<0.05).

Tukey test as a post has the one way analysis in IGFBP-III showed there was a significant difference R-RG (m= 1.46 ±0.62, p= 0.07*), RG-G (m= 2.57 ±0.62, p= 0.01*), and RG-C (m= 2.60±0.62, p= 0.01*), whereas there was no significance difference between R-G (m= 1.31±0.62, p= 0.25), R-C (m= 1.24±0.62, p= 0.28), and G-C (m= 0.17±0.62, p= 0.89) group (P<0.05).

IV. DISCUSSION

There was a significant increase in GH after a four-week resistance training program in our study. The change rates of GH in R, G, RG, and C groups were 120.58%, -7.75%, 94%, and 0.4%, respectively. T-dependence test demonstrated a significant increase in the GH serum level in the R and RG groups (p< 0.05), and Tukey test as a post hoc of one way analysis demonstrated there was no significant difference between R-RG, and G-C groups (P<0.05), and thus, oral glutamine as a supplement had no significant effect on GH serum levels.

Kochańska-Dziurowicz et al. showed that intense exercise led to a significant increase in GH concentration [15]. Whereas Voss et al. demonstrated that a period of heavy, long-term exercise with changes in plasma volume does not significantly increase the GH [16]. GH is widely abused by athletes, especially to increase lean body mass and improve anaerobic exercise capacity, as it is suggested that it improves the athletic performance of professional males [17]. Athletes muscle strength, power, and aerobic exercise capacity are not enhanced by GH administration [18]. Regarding the risks of adverse effects of long-term abuse of GH like acromegaly, and increased morbidity and mortality [18], as well as the use of GH in different clinical purposes like its effect on immunological function [19]; however, resistance training as a stimulator to increase produce GH is the safest method to increase GH. Exercise stimulates and increases GH levels and the advantage of endogenous GH is the lack of side effects, and its role in the reactions caused in other tissues, such as aiding in the recruitment actin of and myosin in muscle fibers. The rest interval between sets is a significant agent on the magnitude of acute GH responses [20]. The intensity of an exercise and the rest time are the main factors that act as a stimulator of the Hypothalamus-Pituitary axis [21], and its effect to recruit actin and myosin (attach – detachment) and maximum shortening velocity of filaments in muscle fiber [22]. Also, the age, sex baseline of other hormones, especially sex hormones, and the disorder of sex releasing hormones are effective factors on GH. GH is also affected by the results induced by exercise in each situation mentioned above.

A recent report presented the effect of glutamine on fatigue [7]; however, no similar investigation was undertaken to control the GH/IGF1 axis in plasma levels by considering oral glutamine consumption.

Some studies demonstrated GH stimulates the synthesis, and regulation of IGF-1 in most tissues. IGF-1 is thought to be a potential to develop skeletal muscle, and regulate muscle hypertrophy [3], [4] .The results of the present study showed a significant change in IGF-1. The change rates of IGF-1 in R, G, RG, and C groups were 13.3%, -2.0%, 15.6%, and 1.5%, respectively. T-dependence test demonstrated a significant increase in the IGF-1 serum level in the R, and RG groups (p< 0.05). Kochańska-Dziurowicz et al. showed IGF-1 concentration did not change after intense exercise [15]. Copeland et al. demonstrates that among female runners, IGF-1 decreased after ultra-marathon race in menopausal women, and remained lower in recovery time [23], also Eliakim et al. reported a 14% drop in circulating IGF-1 in healthy adolescent females after exercise [24]. IGF system changes are associated with catabolic state, and the differences in the results may be due to age, and intensities of physical activity among the studies. Hypo activity of the GH-IGF-1 axis could explain age-related [25]. Interestingly, ovarian hormonal function is an important contributing factor to GH/ IGF-1axis, whereas Milewicz mentioned 17b-estradiol may be as important a contributor to IGF-1 plasma levels, as age in hypo estrogenic, hypogonadotropic hypogonadism women [26]. Sexual differences, sex hormones, and their effects on body composition (the effect of testosterone on muscle mass, metabolic changes, fat distribution due to progesterone) are important factors which are involved in studies with contradictory results. Eliakim et al. emphasized on gender differences in the IGF axis changes, and he noted a significant increase in muscle volume in weight-stable and IGF-1in adolescent males after exercise training [27], whereas Roberts et al. mentioned there was no significant difference in IGF-1 in vastus lateralis muscle biopsies among younger (18-25 years), and older (60-75 years) male groups, both at baseline and after resistance training at an intensity of 80% 1RM. Also in both age groups IGF-1 remained stable throughout the intervention [28].

The result of the study showed a significant change in IGFBP-III. The change rates of IGFBP-III in R, G, RG, and C groups were 38.51%, -12.91%, 11.32%, and -10.54%,

respectively. The T-dependence test demonstrated a significant increase in the IGFBP-III serum level in the RG group ($p< 0.05$). Tukey test, as a post hoc, emphasizes this fact ($p< 0.05$). It seems logical that the increase in GH is associated with IGF-1 and IGFBP-III. In humans, almost 80% of circulating IGF-1 is carried by IGFBP-III. IGFBP-III is regulated mainly by GH, but also to some degree by IGF-1 [29]. In the plasma, 99% of IGFBPs modulate the availability of free IGF-1 to the tissues.

Rosa et al. reported that combined strength and endurance training significantly increased GH levels in both the endurance-strength, and strength-endurance groups compared with the baseline values, and also the IGFBP-III concentrations significantly increased in the endurance-strength group after the exercise training sessions, but did not change significantly in the strength-endurance group [30], and thus, it can be concluded that the design of the training protocol could play an important role in hormonal changes. These results demonstrate that physical activity can have a significant effect on GH, IGF-1, and IGFBP-III levels after exercise depending on the duration and intensity of the workout.

Exhaustive training is associated with a chronic reduction in plasma concentrations of glutamine [9]. The intramuscular concentration of glutamine is known to be related to the rate of the protein synthesis, which regulates human metabolism, alters glycogen depleted muscles, and plays the role of glutamine in promoting glycogen synthesis, so increased gluconeogenesis [6]. Agostini et al. reported increased glutamine availability may contribute to decreased inflammation, which associated with optimal training [9], so this could have an effect on muscle working capacity, and greater tolerate of resistance training, and the effect on the adaptation rate in muscles, which could include the GH/IGF1 axis; however, the results demonstrated glutamine consumption had no significant effect on GH, IGF1, and IGFBP-III. The measure of the glutamine serum level could help explain the results of the present study. The lack of glutamine (intracellular, plasma) measured was a limitation of this study. Another limitation was the lack of control of the subjects' food intake, as some studies noted changes in serum levels of glutamine in starving-feeding, and the role of carbohydrates in maintaining or depleting glutamine resources.

Based upon these findings, the study concluded that resistance training at 80-95% 1RM intensity, increase significantly secretion of GH, IGF-1, IGFBP-III in amateur males, oral glutamine showed no significant difference in the GH/IGF-1 axis, and also there was no significant difference between resistance training, and resistance training along with oral glutamine in GH, IGF-1, and IGFBP-III changes. The study concluded that resistance training is the stimulator to the GH/IGF-1 axis in comparison with glutamine supplements as a peptide to control the GH/IGF-1 axis on protein synthesizing in muscles.

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