

Acute IGF-1, Cortisol and Creatine Kinase Responses to Very Short Rest Intervals Between Sets During Resistance Exercise to Failure in Men

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Abstract: Insulin-like growth factor-1 (IGF-1) peptides exist as single chain polypeptides consisting of about 70 amino acid residues which stimulate muscle hypertrophy via PI-3K-Akt-mTOR signaling. The purpose of the present study was to investigate the effect of a resistance exercise (RE) protocol (4 sets of squat and bench press with 85 % of 1RM) at different rest intervals between sets on serum IGF-1, cortisol (CO), creatine kinase (CK) and blood lactate concentrations. Hence, ten recreationally resistance-trained men (Mean \pm SD, age=20.37 \pm 2.44 years, body mass= 65.6 \pm 26.70 kg) volunteered as subjects that in 4 separate sessions with 48 h from each other performed a RE protocol with 60, 90 and 120 second rest period between sets. Blood samples were collected before (Pre), immediately after (Post) and 30 min after the exercise protocols (30Post). Changes in serum IGF-1 levels were not significant between protocols. However, serum IGF-1 concentration was significantly increased by 3.6 and 23 percent during RT with 60 second rest between sets at Pre and 30Post. Serum CO concentrations were significantly higher in 60 and 90 second rest protocol than 120 second protocol. Changes in serum CK and blood lactate concentrations were not significant between protocols but post exercise levels significantly increased in each protocol. The data indicate that although rest period between sets in RE to failure resulted in a significant change in CO levels, it had no effect on plasma IGF-1, CK and lactate levels. However, post exercise serum IGF-1 concentrations significantly increased only at RE to failure with 60s rest between sets.

Abbreviated: IGF-1, cortisol and creatine kinase responses to very short rest intervals between sets

Key words: Resistance exercise % IGF-1 % Cortisol % CK

INTRODUCTION

It has been well known that the stress of heavy-resistance exercise has a potent effect for both strength development and muscle fiber hypertrophy. This may be due, at least in part, to exercise induced acute increase in serum anabolic hormone [1]. IGF-I is part of the insulin-related peptides. IGF-I can act as a hormone and these effects are GH dependent. However, the majority of IGF-I actions occur primarily as a result of paracrine or autocrine secretion and regulation, which are only partially GH-dependent.

It is now well established that acute exercise lead to an increase in circulating IGF-I levels. Interestingly, several studies demonstrated that the exercise induced IGF-I increase occurred following very short high

intensity exercise (i.e. 90 s) [2], occurred 10 min following the beginning of endurance exercise [3,4] and occurred in exercise of both below and above the LAT [4]. Exercise was also associated with an increase in urinary IGF-I [5].

However, RE is known to be a powerful stimulus for the endocrine system. The hormonal response to RE is dependent on several factors including the number of training sets, number of repetitions, training intensity, training volume and rest between sets [6]. The effect of RE on circulating IGF-I is inconsistent. Several studies found an increase in circulating IGF-I and free IGF-I following strength exercise [7,8]. Moreover, eccentric exercise was associated with increases in muscle IGF-I mRNA suggesting that IGF-I may modulate tissue regeneration after mechanical damage [9]. However, most studies dealing with the acute response of IGF-I to RE

have shown no change in serum IGF-I level [10,11]. These conflicting results reflect probably differences in fitness level of the participants, timing of blood sampling, exercise protocols, etc.

In addition, the effect of RE on basal plasma CO levels is variable and either no change [12-14] or a decrease has been reported [15]; the difference probably related to lower training volumes. Increasing resistance, training volume, or intensity may result in an increase in resting CO levels [16]. A doubling of training volume has also been observed to result increases in CO levels [16].

RE includes eccentric and concentric muscle actions. Eccentric muscle actions, which are capable of producing higher force than isometric or concentric muscle actions, result in significantly greater muscle damage. When muscle is damaged, there is disruption of the sarcolemma allowing muscle proteins such as creatine kinases (CK) and myoglobin to be released from the cell into the blood stream [17]. There are only two studies that examined RE-induced microinjuries by according to the rest intervals between sets and their results are inconsistent [18,19]. Riberiro, *et al.*, didn't observe significant difference in muscle damage induced by a RE protocol (3set×10repetition×10RM load) with different rest intervals (1vs.3 min) [17]. However, Mayhew *et al.*, finding that muscle damage, as measured by CK, was significantly greater 24H after the 1-minute rest-interval bout than after the 3-minute bout in a resistance exercise protocol with 10 sets × 10 repetitions at 65 % of 1RM in leg press [18]. These conflicting results may be due to, at least in part, long rest interval (60 second vs. 180 second), also higher volume and intensity in RE protocol used by previous studies [17,18].

Resistance exercise is characterized by exposing subjects to a very high degree of sudden strenuous all-out exercise. Previous studies evaluated the growth hormone, cortisol and CK to rest intervals ranging from 60 second to 180 second [18-20]. Little data are available on changes in the levels of IGF-I following heavy RE in healthy young subjects. Furthermore, the effect of rest between sets on these responses has not been studied yet. We hypothesized that shorter rest would manifest greater alterations in serum IGF-1, CO and CK levels than the longer rest intervals between sets. Therefore the purpose of this study was to assess acute IGF-1, CO and CK responses to very short rest intervals between sets during resistance exercise to failure in resistance-trained college-age males.

MATERIALS AND METHODS

The Experimental Approach to the Problem: The acute hormonal responses of three resistance training protocols differing by rest periods between the sets (60, 90 and 120 second) were studied with 10 recreationally resistance - trained men. Loading protocols were performed to failure and expected to lead to large acute hormonal responses. We hypothesized that when using short rest periods between the sets in resistance training to failure (maximum repetitions per sets); the endocrine response should be larger along with a greater metabolic stress (i.e., lactate) than that of long rest periods between the sets.

Subjects: Ten experienced resistance-trained college-age males (age, 22 ± 2 years; weight, 84 ± 8 kg; at least 1 years of RE experience) volunteered for this study. Each subject had at least 1 year recreational experience with resistance training and performed at least three resistance training sessions per week during the previous 6 months, but none were competitive strength athletes. The values for 1RM were 105.62 ± 18 kg for bench press and 106.31 ± 19.71 kg for squat. Subjects were informed of the experimental risks and signed an informed consent document prior to the investigation. The Institutional Review Board of the University approved the research protocol. Subjects were on their ordinary diet, not permitted to use nutritional supplementation and did not consume anabolic steroids or any other anabolic agents known to increase performance.

Experimental Design: The subjects were familiarized with the experimental testing procedures during a control day about one week before the actual measurements. Resistance load verification for the experimental bench press and half squat exercises were also determined. All of the subjects went through three strength exercise trials of different rest intervals between sets. The strength exercises lasted from 09 00 hours to 11 00 hours and to avoid any potential carry-over effects and threats of internal validity, each of the three protocols was performed in a counterbalance order by all 10 participants. At least 48 h but not more than 72 h of recovery time was allowed between each training session [20]. During the control day, three blood samples were obtained from each subject. One blood sample was drawn in the morning after 12 hours of fasting and approximately eight hours of sleep for determination of basal serum hormone concentration. Two blood samples were also drawn without exercise at the same time of day that each subject would later under

tack his heavy-resistance loading protocols to determine the normal diurnal variation of serum hormone concentration. During the exercise sessions, blood samples (5 ml) were drawn from an antecubital vein into 10-ml serum Vacutainer tubes at rest (Pre); immediately after (Post) and 30 min after the end of the session (30Post). The experimental design comprised three resistance training protocols within one week, (a) resistance training protocol to failure with 60 second rest between sets (P60), (b) resistance training protocol to failure with 90 second rest between sets (P90) and (c) resistance training protocol to failure with 120 second rest between sets (P120). RT protocols included 4 sets bench press and squat to failure with 4-min recovery between the exercises.

Strength Testing: One repetition maximum (1RM) in bench press and squat was determined according to the method of [8,25]. Warm-up consisted of a set of five repetitions at the loads of 40-50 % of the perceived maximum. An attempt was considered successful when the movement was completed through a full range of motion without deviating from proper technique and form. Spotters were present to provide verbal encouragement and safety for the subjects. To ensure that all subjects were moving at approximately the same velocity for each repetition, each set was timed using a handheld stopwatch. The spotter called out a cadence for the eccentric and concentric phases of each repetition. The repetition velocity consisted of a 3-second eccentric phase followed by a 1-second concentric phase. During the next 3 testing sessions, 4 sets of the squat and bench press were performed with a 60-, 90-, or 120-second rest interval between sets. A counterbalance procedure was used to determine the order of the rest interval between sets for each testing session. Subjects didn't allow continuing with their normal workouts throughout the duration of the study. Also, subjects were instructed not to perform training 48 h before the testing session and subjects were instructed not to change their eating patterns during the study.

Hormonal Analysis: Blood samples (5 ml) were drawn from an antecubital vein into 10-ml serum Vacutainer tubes and after approximately 45 min, serum tubes were centrifuged at 3000 rpm (5000g) for 10 min at room temperature. Serum was separated from blood cells and stored at -20 °C until analyzed. Blood samples were analyzed using commercial Kits for: IGF-1 (Enzym Linked Immunosorbant Assay, BioSource Europ S.A.- Reu De

I'Industrie, 8-1400 Nivelles- Belgium) and Cortisol (Enzym Immunosorbant Assay, RADIM SpA, Via del Mare, 125-00040 Pomezia (Roma) Italia). All blood parameters were determined by duplicate analysis. Inter- and intra-assay coefficients of variances were 7.8% and 11.3% for serum IGF-1 and 6.9% and 6.2% for serum cortisol. Lactate in plasma was analyzed enzymatically using a YSI 1500 Sport (Yellow Springs, USA). The CV's for lactate was <5%. All samples from each subject were analyzed on the same day. CK was assayed spectrophotometrically through the use of commercially available kits (PARS AZMUN CO. TEHRAN, IRAN). The CV's for CK was <3%.

Statistical Analyses: Data are expressed as Mean \pm SD. Statistical evaluation was performed with SPSS 12.0 (SPSS, Chicago, IL) for windows and two-way (3 \times 3) repeated measures ANOVA (rest intervals \times time (T0, T1, T30) were used to compare blood samples for the different programs. Multiple comparisons with confidence interval adjustment by the LSD (Least Significant Difference) method were used as post hoc when necessary. Statistical analysis compared the blood samples for each sequence against resting. The significance level was set at $p < 0.05$.

RESULTS

Changes in serum IGF-1 levels were not significant between protocols. However, serum IGF-1 increased after P60 from 797 \pm 69 up to 826 \pm 149 and 981 \pm 34 ng/ml (Pre, Post and 30Post; respectively), ($P=0.05$). Also, serum IGF-1 increased after P90 at Pre (5.6%), 30Post (10%) and P120 at 30Post (7.6%) but these changes were not statistically significant ($p > 0.05$) Fig.1. Serum CO levels significantly increased immediately after and 30min after in P60 and P90 from 133 \pm 18 up to 225 \pm 45 ng/ml and 152 \pm 39 up to 248 \pm 51 ng/ml, respectively) ($P=0.05$). However, no significant changes in CO levels were observed in P120. In addition, Serum CO concentrations were significantly higher in 60 and 90 second rest protocol than 120 second protocol ($P=0.05$) (Fig.2). CK activities showed a significant increase in all post- and 30min post-exercise values (P60: from 212 \pm 42 up to 279 \pm 66 and 263 \pm 80 U/L; P90: from 218 \pm 23 up to 259 \pm 52 and 259 \pm 58 U/L; P120: from 204 \pm 48 up to 255 \pm 40 and 253 \pm 56 U/L) of three protocols ($P=0.05$) (Fig.3). Significant differences ($P=0.05$) were observed in mean blood lactate from pre- to post-exercise within each protocol (Fig.4). Total work performed comparison between groups (P60, P90 and P120) revealed no significant differences ($p > 0.05$) in P60 (3603.81 \pm 816.05 kg), P90 (4175.50 \pm 939.89 kg) and P120 (4352.06 \pm 996.87 kg).

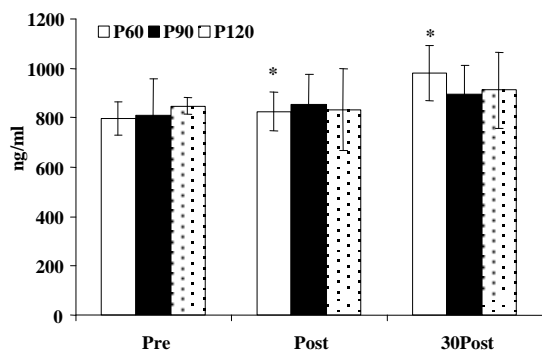


Fig. 1: Serum IGF-1 concentrations in three protocols (60, 90 and 120s rest period between sets) pre-, immediately post- and 30min post-exercise
* Significant difference with pre-exercise

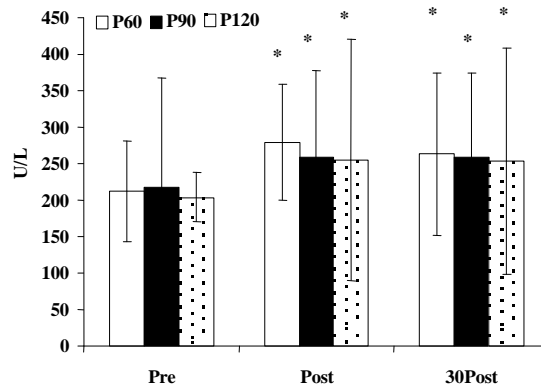


Fig. 3: Serum CK concentrations in three protocols (60, 90 and 120s rest period between sets) pre-, immediately post- and 30min post-exercise
* Significant difference with pre-exercise

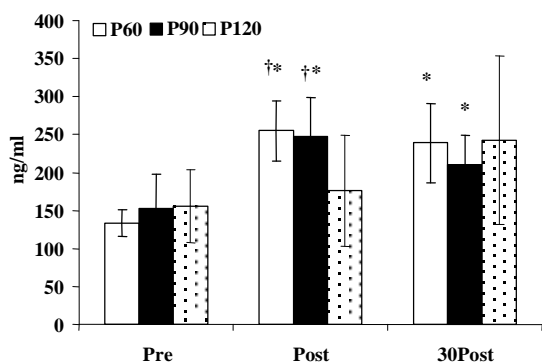


Fig. 2: Serum CO concentrations in three protocols (60, 90 and 120s rest period between sets) pre-, immediately post- and 30min post-exercise
* Significant difference with pre-exercise
† Significant difference with P120

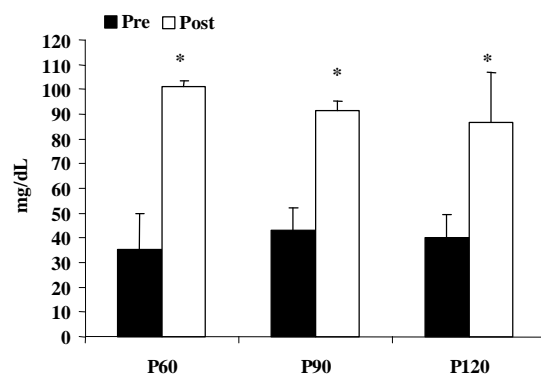


Fig. 4: Blood lactate concentrations in three protocols (60,90 and 120s rest period between sets) pre- and immediately post-exercise
* Significant difference with pre-exercise

DISCUSSION

IGF-1 stimulates muscle hypertrophy via PI-3K-Akt-mTOR signaling [21,22]. Additionally, IGF-1 stimulates the proliferation and differentiation of specialized stem cells located at the periphery of muscle fibers called satellite cells. Satellite cell activation, proliferation and differentiation contribute significantly to muscle growth following long-term training [23]. The acute response of circulating IGF-1 to RE is unclear. The results of the present study indicate that serum IGF-1 concentrations significantly increased after- and 30 min after-RE protocol with 60 s rest interval between sets which is in accordance to Bermon *et al.*, finding increase in circulating free and total IGF-1 concentration immediately- and 6h –after the RE protocol [7]. In similar study, Popove *et al.*, indicating a moderate-intensity (50% of 1RM) leg press with short

rest periods (30s) between sets induced an acute increase in circulating IGF-1 [24]. Also, Boroujerdi and Rahimi investigated RE protocol (5set×10 Reptition×10RM load) with different rest periods between sets (1 vs. 3 min) on IGF-1 and GH concentrations [8]. They observed significant increase in serum IGF-1 concentrations 1h after both protocols. However, Kraemer *et al.*, found no changes in IGF-1 concentration for a period of 24 h after strength exercise designed to give a maximal GH response [10] as well as Nidl *et al.*, found no change in circulating IGF-1 following heavy resistance exercise [11]. These conflicting results reflect probably differences in exercise protocols, fitness level of the participants, timing of blood sampling, etc.

Rest between sets in RE has a special important which is defined as the time period between the ends of training set and commence of the next set so that body

condition of the individual approached to the physiological stance before the activity. Amount of rest between sets affects the metabolism, cardiovascular function, hormonal response, also number of repetition in subsequent sets [6,8,25-27]. However, Based on the results rest interval between sets didn't significantly influence on serum IGF-1 concentrations in young trained men after a RE protocol. This result is in accordance to Boroujerdi and Rahimi, finding no significant difference in serum IGF-1 levels between a RE protocol with different rest between sets (1 vs. 3 min) [8]. Unfortunately, studies focusing on the effect of rest intervals between sets on IGF-1 concentrations are lacking, making it difficult to compare our results.

The mechanism for the transient increase in circulating IGF-I in response to exercise is not readily apparent. One possibility would be the classic mechanism of increased hepatic IGF release due to exercise-induced secretion of GH. Previous study indicated that GH increases significantly mainly in response to high intensity exercise, while IGF-I increase for both low and high intensity exercise. Moreover, circulating IGF-I reaches its peak before the GH peak (i.e. 10 vs. 30 min) [28], while increases in serum IGF-I, due to de novo IGF-I synthesis in the liver and transport to the circulation, occurs several hours after the administration of endogenous GH [29]. In addition, earlier studies showed [3] that exercise led to increases in IGF even in subjects with pituitary insufficiency. These studies suggest that the exercise-associated increase in IGF-I is, in fact, not related to GH and must reflect rapid changes in IGF-I distribution in the circulation due to release from marginal pools or changes in IGF-I removal. In addition, the transient nature of the increases suggests that hemodynamic or metabolic effects of exercise might play a role. Exercise in humans is accompanied by the rapid autotransfusion of hemoconcentrated blood from the spleen into the cellular circulation [30], by increased blood flow to the exercising muscle and by loss of plasma water [31]. Each of these phenomena might explain, in part, an increased IGF concentration by changes in IGF flux and/or volume of distribution. Another possible source for the increase in circulating IGF-I can be a release from the exercising muscle. To test this, Eliakim *et al.*, used a simple approach in which subjects performed a unilateral repeated flexion of the wrist against relatively high resistance, while during- and post-exercise blood samples were collected simultaneously from the basilic vein of both the exercising (representing local release) and resting arm (representing systemic response) [32]. They found a

bilateral, simultaneous increase in IGF-I suggesting that the local exercising muscle was not the source for of the IGF-I increase.

Also, Cortisol secretion responds quite rapidly to various stresses (e.g. exercise, hypoglycaemia, surgery, etc.), typically within minutes. The importance of the CO response to RE is related to its catabolic effects on skeletal muscle. As a result, CO has been the catabolic hormone most frequently analyzed after resistance training [33]. Gotshalk *et al.*, 1997 reported that three sets versus one set of resistance exercises resulted in a greater cortisol increase [34]. In the present study rest intervals between sets affect serum CO concentrations and observed higher level of CO concentrations in RE with short rest intervals (60-90 second) between sets than long rest interval which is in accordance to previous studies [33,35] have demonstrated that shorter rest intervals are associated with an increased CO response after resistance training. However, contrary to our findings, Bottaro *et al.*, reported that CO concentrations were not different among three RE protocol with 30, 60 and 120 second rest durations between sets [20]. The mechanisms responsible to increase CO concentrations in RE maybe due to physiological and psychological stress [36]. Thus, higher levels of blood CO during RE with 60 and 90 s rest periods as compared to 120second protocol, maybe due to an increase in physiological stress in response to a short rest interval.

RE include concentric and eccentric muscle actions. In particular, eccentric muscle actions, which include high levels of force, were shown to result in muscle tissue damage. The CK activity used as a marker of muscle damage [18,19]. The CK activity observed in this study showed that muscle tissue damage occurred well after the exercise in three RE protocols. This result is in accordance to previous studies [18,19] have demonstrated that RE is associated with muscle tissue damage. However, based on the findings there were no significant differences in muscle tissue damage related to rest intervals between sets in RE which is in accordance to Riberiro, *et al.*, They didn't observe significant difference in muscle damage induced by a RE protocol (3set×10repetition×10RM load) with different rest intervals (1vs.3 min) [18]. However, our finding is inconsistent to Mayhew *et al.*, finding that muscle damage, as measured by CK, was significantly greater 24H after the 1-minute rest-interval bout than after the 3-minute bout [19].

In summery, the present data indicate that RE with short rest period (60s) induced increase in serum IGF-1 concentration but serum IGF-1 level didn't significantly increased in an inter-set rest dependent manner. However,

higher levels of serum CO were observed in RE with short rest periods (60-90s) between sets as compared to long rest period (120s). RE protocol imposed to our subjects induced muscle tissue damage as measured by CK activity, which to be independent of rest durations between sets.

CONCLUSIONS

The physiological and mechanical stress caused by the proposed training session cause similar changes in serum IGF-1 levels as well as muscle fiber damage. Serum IGF-1 concentration and CK activity do not depend on the 60, 90 or 120second rest interval between sets in RE but CO concentration depend on the rest period between sets.

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