AN ABSTRACT OF THE THESIS OF

Jane V. Higdon for the degree of Master of Science in Human Performance presented on July 18, 1996. Title: Effects of Acute Heavy Resistance Exercise on Serum Insulin-like Growth Factor-I and Insulin-like Growth Factor Binding Protein 3 Levels in Older Men and Women.

Redacted for Privacy

Abstract approved: Daniel P. Williams

The purpose of the present study was to assess changes in circulating levels of insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) resulting from a single session of heavy resistance exercise, in previously inactive men (n=9) and women (n=6) between the ages of 50 and 60. Acute changes in IGF-I and IGFBP-3 were assessed in order to provide information useful in standardizing blood collection protocols for resistance training studies, with respect to the timing of the last acute exercise session, and to determine whether the acute changes in IGF-I or IGFBP-3 after heavy resistance exercise are sufficient to be considered a putative humoral mechanism contributing to increases in muscular strength and fat free mass, associated with resistance exercise training.

Participants performed 3 sets of 6 repetitions of 6 resistance exercises, at an intensity of 85% of 1-repetition maximum (1-RM) with 3 minutes rest between sets. Serum IGF-I concentrations were measured by radioimmunoassay after acid ethanol cryoprecipitation. IGFBP-3 concentrations were measured using a 2 site immunoradiometric assay. All post exercise values for IGF-I and IGFBP-3 were corrected for plasma volume change, estimated from hemoglobin and hematocrit.
A single bout of heavy resistance exercise in 50 to 60 year old men and women did not result in any significant changes from baseline in plasma volume adjusted-serum IGF-I (143 ± 43 ng/mL, pre-ex; 143 ± 37, post-ex; 138 ± 37, 12h; 137 ± 35, 24h; 140 ± 35, 48h; p= .32) or serum IGFBP-3 (2616 ± 412 ng/mL, pre-ex; 2594 ± 370, post-ex; 2636 ± 390, 12h; 2558 ± 417, 24h; 2617 ± 360, 48h; p= .72) concentrations over a 48 hour period. The changes in IGF-I and IGFBP-3 concentrations over time did not differ by gender.

The apparent lack of change in IGF-I and IGFBP-3 concentrations following a single session of acute resistance exercise does not preclude the possibilities that resistance exercise may augment the mitogenic effects of IGF-I through proteolysis of the IGF-I-IGFBP-3 ternary complex or that resistance exercise may increase tissue sensitivity to circulating IGF-I levels. Data from the present study suggest that post-resistance exercise training samples for basal serum IGF-I and IGFBP-3 could be collected as early as 12 to 24 hours after the last acute bout of resistance exercise.
EFFECTS OF ACUTE HEAVY RESISTANCE EXERCISE ON SERUM INSULIN-LIKE GROWTH FACTOR-I AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 LEVELS IN OLDER MEN AND WOMEN

by

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CONTRIBUTION OF AUTHORS

Dr. Daniel P. Williams was involved in the design, data collection, analysis, and writing of the manuscript. Dr. Christine M. Snow was involved in the design and the analysis of the manuscript. Hormonal assays were performed in the laboratory of Dr. Clifford Rosen, who also assisted in the interpretation of the data. Gianni F. Maddalozzo contributed to the design and assisted with the data collection for the study.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACKGROUND AND SIGNIFICANCE</td>
<td>1</td>
</tr>
<tr>
<td>EFFECTS OF ACUTE HEAVY RESISTANCE EXERCISE ON SERUM INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 LEVELS IN OLDER MEN AND WOMEN</td>
<td>7</td>
</tr>
<tr>
<td>Introduction</td>
<td>8</td>
</tr>
<tr>
<td>Methods and Procedures</td>
<td>10</td>
</tr>
<tr>
<td>Results</td>
<td>17</td>
</tr>
<tr>
<td>Discussion</td>
<td>22</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>27</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Timeline of the Experimental Resistance Exercise Session</td>
<td>12</td>
</tr>
<tr>
<td>2.</td>
<td>Percent change in plasma volume from pre-exercise value immediately after resistance exercise, 12, 24, and 48 hours after the pre-exercise sample</td>
<td>18</td>
</tr>
<tr>
<td>3.</td>
<td>No significant differences from baseline are indicated in plasma volume unadjusted and adjusted concentrations of IGF-I before and immediately after a resistance exercise session, 12, 24, and 48 hours after the pre-exercise sample</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>No significant differences from baseline are indicated in plasma volume unadjusted and adjusted concentrations of IGFBP-3 before and immediately after a resistance exercise session, 12, 24, and 48 hours after the pre-exercise sample</td>
<td>21</td>
</tr>
</tbody>
</table>
Aging is associated with significant decreases in skeletal muscle and fat-free mass (FFM) (24,66), muscular strength (41) and bone mineral density (BMD) (47,51). Diminished muscle mass and strength are associated with an increased risk of falling in the elderly (1), while decreases in BMD are associated with increased risk of hip fracture (22). Falls and fractures represent a significant health risk in the elderly, because they frequently result in extended hospitalization, immobility, and death (67).

Aging, even in adults with clinically normal pituitary function, has been associated with diminished pituitary growth hormone (GH) secretion. Additionally, decreased serum levels of insulin-like growth factors (IGFs) (62,69) and their major serum binding protein, insulin-like growth factor binding protein-3 (IGFBP-3) (12,19) have also been associated with advancing age. Because pathological GH deficiency (resulting in diminished IGF-I and IGFBP-3 levels) and normal aging are both associated with reductions in protein synthesis, FFM and BMD, age-related reductions in FFM and BMD may be mediated by age-related reductions in serum IGF-I and IGFBP-3 levels. (11). However, age related reductions in FFM and BMD, as well as in GH secretion, and IGF levels are confounded by age-related reductions in physical activity (36,54). It is presently unclear whether such reductions in lean mass and endogenous mitogenic hormones like GH and IGF-I are a result of the aging process per se, or the reduction in physical activity that often accompanies aging.

The participation of older individuals in resistance exercise programs has been shown to result in significant increases in muscular strength as well as muscular hypertrophy (9,25,68). Muscular strength is also correlated with regional bone density in older adults (48,51). Several studies have indicated that prevention of age-related declines
in BMD (48, 56, 64) and even moderate increases in regional BMD (16) may be possible with resistance training. Although resistance exercise has been advocated as a means to counteract the age-related loss of FFM and BMD, the efficacy and optimal dose (frequency, intensity, duration) of resistance exercise required to prevent age-related reductions in FFM and BMD are not known. The paucity of information results, in part, from the fact that the mechanisms responsible for resistance exercise-related changes in FFM and BMD have not been determined.

The production and regulation of IGFs appear to play a critical role in the maintenance and augmentation of skeletal muscle mass and bone mass. It is well documented that IGF-I stimulates proliferation and differentiation of bone cells and skeletal muscle cells (33). Along with their binding proteins, IGFs are found in very high concentration in the bone matrix. Osteoblasts also synthesize IGFs, and possess both known types of IGF receptors (61). Serum IGF-I levels may provide a marker for bone IGF-I levels, as serum IGF-I levels are comparable to bone tissue IGF-I in humans and a number of other vertebrates (3). Similarly, cortical bone tissue IGF-I levels, like serum IGF-I levels, decrease with age (50), resulting in a 60% reduction in cortical bone tissue IGF-I between the ages of 20 and 60 years. This reduction in cortical bone tissue IGF-I levels parallels the age-related reduction in serum IGF-I levels (4).

Acute IGF-I infusions, sufficient to create a three-fold increase in serum IGF-I levels, stimulates skeletal muscle protein synthesis in humans (26). Evidence of increased IGF-I in skeletal muscle, such as increased mRNA for IGF-I, has been associated with compensatory hypertrophy (17) and stretch-induced hypertrophy (15, 27) of skeletal muscle in animals, suggesting that IGF-I plays a role in muscular hypertrophy related to the application of increased or altered forces to skeletal muscle. Measuring changes in serum IGF-I after acute resistance exercise may provide insight into possible humoral mechanisms for the maintenance and augmentation of skeletal muscle mass, muscular strength, and bone mass.
The IGF-I present in the circulation and the extravascular space are almost entirely bound to a family of IGFBPs. IGFBPs, including IGFBP-3, are synthesized and secreted by the liver, as well as by extrahepatic tissue, including skeletal muscle and bone. The functions of IGFBPs are essential to the coordination and regulation of the biological activities of IGF-I (33). By far, the most plentiful IGFBP in circulation is IGFBP-3. In the circulation, IGFBP-3 forms a 150 kDa ternary complex with IGF-I and an acid labile subunit (ALS). As a ternary complex, IGFBP-3 increases the half life of IGF-I and prevents its efflux from the vascular space to the tissue, thus limiting its bioavailability. The exact function of this large store of IGF-I bound to the 150 kDa complex is unclear, though several investigators have suggested that it serves as a plasma reservoir of readily available IGF-I (30,33).

In order for the 150 kDa ternary complex to function as a circulatory reservoir of IGF, some mechanism must exist to release IGF from this complex and make it available to tissue receptors. Recent evidence of the specific proteolytic modification of IGFBP-3 suggests a plausible mechanism for making circulating IGF-I available to tissue receptors (30). After proteolytic cleavage of the 150 kDa IGFBP-3 ternary complex, a lower molecular weight (30 kDa) IGFBP-3 fragment with 20 to 30 times less affinity for IGF-I can be detected (6). Both free IGF-I and the truncated IGF-I-IGFBP-3 complex are capable of leaving the circulation and entering the extravascular compartment, where IGF-I is available for interaction with tissue receptors. Increased proteolysis of IGFBP-3 has been observed in the serum of pregnant women (28). Increased IGFBP-3 proteolysis has also been observed in a number of catabolic states (52), including the post-operative period after major surgery (30). However, no published study to date has examined the effect of exercise on IGFBP-3 proteolysis.

In the extravascular space IGFBP-3 modulates the interaction of IGFs with their receptors, indirectly controlling IGF actions. Extravascular IGFBP-3 may directly oppose IGF action by binding IGF and preventing IGF-receptor interaction. In contrast,
extravascular IGFBP-3 may also potentiate IGF effects when it associates with the cell surface, ultimately decreasing its affinity for IGF and making more free IGF available for receptor interaction (33). The complexity of the interaction between IGFs and IGFBP-3, and the multiplicity of the effects of those interactions on tissue growth, make the simultaneous assessment of serum IGFBP-3 and IGF-I a critical component when examining the relationships between IGF-I and the maintenance of bone and skeletal muscle mass. However, the effects of exercise on the relationship between IGF-I and IGFBP-3 are not well known.

Although several studies have found increased basal serum IGF-I levels to be associated with increased physical activity in adults (36,54), resistance training studies have reported no changes in basal serum IGF-I levels in older men or women. The apparent lack of adaptation in basal serum IGF-I levels, in response to resistance exercise may be inconsistent with the resistance training-related increases in muscular strength and skeletal muscle mass (9,22, 68). Studies that have utilized recombinant human growth hormone (rhGH) treatment in combination with resistance training have likewise reported no association between the rhGH treatment-related increases in serum IGF-I levels and the treatment and training-related increases in strength and FFM (70,71). A potential limitation of previous resistance exercise studies is a lack of standardization of the timing of the post-resistance training sample of IGF-I with respect to the last resistance exercise bout. Acute changes in circulating IGF-I in response to resistance exercise have not been examined in older individuals. Additionally, tissue availability of circulating IGF-I is clearly related to the status of circulating IGFBP-3. However, none of the resistance training studies in older adults to date have assessed IGFBP-3 levels, nor has the acute response of circulating IGFBP-3 levels to resistance exercise been examined.

The lack of evidence for resistance training-associated changes in basal serum IGF-I raises the possibility that the acute physiological response of IGF-I and IGFBPs to resistance exercise may be a more important mechanism for explaining effects of resistance
exercise on the maintenance of skeletal muscle and bone mass in older adults, than chronic changes in basal IGF levels. Evidence is increasing that some of the major health related changes produced by physical activity may be related to a repetition of the acute physiological responses during, and shortly after each exercise bout (29). Therefore, resistance training-related changes in body composition may be mediated in part, by repeated, acute, transient changes in IGFs and/or IGFBPs rather than solely by chronic alterations in basal levels.

Previous studies of the acute response of IGF-I to resistance exercise have examined only serum levels of IGF-I, without examining its major serum binding protein IGFBP-3 (37-40). In addition, prior studies of the acute IGF-I response to resistance exercise have examined post-exercise IGF-I levels for a maximum of only 2 hours after the completion of exercise. Such a short observation period may fail to discern GH dependent alterations in IGF-I, as they have been reported to occur from 4 to 12 hours after a GH surge (46). Furthermore, most studies of the acute response of IGF-I to resistance exercise have utilized young subjects. Acute IGF-I responses to resistance exercise have not been well described in men and women over 50 years of age, who are most at risk of debilitating loss of skeletal muscle and bone mass.

Although a variety of research suggests that IGF-I plays an integral role in the maintenance and augmentation of skeletal muscle mass and bone mass resulting from resistance exercise, a number of issues require further clarification. The nature of the relationship between IGFBP-3 and IGF-I in response to resistance exercise has not been well studied. Additionally, acute changes in IGF-I and IGFBP-3 resulting from a single bout of resistance exercise have not been explored for a sufficient time period following the completion of the resistance exercise session. While there is evidence suggesting that the circulating and tissue levels of IGF-I decline with age, acute responses of IGF-I to resistance exercise have not been described in individuals over 50 years of age. The following manuscript reports the results of a study designed to assess the effects of a single
bout of resistance exercise on serum IGF-I and IGFBP-3, over a 48 hour period, in older men and women.
EFFECTS OF ACUTE HEAVY RESISTANCE EXERCISE ON SERUM INSULIN-LIKE GROWTH FACTOR-I AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 LEVELS IN OLDER MEN AND WOMEN

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EFFECTS OF ACUTE HEAVY RESISTANCE EXERCISE ON SERUM INSULIN-LIKE GROWTH FACTOR-I AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 LEVELS IN OLDER MEN AND WOMEN

Introduction

Age-associated decreases in growth hormone secretion (GH) secretion and concomitant declines in circulating insulin-like growth factor-I (IGF-I) and its predominant circulating binding protein, insulin-like growth factor binding protein-3 (IGFBP-3), may be related to age-associated declines in muscular strength (41), skeletal muscle mass (24,68) and bone mineral density (44,47). However, age-related reductions in fat free mass (FFM), bone mineral density (BMD), GH secretion, and circulating IGF-I levels are confounded by reductions in physical activity. Presently, it is unclear whether reductions in FFM, BMD, serum IGF-I, and IGFBP-3 are a result of the aging process per se, or the reduction in physical activity that often accompanies aging (51,55).

Although resistance exercise training in older adults has resulted in increased muscular strength, skeletal muscle mass (9,22,48,67), and in some cases moderate increases in bone mineral density (16,64), basal serum IGF-I has not been observed to increase in older individuals after resistance exercise training (57,68,71). Despite the lack of evidence for resistance training-associated changes in basal serum IGF-I, a role for IGF-I in the mediation of resistance exercise-associated effects on skeletal muscle and bone mass in older adults might be found in the the acute physiological response of IGF-I and IGFBP-3 to a single bout of resistance exercise.

Evidence is increasing that some of the major health related changes produced by physical activity may be related to a repetition of the acute physiological responses during and shortly after each exercise bout (29). Therefore, resistance training-related changes in body composition and muscular strength may be mediated, in part, by repeated, acute, transient changes in IGF-I or IGFBP-3 rather than solely by chronic alterations in basal levels.
Several studies have previously examined IGF-I responses to acute resistance exercise, and have failed to find any consistent pattern (37-40). However, previous studies are limited, in that they were designed to assess a wide range of humoral responses and not specifically to assess the acute response of IGF-I to resistance exercise. While studying acute IGF-I responses to resistance exercise, investigators have generally examined post-exercise IGF-I levels for a maximum of only 2 hours after the completion of a resistance exercise session (38-40). Such a short observation period may fail to discern GH dependent alterations in IGF-I, as IGF-I responses to increased circulating GH have been reported to occur from 4 to 12 hours after a GH surge (46). Although IGFBP-3 modulates IGF-I bioavailability, no previous studies have examined the response of IGFBP-3 to acute resistance exercise. Moreover, most studies of acute responses of IGF-I to resistance exercise have utilized young adult subjects (37-40). Acute IGF-I responses to resistance exercise have not been well described in men and women over 50 years of age, who are more at risk of debilitating loss of skeletal muscle and bone mass.

The purpose of the present study was to assess changes in circulating levels of IGF-I and IGFBP-3 resulting from a single session of heavy resistance exercise over a 48 hour period in previously inactive men and women between the ages of 50 and 60. Information gained from such a study may be useful in standardizing blood collection protocols for serum IGF-I and IGFBP-3 in resistance training studies, with respect to the timing of the last acute exercise session. Ultimately such information may help to determine whether the acute changes in IGF-I or IGFBP-3 after heavy resistance exercise are sufficient to be considered a putative humoral mechanism, contributing to increases in muscular strength and FFM associated with resistance exercise training.

It was hypothesized that serum IGF-I levels would be increased from baseline immediately after and at 12 and 24 hours after a single bout of resistance exercise. An immediate post-exercise increase in serum IGF-I would occur too rapidly to be attributable to exercise-induced pituitary GH secretion (8). In contrast, the hypothesized increases in
serum IGF-I levels at 12 and 24 hours after resistance exercise would be more likely due to the stimulatory effects of increased GH secretion on hepatic and extrahepatic IGF-I synthesis and secretion. Finally, it was hypothesized that IGFBP-3 levels would be significantly increased from baseline at 24 and 48 hours after resistance exercise as a result of GH stimulated synthesis and secretion of IGFBP-3 by hepatic and extra-hepatic tissue.

Methods and Procedures

The present study was an ancillary study to a resistance training study conducted by the Oregon State University Bone Research Laboratory, and made use of a previously recruited sample, which is described briefly below.

Subjects

12 men and 7 women volunteered to participate in the present study. Of the volunteers who participated, 3 men and 1 woman were later excluded from the sample due to missing values. The participants in the present study were recruited from a sample of 56 previously sedentary men and women (27 men, 28 women) between the ages of 50 and 60, who had already been recruited for a 9-month trial designed to evaluate the effect of resistance exercise on muscular strength, bone density, as well as basal IGF-I and IGFBP-3 levels. Subjects were recruited for the larger training study, primarily through newspaper advertisements, and were excluded if they had participated in any exercise program or performed any strenuous endurance or resistance exercise in the past year. Subjects who had any medical conditions affecting their ability to perform heavy resistance exercise or known to affect bone density were also excluded from the study, as well as those individuals taking medications or substances in quantities known to affect bone density. All smokers were excluded. Each subject accepted into the study obtained medical clearance from his or her physician to participate in heavy resistance training. The
menopausal status of all female participants was determined through a questionnaire, though no exclusions were made on the basis of menopausal status alone. Four of the female participants were post-menopausal, whereas 2 of the women had menstruated in the past 2 months and were considered premenopausal. Only those women who were not on hormone replacement therapy were accepted into the present study and the larger training study.

All subjects gave written informed consent to participate in the present study. The protocol was approved by the Oregon State University Institutional Review Board.

Protocol

**Preliminary testing and familiarization procedures:**

A 3-week period was utilized for protocol familiarization and load verification for the exercises to be used in the resistance exercise protocol. Each subject participated in a total of 8 sessions of 60 minutes duration prior to the experimental exercise protocol. The first 4 sessions consisted of orientation and practice sessions, in which participants practiced correct form for each exercise, using minimal resistance. These sessions encouraged the use of the appropriate muscle groups and form for each exercise, in order to reduce the risk of injury during the maximal dynamic strength testing session and the experimental exercise protocol. Exercises were performed using resistance exercise machines (Hammer Strength Training Equipment Co., Cincinnati, OH).

During the fifth session, maximal dynamic strength was measured according to the 1-repetition maximum (1-RM) principle. 1-RM was defined as the maximal weight the individual could lift one time with acceptable form. Acceptable form required that the exercise be executed primarily by the specified muscle groups without the use of momentum or changes in body position (57). If the criteria for acceptable form were not satisfied, the highest correctly lifted weight was assigned the 1-RM value.
The final 3 familiarization sessions, performed over the 7 days after the 1-RM testing session, were similar to the previous 4 practice sessions, in that appropriate form with minimal resistance was emphasized. The final 3 sessions of familiarization allowed for recovery from muscle soreness incurred during the 1-RM testing session and prior to the experimental resistance exercise session.

**Experimental Protocol:**

All subjects taking part in the present study participated in an experimental resistance exercise session of approximately 60 minutes in duration. See Figure 1. Blood was drawn by venipuncture 10 minutes prior to the start of the exercise session, immediately after the exercise session, as well as 12, 24, and 48 hours after the initial resting blood sample.

Fig. 1. Timeline of the Experimental Resistance Exercise Session

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Seated Rest</th>
<th>Baseline Blood Sample</th>
<th>Seated Rest</th>
<th>Exercise Session</th>
<th>Post-ex. Blood Sample</th>
<th>Seated Rest</th>
<th>12 Hour Blood Sample</th>
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<td>+47:50</td>
<td>+48:00</td>
</tr>
</tbody>
</table>

*Time is shown in hours:minutes

All subjects were advised to avoid any strenuous physical activity for the previous 48 hours prior to the experimental exercise session and to avoid ingesting alcohol or caffeine-containing beverages for 12 hours prior to the experimental exercise session.
Subjects arrived at the testing site on the day of the experimental exercise session, having consumed nothing but water for at least 8 hours. Although circadian variation in IGF-I and IGFBP-3 has not been identified in normally active adults (23,34,69), all testing sessions were conducted within a 2 hour period, at the same time, in the early morning (6:30 to 8:30 am), in order to minimize diurnal variation.

A 20-minute equilibration period before the experimental exercise session was used to ensure that resting basal hormonal concentrations were obtained. Subjects rested in a seated position for 10 minutes prior to the initial blood draw, and they rested for 10 more minutes (a total of 20 minutes) after the initial blood draw and prior to the experimental resistance exercise session. Subjects were informed that they would not start exercising until 10 minutes after the resting blood sample was obtained. This 20-minute “equilibration period” has been shown to eliminate any significant anticipatory increases in resting hormonal concentrations (39). All blood samples were obtained with the subject in a seated position. The 10-minute period of rest in a seated position was repeated prior to each of the blood draws at 12, 24, and 48 hours.

The experimental resistance exercise session consisted of 6 resistance exercises, performed by each subject in the following order:
1. Leg Press
2. Chest Press
3. High Lat Pull
4. Leg Extension
5. Shoulder Press
6. Leg Curl

The load for each exercise was set at 85% of that individual’s 1-RM for the specific exercise. Subjects were encouraged to complete 3 sets of 6 repetitions of each exercise, with 3 minutes rest between sets and exercises. The load, number of sets, and repetitions completed for each exercise were recorded for each individual. The entire experimental
exercise session lasted approximately 60 minutes. Water was provided for the subjects to drink ad libitum throughout the exercise session.

The post exercise venous blood sample was drawn immediately after the last resistance exercise set was completed. Following the post-exercise blood draw, subjects resumed their normal daily activities. They were instructed to avoid alcohol and caffeine for the next 12 hours following the initial blood sample, and to avoid any strenuous exercise for the next 48 hours. Additionally, they were instructed to consume nothing but water for 4 hours prior to the scheduled time for the 12 hour sample. Instructions for the subsequent morning blood samples were identical to those for the initial resting blood sample. Compliance to the above instructions was verified by asking the participant when s/he last consumed anything other than water prior to the blood draw.

**Dual energy X-ray absorptiometry (DXA):**

As part of the larger training study, a total body scan was performed using a Hologic QR 1000/W dual-energy X-ray absorptiometer. Bone mineral density (BMD) of the lumbar spine (L2-L4), proximal femoral neck and the entire body were quantified in g/cm². Bone free lean mass, fat mass, and body fat percentage were also assessed using DXA. Coefficients of variation for repeated DXA scans at the OSU Bone Laboratory are less than 1.0% for BMD of hip and lumbar spine, and 1.5% for body composition (60).

**Specimen handling and analyses**

**Blood collection and specimen handling:**

Venous blood samples were obtained through venipuncture. Blood for determining circulating levels of IGF-I and IGFBP-3 levels was collected in serum separator tubes. After collection the tubes of serum were allowed to stand at room temperature for 30
minutes to allow for coagulation. Once coagulated, the blood was centrifuged at 1500 x g for 15 minutes to separate the serum from red blood cells. Following centrifugation the separated serum was aliquotted to cryovials, frozen, and stored at -70 degrees C until it was shipped for analysis. Whole blood for determination of hematocrit and hemoglobin was collected in tubes containing EDTA. Whole blood samples were refrigerated for no more than 2 hours, after which, 2 capillary tubes of whole blood were removed for hematocrit measurement. The remaining whole blood was transferred, frozen, and stored at -70 degrees C for later analysis of hemoglobin concentration.

**Hematocrit and Hemoglobin:**

Hemoglobin was analyzed in duplicate with a Milton Roy Spectronic 401 Single-beam Spectrophotometer, using the cyanmethemoglobin method (Sigma Chemical, St. Louis MO). The intra-assay coefficients of variation (CVs) for the hemoglobin assay ranged from 0.8% to 2.2%. The inter-assay CV was 2.1%.

Hematocrit was analyzed in duplicate with a standard microcapillary technique (21). The percent changes in plasma volume were calculated according to the equations of Dill and Costill (18). All serial, time dependent determinations on whole blood or serum for one individual were processed in a single assay to minimize inter-assay variability.

**IGF-I and IGFBP-3:**

Serum samples for determination of IGF-I and IGFBP-3 were shipped frozen on dry ice, via an overnight shipping service, to the Maine Center for Osteoporosis Research and Education in Bangor, Maine. To prevent technician bias, within subject samples for IGF-1 and IGFBP-3 were coded to blind laboratory technicians to the time of collection. All serial, time dependent determinations for one individual were processed in a single assay to minimize inter-assay variability. Samples for serum IGF-I assay were prepared
for analysis by acid ethanol cryoprecipitation (AEC) as per Breier et al. (7). In this manner, IGF-I is extracted from its binding proteins. Acid ethanol extraction followed by cryoprecipitation has been found to reduce residual IGFBPs to a level that does not interfere with the competitive antigen-antibody binding in the radioimmunoassay (RIA) of IGF-I. The recovery of radio-labelled $^{125}$ IGF-I determined by high performance gel chromatography is 98% for AEC-extracted serum (7). Following AEC, the samples were analyzed by radioimmunoassay with IGF-I kits from Nichols Institute Diagnostics (San Juan Capistrano, CA). Samples were extracted and analyzed in duplicate. The intra-assay CVs ranged from 0.4% to 6.0%. The inter-assay CV was 2.5%. Serum IGF-I, determined from AEC and RIA in this manner, has been dissociated from its binding proteins, and represents total (bound and free) IGF-I levels.

IGFBP-3 was assayed in duplicate using a two site immunoradiometric assay (IRMA) kit (Diagnostic Systems Laboratories, Inc. Webster TX). The intra-assay CVs ranged from 0.9% to 5.9%, and the inter-assay CV was 5.9%. IGFBP-3 analyzed in this manner represents total (bound and whole, bound and truncated, and free) serum IGFBP-3.

Statistical analyses

Multivariate analysis of variance (MANOVA) for repeated measures was used to examine whether values for serum concentrations of IGF -I and IGFBP-3 (both unadjusted and adjusted for plasma volume changes) varied by gender and time. The MANOVA also examined whether any significant gender by time interactions were present. The critical alpha level for significance was set at p<0.05. F ratios with p<0.05 were followed by Duncan’s multiple range post hoc test to determine where the significant differences were located. The Statistical Package for the Social Sciences (SPSS 6.1 for Power Macintosh) was used for the statistical analysis of the results of the present study (65).
Results

Subject Characteristics

The characteristics of the subjects included in the analysis are presented in the table below. Males were taller, heavier, and leaner than females. No significant gender differences in basal serum IGF-I or IGFBP-3 were observed.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
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<tr>
<td>Age, yr</td>
<td>52.8 ± 3.1</td>
<td>53.2 ± 2.7</td>
<td>0.802</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166.8 ± 3.1</td>
<td>180.1 ± 3.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>69.4 ± 10.1</td>
<td>94.5 ± 12.4</td>
<td>0.001</td>
</tr>
<tr>
<td>% Body fat</td>
<td>31.4 ± 8.2</td>
<td>24.0 ± 3.5</td>
<td>0.031</td>
</tr>
<tr>
<td>Serum IGF-I, ng/mL</td>
<td>142 ± 42</td>
<td>144 ± 45</td>
<td>0.923</td>
</tr>
<tr>
<td>Serum IGFBP-3, ng/mL</td>
<td>2814 ± 279</td>
<td>2484 ± 446</td>
<td>0.133</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± SD  
<sup>b</sup>P values derived from t-tests between gender
Estimated Plasma Volume Change

To quantify the effect of plasma volume shifts on circulating IGF-I and IGFBP-3 levels following a single session of resistance exercise, plasma volume changes were estimated from hematocrit and hemoglobin, so that the post-exercise hormonal concentrations could be adjusted for plasma volume. The largest mean change in plasma volume was a decrease of 3.6% observed immediately after the resistance exercise bout. See Figure 2.

![Mean Percent Change in Plasma Volume Over Time](image)

<table>
<thead>
<tr>
<th>Time of Sample</th>
<th>% Change in Plasma Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post Ex</td>
<td>-3.6 ± 6.8</td>
</tr>
<tr>
<td>12 hr</td>
<td>-0.6 ± 7.3</td>
</tr>
<tr>
<td>24 hr</td>
<td>0.6 ± 6.7</td>
</tr>
<tr>
<td>48 hr</td>
<td>-2.5 ± 7.5</td>
</tr>
</tbody>
</table>

Figure 2. Percent change in plasma volume from pre-exercise value immediately after resistance exercise, 12, 24, and 48 hours after the pre-exercise sample. Data are mean ± SD.
**Serum IGF-I:**

The results of the MANOVA indicated that serum IGF-I, unadjusted for plasma volume changes, differed by time (p=0.020), but not by gender (p=0.829), nor by the interaction of gender x time (p=.508). Post hoc comparisons revealed significant pairwise differences between the immediate post-exercise sample and the 12 hour post-exercise sample (mean decrease of 10.2 ng/mL), as well as between the immediate post-exercise sample and the 24 hour post-exercise sample (mean decrease of 11.8 ng/mL). Although the unadjusted serum IGF-I concentrations varied significantly by time, no significant changes in unadjusted IGF-I were observed relative to basal, pre-exercise levels. See Figure 3.

IGF-I, adjusted for plasma volume changes, did not differ by time (p=0.324), gender (p=0.960), nor by the interaction of gender x time (p=0.806).

**Serum IGFBP-3:**

Serum IGFBP-3 levels, unadjusted for plasma volume changes, differed by time (p=0.050), but not by gender (p=.092), nor by the interaction of gender x time (p=.908). Post hoc comparison revealed a significant pairwise difference between the immediate post-exercise sample and the 24 hour post-exercise sample (mean decrease of 150.2 ng/mL). Similar to the time-related changes in unadjusted serum IGF-I levels, no significant changes in unadjusted serum IGFBP-3 levels were observed relative to the basal, pre-exercise levels. See Figure 4.

Although the plasma volume-adjusted IGFBP-3 levels were significantly higher (p=0.019) in females (mean = 2855 ng/mL) than in males (mean = 2437 ng/mL), serum IGFBP-3 levels, adjusted for plasma volume changes, did not differ by time (p=0.724), nor by the interaction of gender x time (p=0.614). The main effect of time and the interaction of gender x time are the experimental effects of interest.
Figure 3. No significant differences from baseline are indicated in plasma volume unadjusted (●) and adjusted (○) concentrations of IGF-I before and immediately after a resistance exercise session, 12, 24, and 48 hours after the pre-exercise blood sample. Data are means ± SD.
Figure 4. No significant differences from baseline are indicated in plasma volume unadjusted (●) and adjusted (○) concentrations of IGFBP-3 before, immediately after a resistance exercise session, 12, 24, and 48 hours after the pre-exercise blood sample. Data are means ± SD.
Discussion

In the present study no significant changes in IGF-I or IGFBP-3 concentrations from baseline were observed after a single bout of heavy resistance exercise, in previously sedentary 50 to 60 year old individuals, over an observation period of 48 hours. The magnitude of acute change from baseline in circulating IGF-I or IGFBP-3 likely to account for chronic resistance training adaptation in skeletal muscle and bone is unknown. However, it is unlikely that the small differences from baseline in serum IGF-I and IGFBP-3 observed over time in the present study could account for the well documented increases in muscular strength and skeletal muscle mass associated with chronic resistance training (22,25,71). The percent change in IGF-I from baseline over the post exercise sample times ranged from 0.5% to 4.9%, while the percent change in IGFBP-3 from baseline ranged from 0.04% to 3.1%. Such small differences are within the measurement error for the assays, and thus might reflect measurement error rather than meaningful biological changes in serum IGF-I or IGFBP-3 concentrations.

It is unlikely that the lack of significant changes from baseline after acute resistance exercise was due to abnormal serum IGF-I or IGFBP-3 levels in the sample studied. Although the 50 to 60 year old age group has not been well studied with respect to normal basal IGF-I and IGFBP-3 concentrations, it would appear from existing data on men and women, 50 to 60 years of age and older, that the mean values for basal serum IGF-I and basal serum IGFBP-3 were comparable to those found by other investigators (4,12,54, 64,72). Basal IGF-I and IGFBP-3 values in the 50 to 60 year olds observed in the present study, as well as those observed by other investigators, are markedly lower than IGF-I and IGFBP-3 levels of younger individuals. This well documented age-related decline in IGF-I and IGFBP-3 has been associated with documented age-related declines in pulsatile pituitary GH secretion. Additionally, exogenous growth hormone administration to otherwise healthy older adults results in restoration of basal serum IGF-I and IGFBP-3 to
young adult levels (46,62,71), providing evidence that serum IGF-I and IGFBP-3 levels are responsive to changes in circulating GH levels.

If acute changes in serum IGF-I and IGFBP-3 related to resistance exercise were growth hormone dependent, the subjects in the present study might lack the critical GH response to resistance exercise necessary to evoke changes in serum IGF-I and IGFBP-3. Pykka et al. (58) found that strenuous resistance activity leads quickly to a significant rise in circulating GH in young men and women. However, this response is markedly attenuated in older men and women (mean age 72 ± 0.8 y). Although resistance training has been observed to result in increased GH secretory response to resistance exercise in young adult men, such responses to resistance training in older adults have either not been observed (57), or have been attenuated compared to young adults (13). Studies of GH secretory response to resistance exercise in 50 to 60 year old men and women are lacking.

Physiological responses of untrained men and women to a single bout of heavy resistance exercise may differ from the physiological responses of resistance exercise trained individuals. The participants in the present study were relatively sedentary and had not participated in any resistance training for a minimum of one year prior to undertaking the experimental exercise protocol. It is possible that resistance trained individuals might demonstrate augmented acute changes in circulating IGF-I or IGFBP-3 as a result of the last bout of resistance exercise. As an individual’s work capacity increases and the absolute intensity of exercise performed during the exercise session is increased, acute responses to physiological or biochemical reactions may be enhanced (29). The acute effects of a single bout of heavy resistance exercise on circulating IGF-I and IGFBP-3 levels in resistance-trained individuals have not been investigated.

The configuration of the experimental resistance exercise protocol itself with respect to load, repetitions, and rest, may not have been optimal for inducing changes in GH, IGF-I, or IGFBP-3 levels. The experimental exercise protocol used in the present study utilized heavy loads (85% of 1-RM), relatively few repetitions (5 to 6 repetitions) and relatively
long rest intervals (3 minutes between sets and exercises) in order to produce high levels of force on selected muscle groups and body segments while limiting physiological effects often associated with endurance exercise (e.g. sustained increases in VO$_2$). Kraemer et al. (38-40) have examined acute GH and IGF-I responses of young adult males and females to single bouts of resistance exercise in which the load, the number of repetitions, and the rest between sets were varied. Although they examined comparable resistance exercise protocols to that used in the present study, only resistance exercise protocols utilizing more moderate loads (approximately 70% 1-RM), increased repetitions (10 repetitions) and shorter rests between sets (1 min) resulted in higher levels of GH during and up to 2 hours after exercise.

Endurance type exercise, may be a more potent stimulus of GH or IGF-I secretion than heavy resistance exercise. Although, resistance training studies have failed to observe increases in basal IGF-I levels in older individuals (57,64,71), there is evidence that endurance training may increase basal serum IGF-I levels in older men (55).

Changes in total IGF-I or total IGFBP-3 may not be necessary to induce a meaningful accumulation of acute exercise effects. Acute resistance exercise might result in increased activity of IGFBP-3 specific proteases, resulting in increased dissociation of IGF-I from the IGFBP-3 ternary complex. Proteolytic cleavage of the IGFBP-3 ternary complex results in a truncated IGFBP-3, which has a greatly reduced affinity for IGF. Free IGF-I and the proteolyzed IGFBP-3-IGF-I complex are capable of efflux from the circulation (33), making IGF-I available for interaction with tissue receptors. Increased specific protease activity toward IGFBP-3 has been observed in pregnancy (28) and a number of catabolic states (52), including the post-operative period after major surgery (34). The mechanisms for the regulation of IGFBP specific protease activity are presently unclear. However, hormonal modulation of IGFBP proteolysis in animals and humans has recently been observed (5, 63). Increased circulating GH, which may be provoked by resistance exercise, has been found to increase IGFBP-3 proteolysis, and may modulate
IGF-I activity indirectly by increasing proteolysis of the IGFBP-3 ternary complex and increasing the bioavailability of IGF-I (63).

If resistance exercise were to induce an increase in IGFBP-3 specific proteolysis, bioavailable IGF-I might increase, while total serum IGF-I might remain unchanged or even decrease as a result of IGFBP-3 proteolysis. The assays for IGF-I and IGFBP-3 in the present study cannot distinguish free IGF-I from protein-bound IGF-I, nor can they distinguish intact IGFBP-3 from IGFBP-3 that has undergone proteolysis. To date, no studies on the effect of exercise on free IGF-I levels or on IGFBP proteolysis have been published. Thus, the effects of exercise on free IGF-I and on IGFBP protease activity should be examined.

Additionally, if sensitivity to IGF-I increased acutely as a result of resistance exercise, the biological action of IGF-I could be enhanced without a concomitant rise in tissue or circulating IGF-I. Although acute changes in IGF-I sensitivity after exercise have not been studied, changes in skeletal muscle sensitivity to IGF-I have been observed in animals (42,43). The status of the IGF-I receptor is also dependent on the hormonal milieu. In vivo, decreased local and circulating levels of IGF-I appear to increase IGF-I receptor gene expression and binding, while in cell culture, increasing IGF-I concentration causes a decrease in receptor number. Other growth factors and steroid hormones can also modulate the expression of the IGF-I receptor in vitro (42). Resistance exercise is capable of inducing acute changes in the circulating hormonal milieu (14,37-40). Although the effects of resistance exercise on IGF-I receptor status awaits investigation, it is possible that significant acute increases in protein synthesis documented in skeletal muscle within 24 hours after a single bout of resistance exercise (45) may be related to increases in IGF-1 receptor binding, or to unknown changes in post-receptor activity, rather than to absolute increases in circulating or tissue IGF-I concentrations.

The lack of acute change in total serum IGF-I and IGFBP-3 levels observed in the present study does not preclude the possibility that resistance exercise augments IGF
bioavailability through IGFBP proteolysis or that exercise augments IGF bioavailability by increasing tissue sensitivity to circulating IGF concentrations. The effect of resistance exercise on IGFBP-3 proteolysis, as well as tissue sensitivity to IGF-I, warrant further investigation.

Failure of the present study to observe any acute change from baseline in serum IGF-I and IGFBP-3 may also indicate that IGF-I is not critical to resistance exercise-related increases in muscular strength and FFM in older individuals. Resistance training studies of older adults have failed to observe changes in serum IGF-I despite finding significant increases in muscular strength and FFM (57,68). Moreover, exogenous GH administration to young and older men participating in heavy resistance training programs has not resulted in increased muscular strength compared to resistance training alone, despite marked increases in serum IGF-I and IGFBP-3 in GH-treated individuals (70,71).

The results of the present study suggest that there are no significant changes from baseline in serum IGF-I or serum IGFBP-3 over a 48-hour period after a bout of heavy resistance exercise in untrained individuals between 50 and 60 years of age. This information may be used to standardize the time of blood collection for basal serum IGF-I and IGFBP-3 levels after the last bout of resistance exercise in a resistance exercise training study, provided that the magnitude of acute changes in serum IGF-I and IGFBP-3 levels observed herein do not differ from those of resistance trained individuals.

Data from the present study suggest that drawing the post-training blood sample as soon as immediately after the last acute bout of resistance exercise would not result in significantly different values from those obtained at rest, if those values are corrected for plasma volume changes. If correction for acute resistance exercise-associated plasma volume shifts are not planned, the post-resistance training samples for serum IGF-I and IGFBP-3 should be delayed until 12 to 24 hours after the last acute bout of resistance exercise.


