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Resistance Training

RESISTANCE TRAINING AND TESTOSTERONE REPLACEMENT INDUCED CHANGES IN BODY COMPOSITION, FREE TESTOSTERONE, IGF-1, and IGFBP-3 IN THE FRAIL ELDERLY

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ABSTRACT

Lambert CP, Bopp MM, Johnson LE, Sullivan DH. Resistance Training and Testosterone Replacement Induced Changes in Body Composition, Free Testosterone, IGF-I, and IGFBP-3 in the Frail Elderly. ***JEP****online* 2007;10(1):48-56. The purpose of this investigation was to examine the effects of two resistance exercise intensities and testosterone replacement on changes in circulating hormone/growth factor concentrations and their relationship to body composition changes in frail elderly individuals. This study design was a 2 x 2 x 2 factorial with Drug (100 mg/wk of testosterone; T or no-testosterone), Resistance Exercise (Low Intensity and High-Intensity) and Time (Pre and Post Intervention). The duration of the interventions was 12 weeks. Subjects for this investigation were sixty-one men with a mean age of 77.8±6.1, a total testosterone concentration of <480 ng/dL, and who were in the recuperative phase of a recent illness. Serum was analyzed for insulin like growth factor-1 (IGF-1), IGF binding protein 3 (IGFBP-3), sex hormone binding globulin (SHBG), and total testosterone. Leg strength and mid-thigh cross-sectional muscle area were also measured pre- and post-intervention. As expected, total testosterone and free testosterone were significantly elevated by testosterone administration (each $p<0.001$). There were trends for a reduction in IGFBP-3 ($p=0.175$) and an increase in IGF-I/IGFBP-3 ratio ($p=0.094$) by testosterone administration. There was a significant time effect with regard to IGF-I with the mean of the four groups increasing over time ($p=0.013$). Significant correlations were observed between the post-intervention

total (r=0.385; p=0.002) and free (r=0.315;p=0.013) testosterone concentrations and the % change in mid-thigh cross-sectional muscle area. The Post-intervention IGF-I/IGFBP-3 ratio correlated significantly with % change in mid-thigh cross-sectional muscle area (r=0.269 p=0.036) and the % change in fat free mass (r=0.261; p=0.05). These data would suggest that elevations in the total and free testosterone as well as in the IGF-I/IGFBP-3 ratio after testosterone administration may be indicators of changes in muscle mass with testosterone administration in older hypogonadal men recovering from a recent illness.

Key Words: Exercise, Aging, Hormone Replacement, Rehabilitation

INTRODUCTION

It is well documented that both muscle strength and muscle mass decline steadily with advancing age (1). Larsson et al. (2) reported that muscle strength is maintained between the ages of 30-50 but after the age of 50 until approximately 70 years of age there is about a 30% drop in muscle strength. It is also known that muscle mass is the primary determinant of age-related changes in muscle strength (3). Although such declines occur even in healthy individuals as they age, it is often the combined effects of acute and chronic illness with aging that lead to the most profound losses. Any reduction in muscle mass potentially contributes to a decline in functional capacities. When the loss of muscle mass reaches the point where the individual is no longer able to compensate, failure to independently perform one or more activities of daily living may occur (4). Progressive resistance muscle strength training has been utilized successfully to increase strength in elderly individuals (5,6,7). Further, it has been reported that lower intensity resistance exercise (40-50% of 1RM) was as effective as higher intensity resistance exercise (80% of 1RM) with regard to strength gains in elderly individuals (8). Resistance exercise acts to increase strength via an increase in neuromuscular activation by way of increased motor unit firing rates and increased recruitment of motor units and by increasing the hypertrophy of existing muscle fibers. Additionally, testosterone replacement therapy alone has been successfully utilized to increase strength in elderly men (9,10,11). Testosterone acts to increase the mass of existing muscle fibers by increasing intramuscular insulin-like growth factor-I (IGF-I) which is involved in the stimulation of muscle protein synthesis. In addition to the paracrine and/or autocrine release of IGF-I testosterone administration results in an increase in circulating growth hormone (GH) and IGF-I concentrations (12) Gentili et al. (12) have suggested that the mechanism by which testosterone increases GH and IGF-I concentrations in the circulation is by increasing GHreleasing hormone secretion and/or by a reduction in somatostatin release. Although resistance training and testosterone administration have been utilized independently in healthy older men with success, to our knowledge, no study has combined these interventions in very frail older men (ie. severely impaired physical function) in the recuperative phase of a recent illness. The purpose of the present investigation was to examine the effects of low intensity (20% of 1RM) and high-intensity (80% of 1 RM) resistance training, alone, or with testosterone replacement, on circulating hormone/growth factor concentrations as well as the relationship between the changes in these humoral factors and changes in body composition in frail elderly individuals with subnormal circulating testosterone concentrations.

METHODS

Subjects

Physicians responsible for the care of patients within the inpatient and outpatient Geriatric Evaluation and Management (GEM) clinics, and the Transitional Care Unit from a Veterans Affairs hospital referred patients who were age 65 or older and had a recent decline in function. This decline in

function was deemed reversible by the referring physician. All of the subjects had either lost a significant amount of weight and/or their gait speed was less than the median for a reference population of healthy elderly adults. Additionally, 70% of the patients enrolled had described their health as fair to poor. All included subjects also had a serum total testosterone <480 ng/dL and the ability to provide informed consent. Subjects were excluded for unstable cardiovascular disease, an abnormal prostate exam suggestive of prostate cancer and/or a history of prostate cancer, and/or a prostate specific antigen >10 ng/mL. Additionally, individuals for whom independent ambulation was unlikely, such as those with permanent neurological disease or disabling arthritis, were also excluded. All volunteers were male and had a mean$\pm$SD age of 77.8$\pm$6.1 with a range of 65.3-93.2.
The requirements and possible risks of the study were explained to the subjects verbally and in writing. Each subject signed the Institutional Review Board approved study consent and the Health Insurance Portability and Accountability Act (HIPAA) consent, prior to beginning study procedures. The subjects' clinical data were then re-reviewed by the study physician and a physical exam with rectal exam was performed.

Experimental Design

The study was 2 X 2 X 2 experimental design with drug (Testosterone or Placebo) X resistance exercise of varying intensity (high-intensity or low-intensity) X time (Pre-intervention and Post-Intervention). The study is described briefly below. Further details of the study design and subjects are provided elsewhere (13).

Resistance Training

Subjects were instructed as to the proper technique for using the exercise equipment. Two exercises were utilized: 1) The chest press, and 2) the leg press. The chest press requires utilization of the pectoralis, front deltoid, and triceps muscle groups; and the leg press requires the use of the gluteal, the quadriceps, and the hamstring muscles. Both of the resistance training machines were pneumatic devices (Keiser Sports Health Equipment, Fresno CA) that provided an isotonic force. During training subjects performed a warm-up set and three sets of eight repetitions. The repetition rate and the amount of rest between sets were adjusted to maintain the subject's heart rate below 110 bpm. Exercise session termination criteria were as follows: 1) chest pain, 2) lightheadedness, 3) severe shortness of breath, 4) a greater than 20 mmHg drop in blood pressure, 5) a sustained elevation in blood pressure >200/100 mmHg, or 6) a heart rate >140 bpm. Subjects were also advised to terminate an exercise session if they felt too weak or ill to continue.

Low-Intensity Training (LIT)

Subjects performed a warm-up set at approximately 10% of one repetition maximum (1RM) (as defined below) and then completed three sets of 8 repetitions at 20% of 1RM. The duration of the LIT resistance training regimen was 12 weeks.

High-Intensity Training (HIT)

For the first week of training subjects trained at 20% of 1 RM. The intensity during week 2 was 50% of 1 RM while during week 3 through week 12 the resistance was set as high as the subject could tolerate for 3 sets with the goal being $\geq$ 80% of 1RM. The duration of the HIT resistance training regimen was 12 weeks in duration.

Testosterone Administration

On the second of the 3 weekly visits for all 12 weeks of the intervention, patients received either testosterone enanthate or placebo. For week one, 50 mg of testosterone enanthate dissolved in sesame seed oil or an equal volume of placebo (sesame seed oil) was administered by intramuscular injection while each of the subsequent weeks, 100 mg of testosterone or an equal volume of placebo was administered.

Procedures

Mid-Thigh Muscle Cross-sectional area

This was determined using a General Electric Medical Systems Computed Tomography scanner (Waukesha, WI). One 10 mm slice was obtained at the midpoint between the patella of the dominant

leg and the right iliac crest prior to the intervention and after the intervention. Pre-intervention and post-intervention scans were obtained from the same location for a given individual via the use of measurements from bony landmarks. The images were then analyzed via personal computer using medical imaging software (SliceOmatic version 4.2, TomoVision, Montreal, Canada). Muscle, adipose, and bone areas were determined to the nearest 0.01 cm^2.

Fat Free Mass via Whole Body Air Displacement Plethysmography

Body volume was determined using this method and with body mass, body density was calculated (D=M/V). Body fat was calculated from body density using the equation of Siri (14). Fat free mass was taken as the difference between total body mass and fat mass.

Muscle Strength Testing

The one repetition maximum (1RM), which is the maximum amount of weight an individual could lift through the full range of motion with correct form, was used to assess muscle strength for both exercises. During the 1RM determination the subject's vital signs were determined frequently and the electrocardiogram was monitored continuously. Prior to the determination of 1RM a warm-up set was performed with 20% of 1RM. The resistance was increased gradually and 30 seconds of recovery separated each contraction.

Biochemical Analyses

Serum sex hormone binding globulin (SHBG) was measured using an enzyme linked immnosorbent assay (ELISA) from Alpco Diagnostics (Windham, NH). IGF-I, and IGFBP-3 were measured using Quantikine® Immunoassay from R&D Systems, Minneapolis, Minnesota. Total testosterone was measured using the Beckman Coulter, Inc. Access Testosterone system that utilizes a competitive binding immunoenzymatic assay. Free testosterone (nmol/L) was calculated from total testosterone and SHBG concentrations according to the law of mass action as described by Sodergard et al.(15). Association constants of SHBG and albumin for testosterone of 1×10^9 L/M and 3×10^4 L/M, respectively, were used for all samples.

Statistical Analyses

As the first step in the analyses, all of the hormone/ growth factor (IGF-1, IGFBP-3, IGF-1 to IGFBP-3 ratio, total testosterone, and free testosterone), body composition (mid-thigh cross-sectional muscle area and fat free mass) and muscle strength data (chest strength and leg strength) were log-transformed. To evaluate the effect of the interventions on IGF-1, IGFBP-3, IGF-1 to IGFBP-3 ratio, total testosterone, and free testosterone, three-way ANOVAs were conducted with Drug (testosterone vs. placebo), Exercise (high-intensity training vs. low intensity training) and Time (pre and post) being the factors. To evaluate the effect of the interventions on the % change of each of the above mentioned hormone/ growth factors, two factor ANOVAs were performed. The difference in the admission and final log-transformed data were taken to represent the % change for the given variable. For each of these analyses, Drug (placebo vs. testosterone) and Exercise (low-intensity vs. high-intensity) were the factors. If an ANOVA revealed a significant effect, the individual groups were compared using Tukey's multiple comparison procedure. The relationship between the % change in each hormone/ growth factor and the % change in each body composition (mid-thigh cross-sectional muscle area and fat free mass) and muscle strength (chest strength and leg strength) outcome variable was examined using univariate statistics (Pearson Product Moment correlations). For each of these analyses, the difference in the admission and final log-transformed data were again taken to represent the % change for the given variable. The same approach was used to assess the strength of the correlations between the post-intervention hormone/growth factors and the % change in each measure of body composition. To determine if there were possible additive effects of the various hormone/growth factors on the % change in mid-thigh cross-sectional muscle area, multi-variable least-squared regression analyses using a forward step-wise procedure were utilized. Two analyses were conducted. For the first, the post-intervention IGF-I to IGFBP-3 ratio, the post intervention total testosterone, and the post-intervention free testosterone were entered as the independent variables. In the second, the % changes (defined as the difference in the admission and final log-transformed

data) in free testosterone, total testosterone, and the IGF-I to IGFBP-3 ratio were the independent variables. In both analyses, % change in mid-thigh muscle cross-sectional area was the dependent variable. All the analyses for this study were performed using SAS for Windows Version 9.1 (SAS Institute Inc. Cary, NC, 2003). Statistical significance for each analysis was defined as $p \leq 0.05$.

RESULTS

Effects of the Interventions on Indicators of Hormone Status

There were significant Drug x Time interactions for both total testosterone (reported elsewhere (13)) and free testosterone with the post-intervention values for the groups that were receiving testosterone being significantly higher than the post-intervention values for the groups not receiving testosterone ($p<0.001$ for each analysis). For IGF-1 there was a significant time effect ($p=0.013$) with the mean post-intervention IGF-1 concentration of the four groups being significantly higher than the pre-intervention mean (Figure 1). There was also a significant time effect ($p=0.005$) observed for the IGF-1 to IGFBP-3 ratio but not for IGFBP-3. Significant drug effects were observed for % change in total testosterone ($p<0.001$) and % change in free testosterone ($p<0.001$) with the mean for those groups receiving testosterone having a significantly greater % change than the mean for those groups not receiving testosterone (Table 1). No significant effects of drug or exercise were seen on the % change in IGF-I, IGFBP-3, or the IGF-1 to IGFBP-3 ratio.

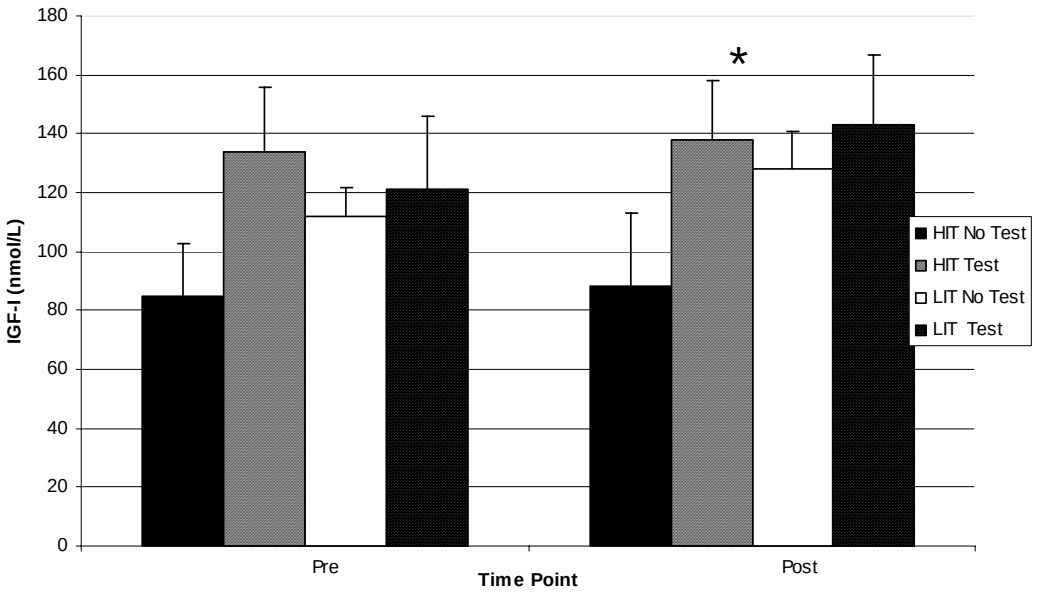

Figure 1. Insulin Like Growth Factor-1 concentration (nmol/L) pre-intervention (Pre) and post- intervention (Post). Asterisk denotes a significant time effect (P=0.013) with the mean of the four groups for the post-intervention values being greater than the mean of the four groups for the pre-intervention values.

Relationships between Hormone Status and Changes in Body Composition, and Strength

As shown in Table 2, the % change in mid-thigh cross-sectional muscle area was significantly correlated with the post-intervention total testosterone ($r=0.39$, $p=0.002$), post-intervention free testosterone ($r=0.32$, $p=0.013$), and the post-intervention IGF-1 to IGFBP-3 ratio ($r=0.27$, $p=0.036$), but not with the % change in any of these hormones. As also shown in Table 2, the % change in leg and arm muscle strength did not correlate significantly with any post-intervention hormones or with the % change in any of the hormones. The post-intervention IGF-1 to IGFBP-3 ratio was significantly correlated with both the post-intervention total testosterone ($r=0.28$, $p=0.032$) and the post-intervention free testosterone ($r=0.26$, $p=0.045$). When post-intervention IGF-1 to IGFBP-3 ratio, post-intervention free testosterone and the post-intervention total testosterone were included in forward

stepwise regression the strongest predictor of the % change in mid-thigh cross-sectional muscle area was the post-intervention total testosterone followed by the post-intervention free testosterone (model R^2 =0.23, p< 0.001). As expected, when the % change in the IGF-I to IGFBP-3 ratio, free testosterone, and total testosterone were used as independent variables, no variables entered the model.

DISCUSSION

It is noteworthy that the post-intervention IGF-1 to IGFBP-3 ratio was significantly correlated with the % change in the mid-thigh cross-sectional muscle area and fat free mass. To our knowledge this is the first study in humans to report a significant correlation between the IGF-1 to IGFBP-3 ratio and a change in any indicator of muscle mass. The IGF-1 to IGFBP-3 ratio may be an important biomarker of an anabolic state in skeletal muscle. Zdanowicz and Teichberg (16) reported that the treatment of rats who were hindlimb suspended for 10 days with an IGF-1, IGFBP-3 complex (twice daily subcutaneous injections at 50 mg/kg per injection) resulted in a reduction in the loss of muscle mass and a reduction in muscle protein degradation. Therefore, there is some evidence for a mechanism by which changes in IGF-I, IGFBP-3 or both of these factors could enhance net muscle protein balance.

We did not, however, find a significant relationship between muscle strength and the IGF-1 to IGFBP-3 ratio. This is consistent with the findings of Storer et al. (17) who found a non-significant (r=0.18 and P=0.22) relationship between the change in leg press strength and the serum IGF-1 concentration in individuals receiving testosterone but who were not resistance training. Our finding of a non-significant relationship between strength and the IGF-I to IGFBP-3 ratio but a significant relationship of fat free mass and muscle mass with this ratio is likely explained by the complexity of the factors that result in the expression of strength. Strength is to a large extent dependent on neurological factors: motor-unit recruitment and motor-unit firing rates. To our knowledge, androgens do little if anything to influence these neurological factors involved in the expression of strength. Thus, it is not surprising that there was no significant relationship between strength and the IGF-1 to IGFBP-3 ratio but a significant relationship between fat free mass and muscle mass and this ratio.

The post-intervention IGF-1 to IGFBP-3 ratio was also strongly correlated with the post-intervention concentrations of both free testosterone and total testosterone. Androgen administration increases growth hormone (GH) concentrations in individuals with hypogonadism (18-20). Further, elevated GH concentrations in acromegaly result in an increase in the circulating IGF-1 to IGFBP-3 ratio (21). In contrast, aging results in a decrease in GH and leads to a reduction of the IGF-I to IGFBP-3 ratio (19, 20). These facts are consistent with the findings from this study that indicate that testosterone induces an increase in the IGF-1 to IGFBP-3 ratio by both increasing IGF-1 and suppressing IGFBP-3. The increase in the ratio of IGF-1 to IGFBP-3 indicates greater availability of free IGF-1.

Another important finding of this investigation was that the % change in mid-thigh cross-sectional muscle area was correlated with *post-intervention* total testosterone concentration but not to % change in total testosterone. This suggests that it is only the absolute concentration of circulating testosterone and not its % change that is responsible for how much skeletal muscle hypertrophy will occur with testosterone replacement. Additionally, it would appear that the effect of free or total testosterone on muscle mass was of equal significance to that of the IGF-I/IGFBP-3 ratio. The strength of the correlations between the final serum concentrations of total and free testosterone and the percent change in cross-sectional muscle area were of similar magnitude as the correlations between IGF-I/IGFBP-3 and cross-sectional muscle area.

The study groups did not differ significantly with regard to the change in IGF-1. However there was a time effect for IGF-1 with the mean of the four groups increasing over time. The general increase in IGF-1 for the groups may have been due to the subjects in the LIT and HIT groups participating in resistance exercise training and/or that the subjects frequently interacted with study personnel and were otherwise undergoing treatments by their primary healthcare teams that were helping them recover from their recent illness. Indeed, resistance exercise training has been shown to increase the circulating growth hormone concentration (22). Further, Melikoglu et al. (23)reported that 7 and 15 days of dynamic exercise (treadmill walking) significantly increased the circulating IGF-1 concentration but resulted in no change in the IGFBP-3 concentration in rheumatoid arthritis patients. However, there was no change in IGF-1 concentrations in rheumatoid arthritis patients who performed range of motion exercises. However, the exact reason for the general increase in IGF-1 over time in the present investigation cannot be stated with certainty.

Others have examined testosterone replacement in older men with low testosterone concentrations. Urban et al. (11) administered 100 mg/wk of testosterone enanthate for four weeks to older individuals (mean age 67 years) who had a serum total testosterone concentration < 480 ng/dL and found significant increases in strength of the quadriceps and hamstrings as well as a significant increase in mixed muscle protein synthesis of the vastus lateralis. In a study from the same laboratory, Ferrando et al. (24)reported that 6 mo of testosterone administration to older men with a serum total testosterone concentration < 480 ng/dL resulted in significant strength improvements of various muscle groups and a substantial improvement in net phenylalanine balance, which is indicative of an anabolic response. Additionally, total lean body mass increased by 4.2 kg. The present study is unique in that it is the only study to examine the combination of resistance exercise and testosterone replacement in frail older men in the recuperative phase of a recent illness. Linderman et al. (25) reported that growth hormone administration alone did not attenuate the loss of muscle mass induced my hind-limb suspension; however, when growth hormone was combined with resistance training the loss of muscle mass was attenuated. Thus, the study of Linderman et al. (25) with the present data support combining exercise with hormone administration for increasing muscle mass. As described elsewhere, this study demonstrated a non-significant association between testosterone administration and strength gains (13) As presented in table 2, the data analyses also reveal a non-significant correlation between the final testosterone concentration and strength gains. The lack of a significant effect of testosterone replacement on muscle strength could possibly be due to a number of factors. The age of the participants in this investigation was considerably greater than the other two investigations cited above. It is possible that older elders are less responsive to testosterone (at the dosages used in this investigation) than younger individuals or that there is greater neuromuscular impairment the greater the age one achieves which would reduce the benefit of testosterone replacement. Many factors could explain why increasing age may impair the response to testosterone. Additionally, isokinetic testing was used in the investigation of Urban et al. (11) where as variable resistance one-repetition maximum testing was used in the present investigation. There is likely more inherent variability in one repetition maximum testing than isokinetic testing. There are likely other factors to consider when one is studying individuals who are recovering from a recent illness such as the concentration of circulating cytokines. These proinflammatory mediators, which impair muscle protein synthesis and accelerate muscle protein degradation, were likely higher in the present study subjects than those of other studies using individuals who were not recovering from a recent illness.

CONCLUSIONS

The present study found an important relationship between the % change in the mid-thigh cross-sectional muscle area and the post-intervention IGF-I to IGFBP-3 ratio, total testosterone, and free testosterone. This finding is important as there are few if any markers that can be measured in blood that indicate a change in anabolic state specific to skeletal muscle. Additionally, it appears that a higher total testosterone concentration in serum (post-intervention total testosterone in this study) is a better predictor of the increase in muscle mass than the % change in total testosterone concentration after testosterone administration.

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