Effects of Testosterone Replacement and/or Resistance Training on Interleukin-6, Tumor Necrosis Factor Alpha, and Leptin in Elderly Men Ingesting Megestrol Acetate: A Randomized Controlled Trial

Charles P. Lambert, Dennis H. Sullivan, and William J. Evans

Nutrition, Metabolism, and Exercise Laboratory, Donald W. Reynolds Center on Aging, University of Arkansas for Medical Sciences, Little Rock, Geriatric Research, Education and Clinical Center, Central Arkansas Veterans Healthcare System, Little Rock.

Background. Megestrol acetate (MA) has been used to stimulate weight gain in elderly populations with the majority of the gain being adipose tissue. Significant inverse correlations have been reported between weight gain with MA and reductions in circulating the tumor necrosis factor (TNF) alpha receptors. In addition, MA has been shown to reduce circulating interleukin 6 (IL-6) concentrations. We attempted to increase gains in fat-free mass with MA using resistance training and/or testosterone replacement and examined the effects on circulating IL-6, TNF alpha, and leptin in elderly men.

Methods. All subjects received MA and were randomly assigned to one of four groups: placebo (P); injections of resistance training (T); injections of testosterone (T); or RT + T. Cytokines were assayed by enzyme-linked immunosorbent assay (ELISA) at baseline, 12 weeks of the intervention (Post).

Results. IL-6 decreased (p < .03) over time for the T and P groups when compared to the RT + T and RT + P groups. A time effect (p = .013) was observed for TNF alpha with Post but not Pre differing significantly between the T and P groups. A positive correlation was observed (r = .60, p < .05) between changes in fat mass and the change in leptin.

Conclusion. IL-6 was reduced by MA except when RT was undertaken; TNF alpha was reduced over time regardless of group; leptin was higher in individuals not on T than those on T; and change in plasma leptin was correlated with the change in fat mass.

MEGESTROL acetate (MA), a synthetic progestin, stimulates appetite and weight gain in individuals with cancer and AIDS (1–6), and in overweight elderly men and women (7,8). We previously reported that MA stimulated weight gain in a group of chronically underweight but medically stable older men (9). The weight gain seen in these men consisted entirely of fat and a loss of skeletal muscle mass. In this study we also observed that testosterone replacement did not prevent the loss of muscle due to MA, but resistance exercise did preserve skeletal muscle mass during MA-stimulated weight gain. The lack of an effect of testosterone was despite the fact that testosterone administration has been shown to increase muscle mass in older individuals (10). We now report on the effects of MA, testosterone, and resistance exercise on circulating cytokines in these subjects.

Ye et al. (11) reported that MA-induced weight gain in individuals with geriatric cachexia was inversely correlated to a reduction in tumor necrosis factor (TNF) alpha receptors (indicators of TNF alpha concentrations). Further, changes in fat-free mass were negatively correlated with the TNF alpha receptor p75. Thus, at least part of the weight/fat-free mass changes with MA may be related to the reduction in inflammatory cytokines.

Leptin is a hormone/cytokine released from adipose tissue, which may have an effect on appetite. Circulating leptin levels increase with weight gain and decrease with weight loss (12). Further, in aging, a decline in circulating testosterone concentrations is associated with an increase in leptin concentrations (13,14), and testosterone treatment has been shown to decrease leptin concentrations (15,16). We (9) and others (7,8) have reported that MA induced fat gain in elderly individuals. However, the relationship between MA ingestion and circulating leptin concentrations has not been previously evaluated.

The purpose of this investigation was to evaluate the effects of MA ingestion alone and combined with testosterone replacement and/or resistance training on the circulating concentrations of interleukin-6 (IL-6), TNF alpha, and leptin. Further, it was of interest to correlate the changes
Table 1. Descriptive Characteristics for Study Participants

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (y)</th>
<th>Height (m)</th>
<th>Body Mass (kg)</th>
<th>Body Mass Index (kg/m²)</th>
<th>Body Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (n = 7)</td>
<td>64.0 ± 5.3</td>
<td>1.77 ± 0.7</td>
<td>66.5 ± 9.4</td>
<td>21.2 ± 2.9</td>
<td>20.2 ± 11.9</td>
</tr>
<tr>
<td>RT+P</td>
<td>67.0 ± 6.1</td>
<td>1.77 ± 0.8</td>
<td>70.6 ± 12.2</td>
<td>22.5 ± 2.8</td>
<td>22.0 ± 10.3</td>
</tr>
<tr>
<td>T (n = 8)</td>
<td>66.6 ± 3.7</td>
<td>1.76 ± 0.5</td>
<td>75.2 ± 8.1</td>
<td>24.3 ± 1.8</td>
<td>26.6 ± 2.2</td>
</tr>
<tr>
<td>RT+T</td>
<td>66.9 ± 5.5</td>
<td>1.74 ± 0.7</td>
<td>69.8 ± 11.2</td>
<td>22.9 ± 2.9</td>
<td>19.1 ± 2.9</td>
</tr>
</tbody>
</table>

BMI = body mass index; P = placebo; RT = resistance training; T = testosterone.

in these cytokines to changes in bodyweight, fat mass, and muscle mass.

METHODS

Experimental Subjects

This study was approved by the Human Research Advisory Committee at the University of Arkansas for Medical Sciences. All subjects gave written consent prior to their participation. Men with a body mass index of <=25 kg/m² between the ages of 60 and 85 were recruited for the study. Subjects were medically stable, generally healthy (all medical problems were under control and medication dosage was stable), and weight stable for the previous 2 months. After completing a preliminary medical history form and signing consent forms, subjects had the following tests performed: (a) a 12-lead electrocardiogram (EKG); (b) a screening blood draw to assess routine clinical measures; (c) a health history and physical. Subjects were excluded for the following reasons: metastatic cancer, exertional angina, or any condition that prevented resistance training. The descriptive statistics for the study participants (mean ±SD) are presented in Table 1.

Study Design

MA has consistently been shown to stimulate appetite and weight gain. However, it reduced the circulating testosterone concentration (9). The purpose of this study was to evaluate possible correlations between proinflammatory cytokines and body composition changes induced by MA in combination with testosterone and/or resistance training (RT). Four groups were studied in this investigation. All subjects received MA. Group P received a placebo injection (saline) for 12 weeks, group RT+P received weekly placebo injections and RT for 12 weeks, group T received testosterone replacement for 12 weeks, and group RT+T received testosterone replacement and resistance training for 12 weeks.

Interventions

Megestrol acetate ingestion.—For all groups, oral daily ingestion of MA (800 mg/day) was initiated at the beginning of the study and continued for the duration of the study. Subjects were asked to ingest the MA with breakfast.

Resistance training.—For the groups receiving MA and resistance training (RT+P) and MA, testosterone, and resistance training (RT+T), Keiser pneumatic resistance training machines (Keiser Sports Health Equipment, Fresno, CA) were used. Two upper body exercises (chest press and arm pull) and three lower body exercises (leg press, leg extension, and leg curl) were performed Monday, Wednesday, and Friday. Training was performed at 80% of one repetition maximum (1 RM) for three sets with the first two sets consisting of eight repetitions and the final set being performed to the point where a full repetition could not be completed. When 12 repetitions could be completed on the third set, the resistance for all three sets was moved up 5%–10% for the next session.

Testosterone administration.—Testosterone was administered in a double-blind placebo-controlled fashion. For groups that received testosterone (T) or testosterone and resistance training (RT+T), a once-weekly intramuscular injection of testosterone enanthate (100 mg) was administered starting on the first day of training and repeated on the same day of the week for 11 more weeks. For groups P and RT+P, the same volume of isotonic saline was injected once weekly to keep the subjects blinded from the intervention.

Measurements

Measurements were made prior to the interventions (Pre), after 6 weeks of the interventions (Mid), and after 12 weeks of the interventions (Post).

Whole body plethysmography.—Body weight was measured to 0.01 kg on a calibrated scale, and body density was determined by whole-body plethysmography using the Bod Pod system (Life Measurement Instruments, Concord, CA). Fat mass and fat-free mass were calculated from body density using the formula of Siri (17): %body fat = 4.950/Db - 4.50. The whole body plethysmography results for these subjects have been previously reported (9).

Computed tomography (CT).—CT scans of the dominant thigh were obtained at its greatest circumference. A GEI Scanner (Milwaukee, WI) was used, operating at 120 kV and 200 mA. A 10.0-mm slice was obtained, and the scanning time was 1 s.

From the CT image, the cross-sectional areas of fat, muscle, and area occupied by the quadriceps femoris muscle group and the knee flexors taken together were obtained using the Slicomatic program (Tomovision, Montreal, Canada). The range of Hounsfield units used to assess the quantity of fat were −250 to −40, and for muscle were −30 to 150. The CT results have also been previously reported (9).

Cytokine and hormone measures.—Venous blood was sampled from an antecubital vein at 7:00 a.m. after subjects awoke. All blood samples were obtained after the subject had been in the supine position for at least 15 minutes. Blood samples were obtained Thursday morning after a Wednesday morning injection of testosterone for the Mid time point and Thursday morning 8 days after the final
injection for the Post time point. Serum IL-6, TNF alpha, and plasma leptin were measured using enzyme-linked immunosorbent assay kits (R&D systems, Minneapolis, MN). Equations from standard curves were determined using Data Fit 8.0 (Oakdale Engineering, Oakdale, PA). A linear equation was used for plasma leptin, while four parameter logistic equations were used for serum IL-6 and TNF alpha.

Statistical analyses.—Three factor analyses of variance (hormone status × resistance training status × time) with repeated measures on the time variable (Pre, Mid, Post) were used. When a significant effect was observed, the appropriate Tukey post hoc analysis was used. In addition, the Pearson product moment correlation was performed on selected data. Data were considered significant at or below an alpha level of ≈ .05.

RESULTS

A significant resistance training by time interaction was observed for IL-6 (p = .03) with a decrease in IL-6 being observed over time for the T and P groups when compared to the RT+T and RT+P groups (Figure 1). A significant time effect was observed for TNF alpha (p = .013) with the Mid and Post values both being significantly lower than the Pre value regardless of group (Figure 2). A significant hormone by time interaction (p = .03) was observed for plasma leptin with individuals not on testosterone (P and RT+P) having a significantly higher concentration than those individuals on testosterone (T and RT+T) at the Pre time point (Figure 3). In addition, a significant time effect (p < .0001) was observed for plasma leptin with the Mid and Post values being significantly higher than the Pre value regardless of group. A significant positive correlation was observed (r = .60; p < .05; Figure 4) between the change in fat mass and the change in leptin concentration from Pre to Post (Table 2). No significant results were observed when the change in muscle mass, the change in fat mass, or the change in body weight were correlated with TNF alpha changes (Table 2). No significant correlations were observed between the change in IL-6 and the change in muscle mass (r = .22; p > .05), fat mass (r = −.07; p > .05), or body weight (r = −.07; p > .05). For clarity, previously published results (9) from this study are presented in Table 3.

DISCUSSION

We previously reported that MA ingestion resulted in a substantial gain in body fat and a concomitant decrease in muscle size (9). MA decreased circulating testosterone concentrations to castrate levels; testosterone replacement elevated the circulating testosterone concentration but did not attenuate muscle mass loss. In this investigation, IL-6 was reduced by an average of 5.2 pg/ml by MA ingestion in the nonresistance training groups. Similarly, Yeh et al. (11) reported that the IL-6 concentration was reduced by 3.63 pg/ml as a result of MA ingestion alone. In the present investigation, resistance training significantly attenuated
Figure 2. Serum tumor necrosis factor (TNF) alpha concentrations (pg/ml). Asterisks denote a significant time effect with values for the Mid and Post time points being significantly ($p = 0.013$) lower than the Pre values. RT = resistance training; T = testosterone; P = placebo.

Figure 3. Plasma leptin concentrations (ng/ml). The asterisk denotes a significant hormone effect with individuals not on testosterone (P [placebo] and RT [resistance training]+P) having a significantly higher ($p = 0.03$) concentration than individuals on testosterone (T and RT+T).
the decline in IL-6 observed relative to the nonresistance training groups. Although there are no available data on the effects of chronic resistance training on IL-6 concentrations, muscle damage as a result of resistance exercise has been demonstrated to result in a systemic acute phase response characterized by increased circulating neutrophils (18) and IL-1 beta concentrations (19). In addition, supramaximal intermittent exercise resulted in an elevation of IL-6 15 minutes and 2 hours after the exercise (20). It is unknown whether the 1-repetition maximum testing (which is both supramaximal and intermittent) performed 3 days before blood sampling, in the present investigation blunted the effect of MA in reducing IL-6 concentrations. Alternatively, the attenuation of the IL-6 reduction with MA in individuals who resistance-trained may be due to a chronic training effect.

Recently, Visser et al. (21) reported that IL-6 and TNF alpha concentrations were inversely related to muscle mass and muscle strength in 3075 elderly individuals. The administration of IL-6 or TNF alpha to rats has been shown to increase skeletal muscle protein breakdown (22,23). Thus, it is generally believed that reductions in elevated IL-6 and TNF alpha concentrations may decrease muscle mass loss. In the present study, for individuals who resistance-trained with MA (RT+P), muscle mass did not significantly change, while in individuals who resistance-trained and received testosterone with MA (RT+T) there was an increase in muscle mass. These changes occurred despite no change in IL-6. In both groups of individuals that did not resistance-train (P and T), there was a large decline in muscle mass, which was accompanied by a decline in IL-6. Thus, based on the above relationships, there was no significant inverse relationship (Table 2) between the change in the IL-6 concentrations and the change in muscle mass. This may be due to only a moderate elevation of IL-6 and the modest reduction with MA administration.

There was a significant time effect for TNF alpha with the mean of all groups showing reduced concentrations at Mid and Post when compared to Pre. Thus MA, regardless of testosterone and/or resistance-training status, resulted in a significant decline in TNF alpha. Yeh et al. (11) reported statistically significant negative correlations between the change in TNF alpha receptor concentration (a measure of the TNF alpha concentration) and weight gain, as well as the change in TNF alpha receptor concentrations and fat-free mass changes. Despite the decline in the TNF alpha concentration across groups (all of which gained weight) in the present investigation, there was not a significant negative correlation between TNF alpha changes and weight gain. It appears that the change in TNF alpha concentrations was not related to change in muscle mass as there was a weak correlation between these two variables. The individuals who did not resistance-train (P and T) had a decline in muscle mass, those that did resistance-training on placebo (RT+P) had stable muscle mass, and individuals who resistance-trained on testosterone (RT+T) had a significant gain in muscle mass. This lack of a significant negative relationship between TNF alpha and muscle mass changes may be due to the modest reduction in this cytokine by MA. Measurement of the concentration of TNF alpha in skeletal muscle, which is the site of cytokine effect (24), may be a better predictor of muscle mass changes (25).

Plasma leptin was significantly greater for individuals not receiving testosterone (P and RT+P) than for individuals receiving testosterone (T and RT+T). Recently, we reported that gains in whole-body fat mass and peripheral fat mass (thigh fat mass) were significantly reduced in individuals who received testosterone compared to individuals who did not receive testosterone (9). Indeed, when we correlated the change in leptin to the change in whole-body fat mass regardless of study group we observed a statistically significant correlation of .60. Leptin is a cytokine/hormone released from adipocytes, which signals the brain to stop energy intake (12). In healthy individuals, an increase in adipose mass will result in greater circulating leptin concentrations. These mechanisms may have played an important role in the present investigation.

In addition to the significant hormone status by time interaction, with P and RT+P having higher leptin concentrations than T and RT+T, there was a significant

| Table 2. Pearson Product Moment Correlations Between Body Weight, Fat Mass, Muscle Mass Changes, and Changes in Leptin, IL-6, and TNF Alpha |
|---------------------------------|----------------|----------------|----------------|
| Measurement | Body Weight Change | Fat Mass Change | Muscle Mass Change |
| Leptin change | 0.33 | 0.60 | -0.12 |
| IL-6 change | -0.07 | -0.07 | 0.22 |
| TNF alpha change | 0.18 | 0.21 | -0.12 |

$^p < .05$

IL-6 = interleukin-6; TNF = tumor necrosis factor.

| Table 3. Change in Body Weight, Muscle Mass, and Fat Mass |
|---------------------------------|----------------|----------------|----------------|
| Group | Change in Body Weight (kg) | Change in Muscle Mass (cm$^2$) | Change in Fat Mass (kg) |
| P | 5.12 (1.02) | -5.20 (1.62) | 6.05 (1.00) |
| RT+P | 4.34 (1.12) | 0.61 (1.41) | 5.71 (1.43) |
| T | 2.34 (1.62) | -4.44 (1.66) | 3.53 (0.69) |
| RT+T | 4.25 (3.38) | -4.51 (1.69) | 3.34 (1.11) |

$p$ Value

Effect = .10

Effect = .0006

Effect = .03

$P$ = placebo; $RT$ = resistance training; $T$ = testosterone.
time effect, with the Mid and Post values for all groups combined having significantly higher leptin concentrations than the Pre value. This is logical as all groups gained a significant amount of fat mass and all groups were ingesting the synthetic progestin. However, Messinis et al. (26) reported that the addition of progesterone to estradiol treatment increased serum leptin concentrations, whereas estradiol by itself did not have an effect in women undergoing ovarioectomy. In addition, Messinis et al. (27) reported that progesterone was significantly correlated with leptin, independent of body mass index and estradiol concentrations, in ovulating women. Thus, it is possible that there was a direct effect of MA (a synthetic progestin) on leptin concentrations in the present investigation.

In conclusion, it appears that MA ingestion has a direct effect on circulating levels of the inflammatory cytokines IL-6 and TNF alpha. However, it appears that resistance training, a stimulus to increase muscle mass, also increases IL-6, thus causing a dissociation between the change in muscle mass and the change in IL-6. A more closely linked relationship between elevated inflammatory cytokines and a loss of muscle mass can likely be found if skeletal muscle inflammatory cytokine concentrations are determined (25), since skeletal muscle is the site of cytokine action in muscle wasting (24).

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Address correspondence to Charles P. Lambert, PhD, Nutrition, Metabolism, and Exercise Laboratory, Donald W. Reynolds Department of Geriatrics, University of Arkansas for Medical Sciences, Little Rock, AR 72205, E-mail: lambrecht@uams.edu

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