Growth Hormone and Sex Steroid Effects on Serum Glucose, Insulin and Lipid Concentrations in Healthy Older Women and Men

Short title: GH, Sex Steroids Glucose and Lipids in the Elderly

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Grant Support
This work was supported in part by the Intramural Research Program of the National Institute on Aging (NIA); National Institutes of Health Research Grant RO-1 AG11005 (to MRB) and General Clinical Research Center Grant MO-1-RR-02719, from the National Center for Research Resources, National Institutes of Health, Bethesda, MD 20892; Department of Veterans Affairs and Veterans Affairs Medical Center Baltimore Geriatrics Research, Education, and Clinical Center; Claude D. Pepper Older Americans Independence Center Grant P60-AG-12583 at the University of Maryland; and the Research Service, Washington DC Veterans Affairs Medical Center. Dr. Münzer was supported in part by the Fogarty International Fellowship Program of the National Institutes of Health

Disclosure Statement
The authors have nothing to declare

Precis: Chronic GH administration to healthy older individuals increases insulin resistance with moderately beneficial effects on lipids.

Word counts: abstract 250; text body: 3439; Tables: 1, Figures: 2

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Abstract

Context: With aging, GH, IGF-I and sex-steroid concentrations and glucose tolerance decrease, and body fat and serum lipids increase.

Objective: Assess GH and/or sex steroid administration effects on serum glucose, insulin, insulin sensitivity and lipids in older individuals.

Design: Double masked, 2x2 factorial, placebo-controlled, double dummy design.

Intervention: GH and/or sex steroid (transdermal estradiol plus oral medroxyprogesterone acetate in women=HRT, testosterone enanthate=T in men) administration for six months.

Participants: Healthy, community dwelling women (n=57) and men (n=74), ages 65-88 y (mean 72 y).

Main Outcome Measures: Serum glucose, insulin and insulin sensitivity (QUICKI and ISI) before and during an OGTT, and lipid profiles.

Results: In women, GH did not alter OGTT 120 min or 2-hour area under the curve (AUC) glucose values, but increased 120 min insulin and AUC insulin. There were no significant effects of HRT or GH+HRT. ISI and QUICKI decreased after GH. In men, GH increased 120 min and AUC glucose and insulin AUC. GH+T increased 120 min glucose, and glucose and insulin AUCs. T alone did not affect glucose or insulin. ISI decreased after GH and GH+T, whereas QUICKI decreased after GH. GH in women and men, and GH+T in men decreased QUICKI by 4 weeks. In women, HRT decreased total cholesterol and LDL-cholesterol, and GH decreased LDL-cholesterol. In men, total cholesterol decreased after T and GH+T. LDL-cholesterol decreased after GH and GH+T. GH increased serum triglycerides.

Conclusions: GH administration to healthy older individuals for six months increased insulin resistance with moderately beneficial effects on lipids.

Keywords: Growth hormone, sex steroids, glucose, insulin, lipids, aging
Introduction

Normal aging is associated with decreased GH secretion, and circulating IGF-I (1) and sex-steroid (2) concentrations, in women and men. In addition, aging predisposes to increases in total and visceral body fat (3). These changes are frequently accompanied by decreased glucose tolerance (4), elevated serum cholesterol (5), and an increased prevalence of the metabolic syndrome (6), changes which contribute to increased risk for cardiovascular disease.

Administration of GH (7, 8), sex-steroids (9), or sex-steroids in combination with GH to healthy older women (10, 11) has been reported to elicit beneficial effects on body composition and lipid concentrations (12). Similarly, administration of GH (13, 14), T (15), or GH plus T (11, 14) to healthy older men, or GH to frail older men (16), decreases total body and regional abdominal fat mass and increases lean body mass. Despite these favorable alterations in body composition and lipids, several studies have reported that GH administration elevates fasting glucose (17) and insulin concentrations (8).

Although GH is widely, and inappropriately, used as an anti-aging therapy (18, 19), relatively few studies have assessed the combined effects of GH plus sex-steroids on glucose tolerance and insulin sensitivity in healthy older individuals. We previously reported that a significant proportion of older men, but not women, treated with GH or GH combined with sex-steroids, developed criteria for impaired fasting glucose or frank diabetes mellitus (14). In another study, six-months of GH administration increased fasting and two-hour glucose and insulin concentrations in postmenopausal women (8). In the current investigation, we systematically examined the effects of GH, without or with concomitant sex steroid administration, on circulating glucose, insulin and lipid concentrations, and insulin sensitivity, in healthy, ambulatory, community-dwelling individuals age 65-years and older.

Materials and Methods

Study Population

Our study population and protocol design have been described previously (14). Participants were recruited by mailed brochures and newspaper advertisements. All were non-diabetic by fasting blood glucose
concentration using the ADA criteria current at the time of study, 65-years of age or older, and healthy by history, physical examination, routine serum chemistries, and graded treadmill exercise stress ECG testing. Subjects did not smoke, drank no more than 30g of alcohol/day, and took no medications known to interfere with GH–IGF-I axis activity, gonadal steroids, or glucose, insulin and lipid concentrations. None of the women had taken HRT for at least three-months prior to study. None of the men had taken T replacement prior to study entry. To be eligible for the study, the subjects had to have age-related reduction of circulating IGF-I concentration ≥1 SD below the mean for values in healthy adults aged 20-35 years, i.e. ≤ 230 µg/L (20), and for men, serum T ≤ 16.3 nmol/L (21).

The study was approved by the combined Institutional Review Board of the Johns Hopkins Bayview Medical Center (JHBMC) and the Intramural Research Program, NIA. Written informed consent was obtained from each participant.

**Study Protocol, Hormone Administration and Assays**

A detailed description of the study protocol, hormone doses employed, and assays has been published previously (14). Briefly, we administered GH and/or sex steroid, *(transdermal estradiol and oral medroxyprogesterone acetate in women, intramuscular testosterone enanthate injections in men)* to healthy older women (n=57) and men (n=74), ages 65-88 y (mean 72 y) using a 2x2 factorial, placebo-controlled, double dummy design. In the current study, we assessed serum glucose and insulin before and during a 2-hour 75 g oral glucose tolerance test (OGTT), as well as serum lipids, at baseline (week 0) and after 26 weeks of hormone administration. In addition, we calculated insulin sensitivity by both the Insulin Sensitivity Index (ISI) (22) and the QUICKI algorithm (23) at baseline and at week 26. During the study protocol, we also assessed insulin sensitivity by monthly measurements of fasting glucose and insulin and use of the QUICKI algorithm. Plasma insulin and glucose concentrations were measured in the Johns Hopkins Bayview GCRC Core Laboratory. Insulin concentrations were determined by RIA (Linco Research Inc., St. Louis, MO). The assay had a sensitivity of 1.2 pmol /L with a linear range from 12-1200 pmol/L. Intra-assay and inter-assay CV’s were 2.1 and 2.8% respectively. Glucose concentrations were measured using an automated glucose-oxidase assay
(Beckman Diagnostics, Fullerton, CA), with intra-assay and inter-assay CV’s of 2.8% and 2.1% respectively. Serum cholesterol and triglyceride concentrations were measured enzymatically on a Hitachi 704 analyzer (Boehringer-Mannheim, Indianapolis, IN) using reagents supplied by the manufacturer at the Lipoprotein Analytical Laboratory at Johns Hopkins Hospital, Baltimore, MD using CDC standards. The intra- and interassay CVs were <1.4% for cholesterol and < 2.5% for triglycerides. HDL cholesterol was measured using a variation of the heparin-MnCl$_2$ method, with intra- and inter-assay CVs both <3.6%. LDL cholesterol concentration was calculated using the equation of Friedewald (24).

**Statistical Analyses**

Sex-specific ANCOVAs adjusted for age, value of the outcome measure at baseline, and treatment group were used to determine if any of the changes in the three active treatment groups (26-weeks minus baseline) were different from the change in the double placebo group. Post hoc analyses, using the method of Dunnett-Hsu (25), were employed to identify the groups whose changes were different from that seen in the double placebo group. For each of our regressions, the Dunnett-Hsu procedure kept the probability of a type-I error at 5% even though we performed three comparisons (comparisons of GH+HRT to double placebo, GH+placebo to double placebo, and placebo + HRT to double placebo). At week 0 and at week 26, times at which our subjects underwent oral glucose tolerance tests, we calculated the insulin sensitivity index using the ISI (22) and QUICKI. In addition, we used the QUICKI index ($1/([\text{Log (Fasting Insulin, } \mu\text{U/ml}) + \text{Log (Fasting Glucose, mg/dl)}])$) to estimate insulin sensitivity at weeks 0, 4, 8, 13, 17, 21, and 26, as participants in this study did not undergo oral glucose tolerance tests at weeks 4, 8, 13, 17, or 21. Sex-specific repeated measures ANOVAs adjusted for time, group, and a group-by-time interaction were used to assess changes in insulin sensitivity over time. Three models, each with a distinct covariance structure (unstructured, compound-symmetry, and first-order autoregressive) were run to determine the covariance structure that best accounted for the serial autocorrelation of the data. A modification of Akaike’s Information Criterion (AIC) (26) that includes a correction for small sample size was used to choose the model with the best covariance structure. To be eligible for inclusion in these analyses, a subject had to have her or his QUICKI determined at five or more of the seven time points assessed. Impaired glucose tolerance and diabetes mellitus were defined based on the 2003 ADA criteria.
Glucose and insulin concentrations were measured at 0, 30, 60, 90, 120 minutes during the oral GTT. The trapezoidal rule was used to calculate the area under the curve (AUC) for glucose and insulin from 0 to 120 min. Fasting serum insulin and AUC for glucose and insulin were skewed to the right and were log_{e} transformed prior to analyses. Data are expressed as the mean ± standard error of the mean (SE), all conventional values were transformed to SI units. SAS 9.1 (SAS Institute Inc., Cary, NC) was used to analyze the data.

Results

We studied 57 women (Placebo n=14, GH n=13, HRT n=14, GH+HRT n=16) and 74 men (Placebo n=17 GH n=17 T n=21, GH+T n=19), with a mean age of 71±0.4 (±SE) yrs and body mass index of 26 kg/m^2. The effects of sex-steroid administration on serum E_2 and T concentrations, and of GH on serum concentrations of IGF-I and IGFBP-3 and body composition in these women and men, have been described previously (14).

There were no significant differences in mean age, weight, or BMI at baseline among the various subgroups of women or men (14).

**GH and Sex Steroid Effects on Glucose and Insulin concentrations during an OGTT (Table 1)**

In women, there were no significant effects of GH, HRT, or GH+HRT on fasting glucose concentrations. GH alone increased fasting insulin concentrations (p<0.01). Glucose and insulin concentrations at the 120-min time point of the OGTT were not significantly affected by any hormone intervention. GH did not significantly alter OGTT glucose AUC, but increased the insulin AUC (p<0.001). There were no significant effects of HRT or GH+HRT on glucose or insulin AUC’s.

In men, fasting glucose and insulin concentrations did not change significantly in any of the hormone administration groups, whereas the OGTT 120-min glucose concentrations increased after GH (p<0.05) and GH+T (p<0.001) but not after T. Insulin concentration at 120-min also increased after GH (p=0.02) and there was a nonsignificant trend for an increase in insulin after GH+T (p=0.08). Glucose AUC’s increased after GH (p= 0.02) and GH+T (p<0.0001), and insulin AUC’s increased after GH (p= 0.03) and GH+T (p=0.04).

**GH and Sex Steroid Effects on Insulin Sensitivity Index (ISI) and on QUICKI at Baseline (Week 0) and Week 26**
In women, ISI decreased significantly (-2.5, p=0.0003) after GH but not after GH+HRT (-0.5, p=0.66) or HRT (-0.2, p=0.96). Similarly QUICKI decreased after GH (-0.015, p=0.003) but not after GH+HRT (+0.0005, p=0.86) or HRT (+0.003, p=0.49). In men, ISI decreased after GH and GH+T (both by -1.3, p=0.005) but not after T (-0.5, p=0.23). In comparison QUICKI decreased after GH+T (-0.007, p=0.035) but not after GH (-0.0006 p=0.0563) or T (-0.002, p=0.7).

*Longitudinal Assessments of Insulin Sensitivity by QUICKI (Figure 1)*

Forty-two women contributed 278 QUICKI measurements (average 6.6 measurements per women) and 66 men contributed 449 measurements (average 6.8 per man) to the analyses. At week 0, QUICKI estimates did not differ between sex or group assignments. In women receiving placebo medications, insulin sensitivity (QUICKI) decreased significantly compared with baseline at weeks 4 (-3.4%, p 0.023), 8 (-3.2%, p 0.03) and 17 (-3.0%, p 0.04) with no significant changes from weeks 4 to 17. Administration of GH alone significantly decreased insulin sensitivity compared both with baseline and placebo treated women at weeks 4 (-9.6% p=0.001), 8 (-7.6% p=0.009), 13 (-9.8% p=0.001), 17(-7.0% p=0.002), 21 (-8.6% p=0.002), and 26 (-7.4% p=0.005), and these changes were similar over time. There were no significant effects of HRT or GH+HRT on insulin sensitivity over time.

In men receiving placebo, insulin sensitivity (QUICKI) decreased by 2.8% (p<0.05) at week 4 and by 3.1% (p=0.04) at week 13, but these decreases did not differ from week 0 when adjusted for multiple comparisons. GH decreased QUICKI at weeks 4 (-5.8%, p=0.01), 8 (-7.3% p=0.005) 13 (-5.2% p=0.02), 17 (-5.1% p=0.02) and 21 (-6.7%, p<0.005), respectively, but these changes differed significantly from placebo only at week 8. There was no significant effect of T alone on QUICKI. In comparison, GH+T decreased the QUICKI by 7% at week 4 (p=0.01), by 6.6% at week 8 (p=0.003), by 6.5% at week 13 (p=0.001), by 5.4% at week 17 (p=0.003) and by 5.4% at week 21 (p=0.016). These decreases in QUICKI were significantly different from placebo at weeks 4, 8, 13 and 17.
Hormone Effects on Serum Lipids (Figure 2)

In women, total cholesterol decreased after HRT (p=0.03), but not after GH or GH+HRT. LDL-cholesterol decreased after HRT (p=0.01) and GH (p=0.01), with a trend to decrease after GH+HRT (p=0.06). Serum HDL-cholesterol and triglyceride concentrations were unaffected by hormone administration. In men, total cholesterol decreased after T (p=0.02) and GH+T (p=0.004), whereas LDL-cholesterol concentrations decreased after GH (p=0.05) and GH+T (p=0.01), and triglycerides increased after GH (p=0.05).

Discussion

Multiple studies have reported GH effects on fasting glucose concentration (28, 29) in older men, and on glucose and insulin concentrations in older women (30). However, these reports were mainly focused upon safety monitoring, as short- and long-term GH administration had been reported to worsen, and long term GH administration to improve, insulin sensitivity and glucose tolerance (31).

In women in our study, administration of GH alone, but not HRT or GH+HRT, increased fasting insulin concentrations and insulin AUC’s during an OGTT. Our results confirm other studies, reporting normal fasting glucose concentrations but elevated fasting insulin after short term GH administration in younger women with abnormal glucose tolerance (32) and after daily GH injections to obese women (8). In the current study, the 120 min insulin concentrations in women receiving GH almost doubled when compared with the pre-treatment 120 min insulin in the same group. However, this increase was not statistically different from the corresponding increment in the placebo group, perhaps due to the small sample size. Co-administration of HRT plus GH appeared to attenuate the GH mediated blunting of insulin sensitivity, similar to the reported effects of HRT+GH in women with adult growth hormone deficiency (33) and with a previous study that reported a reduced incidence of diabetes in a large cohort of postmenopausal women treated with estrogens and progestin over a longer period of time (34). Taken together these data suggest that HRT preserves or enhances insulin sensitivity, perhaps in part by suppression of IGFBP-3 and the acid labile subunit in GH treated women (35).

In men, fasting concentrations of glucose and insulin remained unchanged after GH, T and GH+T. This finding is similar to those previously reported after short-term therapy with GH and/or T (28) or after six-months
of GH, T or GH+T (36). In contrast to the present study, the latter two protocols were performed using lower GH and T doses or GH titration regimens. In our men two-hour glucose and insulin concentrations during an OGTT increased in the GH group, whereas after GH+T, glucose but not insulin increased significantly, and both GH and GH+T increased glucose and insulin AUC’s. Although testosterone has been reported to increase insulin sensitivity in nonelderly abdominally obese (37) and in hypogonadal men (38), in the current study testosterone exerted no significant effect on insulin sensitivity in older men. These data suggest an age associated discordance in the effect of testosterone on insulin sensitivity in men, perhaps owing to differences in fat or muscle tissue responsivity. In addition, the attenuated insulin response at 120 min in the GH+T group might have resulted from an amplified GH secretory burst mass (39).

In women, effects of GH on QUICKI and ISI were similar indicating an increase in insulin resistance after 26 weeks of GH administration. In contrast, we found a significant decrease in ISI in men after GH, whereas the change in QUICKI was not significant. Whether the latter finding is explained by the wider range of data points embedded within the ISI versus QUICKI algorithm, and the correspondingly greater correlation of the ISI with findings from the glucose clamp technology, remains to be established (22).

Our analysis of serial QUICKI data suggests that GH in older women, and GH and GH+T in older men, significantly increases insulin resistance as soon as four weeks after hormone administration, and that the increased resistance does not change further over time. The decrease in QUICKI we observed in our men after GH+T was of similar magnitude to that reported recently in younger abdominally obese men who received escalating daily GH doses for 50-weeks (40). Increased insulin resistance may enhance the risks for development of glucose intolerance and diabetes mellitus. We administered sex-steroid and growth hormone for six–months. Whether longer-term GH administration would result in beneficial effects on glucose and insulin homeostasis in healthy older individuals, as reported in patients with adult growth hormone deficiency (41), remains to be determined. Taken together our results indicate an increase in insulin resistance after GH administration similar to that observed in obese women and men treated with growth hormone (42).

HRT administration to our women decreased total and LDL-cholesterol concentrations, whereas GH+HRT exerted significant effects only on LDL. Many other studies have found beneficial effects of
exogenous estrogens on total cholesterol and LDL-cholesterol concentrations in older postmenopausal women (43), and have linked these changes to improved cardiovascular outcomes (44). However no such improvement was observed in postmenopausal women with established coronary artery disease, (45) and in view of the discussion of the risk-benefit relationship of estrogens (46), their use in older women remains to be established. GH administration to our women and men decreased concentrations of LDL-cholesterol, but not total cholesterol. In their analysis of data from randomized GH trials in older persons, Liu et al (19) reported average decreases of total cholesterol concentration of -0.29 mmol/L, and of LDL-cholesterol concentration of -0.12 mmol/L. The changes we observed were within the expected ranges, of modest magnitude, and would not be predicted to have a major clinical impact upon long-term cardiovascular risk. Testosterone administration reduced LDL-cholesterol in our men. Effects on lipids have been reported to depend on the dose, route and duration of T administration. Transdermal T and testosterone enanthate given daily up to one year elicited no significant effects on total cholesterol (47, 48), whereas 36-months of transdermal T decreased cholesterol (49). A recent meta-analysis of randomized clinical trials supports the hypothesis that the route of T administration and the baseline T concentration are important determinants of response (15). Our findings indicate that intramuscular injections of low dose T-enanthate to older men with age-related decreases in serum-T concentrations reduced total cholesterol to an extent like that observed in similarly treated younger patients with hypogonadism and diabetes mellitus. Co-administration of GH plus T exerts additive effects on serum lipid concentrations in abdominally obese men (50) but not in older men. In one recent trial, administration of GH, T and GH+T to men age 65 and older did not affect total cholesterol, LDL-cholesterol, HDL-cholesterol or triglyceride concentrations (51). In contrast, in the present study, we found the largest decrease in total cholesterol, and LDL cholesterol, in men treated with GH+T. Given that in our men receiving GH+T, total body fat mass (14) and abdominal visceral fat mass (11) decreased we hypothesize that the observed reduction in total and LDL-cholesterol concentrations in our GH+T treated men resulted from the disproportionately greater reduction in fat mass in men treated with GH+T, rather than with either hormone alone.
We found that GH administration increased serum triglycerides in men, but not in women. Because GH increases lipolytic activity (52) we speculate that there is a sexually dimorphic response of triglycerides to GH in healthy older persons like that reported in individuals with adult GH-deficiency (53) and that the increased GH-binding protein in older women (54) may be a contributing factor.

Our study has several limitations. First, the small sample size might have reduced the statistical power to detect significant changes compared with the placebo treated groups. Second, we assessed all of the metabolic outcomes at two-time points, before and after six-months of hormone intervention, whereas the estimates of change in insulin resistance by QUICKI were performed at multiple time points. Thus, we were unable to relate all the measured changes in QUICKI to concomitant changes in glucose and insulin responses to an OGTT, or to lipid concentrations, at the intermediate time points. Thirdly, our study was of relatively short duration. Longer-term studies in GH deficient, nonelderly subjects have demonstrated only moderate increases in glucose after two-years, followed by a decline within the normal range (55), and GH or GH in combination with pioglitazone increased fasting blood glucose after four-weeks but not glucose and insulin AUCs after 40-weeks (56). Thus, it is possible that insulin and glucose responses to an oral glucose load might return to normal over time. However, given that aging \textit{per se} adversely affects glucose tolerance independent of body composition (57) and that older patients with known GH deficiency develop impaired glucose tolerance during GH therapy (58) the likelihood of such a long-term beneficial change seems low. We were not able to control rigorously for dietary input or physical activity throughout the study period, although all participants were advised not to change their diets or physical activity. Thus, changes in either or both may have influenced our results. However we did provide a defined diet on the night before the OGTT in all participants. Although our study period of six-months was too short to infer the relevance of the observed changes in laboratory parameters to cardiovascular risk, much longer term GH therapy has not been shown to alter atherosclerotic changes in patients with adult GHD (59).

In summary, GH administration to healthy older women and men for six months increased insulin resistance and exerted moderately beneficial effects on serum lipids. In women, HRT appeared to preserve insulin sensitivity when co-administered with GH, whereas T given to men exerted no obvious effects on insulin sensitivity. Whether longer term GH administration to healthy old women and men would elicit a more
profound reduction of cardiovascular risk factors, as observed in GH-deficient patients (60) and the potential effects on development of diabetes mellitus and cardiovascular disease, remain to be determined.

**Acknowledgments**

The authors thank the study participants for their extraordinary and unselfish devotion to advancing knowledge of the aging process; the nursing staff of the Johns Hopkins Bayview Medical Center’s GCRC for their assistance in the conduct of our study; the staff of the GCRC Core Laboratory for the glucose and insulin measurements; Genentech, Inc., and Novartis Pharmaceuticals for their generous provision, respectively, of recombinant human GH and transdermal estradiol; and Drs. Luigi Ferruci, Josephine Egan and Dan Longo for their constructive critiques of this manuscript. Dr. Münzer now receives grant support from the Robert Bosch Foundation, Stuttgart, Germany.
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Figure Legends

Figure 1.
Serial measurements of QUICKI calculated at baseline (week 0), and after 4, 8, 13, 17, 21 and 26 weeks of hormone administration in healthy older women (upper panel) and men (lower panel). Overall there were significant treatment or group by time effects (women p=0.0379, men p=0.0044). Data are adjusted for group and group by time interaction. An asterisk indicates significant differences versus the placebo group at baseline adjusted for multiple comparisons using the method of Dunnett-Hsu. GH: growth hormone, HRT: hormone replacement therapy, T: testosterone.

Figure 2.
Change from Baseline (mmol/L±SE) of total cholesterol, LDL cholesterol, HDL cholesterol, and serum triglycerides in healthy older women (upper panel) and men (lower panel) after 6 months of hormone administration. An asterisk indicates significant differences versus the placebo group (white bars). HRT in women and T in men are depicted as grey bars, GH in women and men as dashed bars, and GH+HRT in women and GH+T in men, as solid bars.
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<td>26 Weeks‡</td>
<td>1491.2</td>
<td>156.8</td>
</tr>
<tr>
<td>Change‡</td>
<td>145.5</td>
<td>156.8</td>
</tr>
<tr>
<td>P Value for Change</td>
<td>0.88</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Number of participants with complete data at baseline
† Adjusted for group and age
‡ Adjusted for age, group, and baseline value