Influence of obesity and body fat distribution on growth hormone kinetics in humans

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The mean 24-h plasma growth hormone (GH) concentration and its level in response to virtually every known GH secretagogue are considerably reduced in obese humans (25, 40, 41). The mechanisms underlying this phenomenon are unclear. The concentration of GH (or any other hormone) in plasma is governed by its specific kinetic features, i.e., distribution volume, clearance characteristics, and production rate. A number of studies suggest that the metabolic clearance of GH is increased in obese humans (24, 38, 41). In mildly obese men, body mass index (BMI) was shown to be a negative determinant of plasma GH half-life, determined by deconvolution of spontaneous plasma GH pulse patterns (24). In an elegant study using deconvolution analysis of 24-h GH plasma concentration profiles, a dual defect in GH secretion and half-life was suggested to subserve the hyposomatotropism of massively obese men (41). Also, in an experiment in normal weight men and women that employed continuous GH infusion, BMI was a positive correlate of plasma GH clearance (38). However, BMI may not be a completely accurate indicator of body fat mass (22, 33, 34). Body fat mass was not measured in any of these studies.

The metabolic effects of excessive fat storage are dependent on its location in various body fat stores. Visceral fat accumulation has far more metabolic sequelae than subcutaneous fat storage (8). Thus it is conceivable that body fat distribution affects GH metabolic clearance. The waist-to-hip circumference ratio (WHR) is a measure of relative fat storage in upper body (abdominal) vs. lower body (gluteal/femoral) compartments. A high WHR reflects predominant storage in upper body compartments. Two studies suggest that the WHR, in addition to BMI, might be a determinant of plasma GH concentrations. An inverse correlation of WHR with spontaneous and GH-releasing hormone-induced plasma GH peak levels was observed in mildly obese older men (12). Diminished GH secretion in upper body obesity might explain this observation, because 24-h GH production rates were found to be inversely correlated to WHR in severely obese humans (30). In addition, increased GH clearance or distribution volume might contribute to low circulating plasma GH concentrations in upper body obesity. However, it is unknown to date whether fat storage in upper body compartments affects GH clearance and/or distribution volume.

This study was conducted to examine whether an increased body fat mass is associated with increased GH clearance and/or GH distribution volume. Furthermore, we aimed to investigate whether body fat distribution affects these kinetic parameters. To this end, a bolus injection of recombinant human GH was administered during a continuous infusion of somatostatin to suppress endogenous GH secretion. GH kinetics were investigated with noncompartmental analysis of plasma GH curves. GH peak values in response to GH infusion and plasma half-life of GH were not significantly different between normal weight and obese subjects. In contrast, GH clearance was 33% higher in LBO women and 51% higher in UBO women compared with clearance in normal weight controls. The difference in clearance between LBO and UBO was not statistically significant. Altered GH clearance characteristics contribute to low circulating GH levels in obese humans. Body fat distribution does not appear to affect GH kinetics.

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Their kidney function was normal as indicated by plasma creatinine values within the normal range. They had maintained a stable body weight for ≥3 mo before the study. The two groups of obese women were similar in BMI, and all women were similar in age. Written informed consent was obtained. The study was approved by the Ethics Committee of Leiden University Medical Center.

Study design. All studies were performed in the follicular phase of the menstrual cycle. The subjects were admitted to the research center at 8:00 AM after an overnight (≥8-h) fast. After voiding, the subjects stayed semirecumbent for the duration of the experiment. Body composition was measured using bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK), and electrodes were placed at the right hand and foot (26). A cannula was placed in an antecubital vein for blood sampling. A second cannula was placed in a contralateral antecubital vein for infusion. One hour after insertion of both catheters, a continuous infusion of somatostatin-14 (SMS, Ferring, Hoofddorp, The Netherlands) was started (0.83 μg·min⁻¹·m body surface area⁻²) and continued for 150 min throughout the study. At 60 min, a single bolus of 100 μU of 22-kDa GH (Eli Lilly, Nieuwegein, The Netherlands) was administered intravenously at a constant infusion rate over 5 min by means of a calibrated infusion pump (Harvard Apparatus, Edenbridge, UK).

During the first 60 min of SMS infusion, blood samples were drawn every 10 min. After rhGH administration, blood was sampled every 5 min during the 1st h and thereafter every 10 min until the end of the test.

Blood sampling and assays. Blood samples were collected in heparinized tubes. All samples were centrifuged within the hour of sampling, and plasma was stored at −40°C until assay.

(Free) plasma hGH concentrations were determined with a 22-kDa specific immunofluorometric assay with high sensitivity (Delphia hGH kit, Wallac Oy, Turku, Finland). The detection limit was 0.03 μU/l (0.0115 mg/l). The intra-assay coefficients of variation ranged from 1.6 to 8.4% over the GH concentration range of 50–0.25 μU/ml and >30% for GH concentrations of <0.1 μU/ml. GH binding protein (GHBP) was measured in a basal blood sample by fast liquid chromatography using superose 12 gel filtration (31).

Calculations. GH kinetics was investigated with noncompartamental methods using WinNonlin V1.1 (Scientific Consulting, Apex, NC). Noncompartamental methods were chosen because they require fewer assumptions. The area under the curve (AUC) was calculated using the linear trapezoidal rule and extrapolated to infinity using the terminal half-life estimated with log-linear regression. Adequacy of the log-linear regression for determination of the terminal half-life is the only assumption necessary for noncompartamental modeling. The number of points to be included for this estimate was automatically determined by the program on the basis of the largest adjusted r². In three NW subjects, the terminal part of the curve tended to flatten out, resulting in unlikely half-life estimates. For these subjects, this terminal part of the curve was disregarded in the calculations. The part of the AUC attributable to nonzero prevalues (AUCpre) was estimated by use of the calculated terminal half-life and the prevalue (AUCpre = prevalue/½). This part was subtracted from the initial AUC, resulting in the corrected AUC to be used for clearance calculation [clearance = dose/(corrected AUC)]. Additionally, compartamental modeling was used to obtain two-compartment model parameter estimates. In this analysis, the flattened part of the curves were also disregarded.

Statistical analysis. GH kinetic parameters were compared between groups using unpaired Student’s t-tests. Anthropometric descriptives were correlated with clearance by use of Pearson’s correlation coefficients. All data are presented as means ± SD, unless otherwise specified. 95% Confidence intervals of the difference between means (95% CI) are given if indicated. Statistical calculations and data management were performed using SPSS for Windows V6.1.2 (SPSS, Chicago, IL).

RESULTS

The characteristics of the subjects that were enrolled in the study are shown in Table 1. SMS infusion adequately suppressed endogenous GH secretion in all groups. GH levels fell below 0.75 mU/ml in all participants except one subject in the control group. The average GH profiles for each group after intravenous injection of 100 μU of exogenous GH are given in Fig. 1.

The peak GH concentrations (Cmax), although 40% lower in the UBO group compared with the controls and the LBO subjects, were not significantly different between groups (95% CI: −0.1, 8.7 for UBO vs. NW and −2.6, +10.9 for LBO vs. UBO; Table 2). The apparent volume of distribution (Vd) of GH tended to be larger in obese subjects, although the difference with NW controls did not reach statistical significance (Table 2). As a result of increased clearance and a larger Vd, plasma GH terminal half-life was not different between groups. Plasma GH terminal half-life was not significantly different in obese and NW women (Table 2).

Compartmental analysis indicated clearance, volume, and terminal half-life estimates comparable to the noncompartamental results (Table 3). Data are presented as medians and ranges because of outliers in

Table 1. Subject characteristics

<table>
<thead>
<tr>
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<th>NW</th>
<th>LBO</th>
<th>UBO</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>33.3 ± 10.9</td>
<td>33.4 ± 6.2</td>
<td>36.1 ± 7.5</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.69 ± 0.04</td>
<td>1.65 ± 0.07</td>
<td>1.69 ± 0.06</td>
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<tr>
<td>Weight, kg</td>
<td>62.8 ± 6.85</td>
<td>94.3 ± 12.4*</td>
<td>99.4 ± 9.50*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.0 ± 2.2</td>
<td>34.6 ± 3.7*</td>
<td>34.9 ± 4.4*</td>
</tr>
<tr>
<td>Fat percentage</td>
<td>28.1 ± 5.5</td>
<td>44.0 ± 4.5*</td>
<td>44.4 ± 4.7*</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>73.0 ± 8.2</td>
<td>91.3 ± 6.7*</td>
<td>110.6 ± 9.6†</td>
</tr>
<tr>
<td>WHR</td>
<td>0.74 ± 0.07</td>
<td>0.75 ± 0.04</td>
<td>0.98 ± 0.08*</td>
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</table>

Data are means ± SD, n = 8 women in each group. NW, normal weight; LBO, lower body obese; UBO, upper body obese; BMI, body mass index; WHR, waist-to-hip circumference ratio. *Significantly different from NW women; †significant difference between LBO and UBO.
the terminal half-life estimates for the NW group. Compartmental analysis resulted in parameter estimates that were similar to those obtained by noncompartmental analysis. Therefore, only noncompartmental results are discussed.

GHBP concentration in plasma was measured in all subjects except three LBO women. The obese groups in which GHBP concentrations were compared were still similar in BMI and age (data not shown). Plasma GHBP concentrations tended to be higher in UBO women (compared with LBO and NW controls). The differences between groups did not reach statistical significance (Table 2).

**DISCUSSION**

We studied the kinetics of exogenous recombinant 22-kDa variant hGH by intravenous administration of a GH bolus while endogenous GH secretion was suppressed by somatostatin. 22-kDa hGH is the GH variant that prevails in human serum (3). The dose and type of administration were chosen to mimic a physiological GH pulse concentration, which is of importance in view of the fact that plasma GH levels affect GH clearance (18, 38).

The plasma clearance of 22-kDa rhGH was shown to be enhanced in obese women compared with NW control women. UBO, as indicated by a high WHR, was associated with GH clearance values that were similar to those in peripheral obesity in women who were similar with respect to their amount of adipose tissue. GH clearance was clearly correlated with various indicators of adipose tissue mass. In apparent contradiction with these findings, plasma GH half-life was not significantly different between obese and NW women. However, small concomitant differences in Vd of GH between obese and NW women may explain these data. Increased clearance in the presence of a larger Vd leads to a similar half-life. Vd indeed tended to be larger in obese subjects (Table 2).

Plasma GH half-life in NW subjects was in keeping with previously published data obtained by others (16, 18, 41). In contrast to the present results, one study, employing deconvolution analysis of 24-h GH plasma profiles, did report a small but significant reduction of GH half-life in obese men (41). The apparent discrepancy between these data might be explained by the fact that massively obese subjects (average BMI 46 kg/m²) were studied in the latter experiment. It is conceivable

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<tr>
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<th>LBO</th>
<th>UBO</th>
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<tr>
<td>Prevalues, just before GH bolus, mU/l</td>
<td>0.67 ± 0.99</td>
<td>0.31 ± 0.25</td>
<td>0.08 ± 0.05*</td>
</tr>
<tr>
<td>Max GH concn, mU/l</td>
<td>11.51 ± 5.64</td>
<td>11.39 ± 8.81</td>
<td>7.22 ± 1.38</td>
</tr>
<tr>
<td>AUC, mU<em>l-1</em>min</td>
<td>180 ± 59</td>
<td>131 ± 33*</td>
<td>113 ± 18*</td>
</tr>
<tr>
<td>Clearance, ml/min</td>
<td>603 ± 175</td>
<td>803 ± 190*</td>
<td>908 ± 154*</td>
</tr>
<tr>
<td>Apparent Vd, l</td>
<td>16.6 ± 6.3</td>
<td>21.9 ± 7.8</td>
<td>24.1 ± 8.2</td>
</tr>
<tr>
<td>Terminal half-life, min</td>
<td>20.9 ± 4.1</td>
<td>20.2 ± 4.0</td>
<td>18.5 ± 4.6</td>
</tr>
<tr>
<td>GHBP, pmol/l</td>
<td>675 ± 208</td>
<td>718 ± 160</td>
<td>843 ± 126</td>
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Values are means ± SD, n = 8 in each group, except n = 5 in LBO group for growth hormone (GH) binding protein (GHBP). AUC, area under the curve; Vd, volume of distribution. *Significantly different (P < 0.05) from NW.
that GH clearance in massively obese people is enhanced to such an extent that terminal half-life is affected. The clear linear relation between body fat mass and GH metabolic clearance that we observed is in line with this notion.

Body fat distribution does not appear to affect GH kinetic parameters to a significant extent. Thus, despite the fact that visceral fat is clearly more active (in a metabolic sense) than subcutaneous (gluteofemoral) fat, GH clearance is not increased in UBO women compared with their LBO controls. Also, GH \( V_d \) was not different between these groups. The results of the study showing an inverse correlation between plasma GH levels and WHR (12) may be confounded by the percentage of body fat, which tends to be higher in unselected UBO people. Alternatively, UBO might be characterized by reduced endogenous GH secretion to explain for lower plasma GH concentrations (30). The latter explanation seems more likely, because the GH secretion rate is a major determinant of plasma GH concentrations.

It is unlikely that differences in peak GH response to bolus injection have brought about differences in clearance estimates between our study groups. GH clearance, calculated from the plasma GH decay curve after discontinuation of a continuous GH infusion, is dependent on initial plasma concentrations such that low plasma levels are associated with increased clearance (18). Therefore, we wondered whether the fact that the peak plasma GH concentration in our UBO subjects was slightly (but not significantly) lower than that in the other two groups would have contributed to their increased clearance. We do not believe so, because the difference in peak GH levels between UBO and NW subjects in our experiment was very small (11 vs. 7 mU/l). A sevenfold decrease of initial GH levels appears to be necessary to have clearance values (18). In LBO subjects, GH clearance was clearly increased compared with NW women, whereas peak GH concentrations were virtually the same in these groups (11.51 vs. 11.39 mU/l).

We think that it is unlikely as well that differences in persistent GH secretion have significantly affected the main outcome of this study. Persistent pulsatile and/or basal GH secretion during SMS infusion could affect the GH kinetics parameter calculations. However, we believe that it did not do so in this study for three reasons. First, although some GH peaks appear to occur during SMS infusion in healthy NW humans (11), we did not observe peaks in our individual GH decay curves (data not shown). Second, basal GH secretion during SMS infusion was shown to be negligible, leading to plasma GH levels of 0.065 mU/l (11), which is only 0.7% of the peak levels measured in this study. Finally, GH inhibits its own secretion. Thus, when exogenous GH is injected, endogenous GH secretion is expected to be even lower than during SMS infusion alone. Despite all this, it is difficult to completely exclude the possibility that persistent (basal) GH secretion has flattened out the last part of GH decay curves in some subjects. In addition, assay variability may have scattered the final part of the concentration profiles (>30% for GH concentrations <0.1 mU/l). Because this final part of the curve was extrapolated to infinity to determine AUC, this could affect clearance estimates. However, the part of the AUC that resulted from extrapolation did not exceed 16% of total curve area in any case (data not shown). Thus clearance estimates were never affected to a major extent. For all these reasons, we believe that persistent GH secretion was not a factor affecting the overall estimates in the present study.

Our results are in line with studies that revealed an increased GH clearance in obese monkeys (15). They suggest that low circulating GH levels in obese women are partly due to increased GH clearance rates. An increased \( V_d \) may contribute as well. However, it needs to be kept in mind that GH secretion, which was not measured in this study, is a major determinant of plasma GH concentrations. Thus, in addition to increased clearance, diminished GH secretion is likely to contribute to low circulating GH levels in obese humans (41). There is no way to deduce from our data whether the changed GH kinetics are a primary defect, preceding the development of obesity, or a secondary sequela of increased adipose tissue mass. In any case, low circulating GH levels may have profound metabolic effects that predispose to obesity and/or contribute to the maintenance of large fat stores. GH increases basal metabolic rate (37), facilitates catecholamine-induced lipolysis (7), promotes protein synthesis (13), and stimulates cholesterol removal from the circulation by activating low-cholesterol lipoprotein receptors (36). GH-deficient adults are characterized by obesity, decreased lean body mass, and dyslipidemia (14). Many obese patients (in particular those with UBO) exhibit the same features (except for a decreased lean body mass). It is conceivable that reduced circulating GH concentrations play a role in the pathogenesis of obesity itself and/or its adverse metabolic sequelae.

Increased glomerular filtration of GH may play a role in the enhanced plasma GH clearance in obese subjects. About 50% of GH clearance from the circulation takes place in the kidney (18). Studies in rats have shown that glomerular filtration, tubular reabsorption, and peritubular breakdown are together the main route for GH removal by the kidney (28). A linear relationship between the glomerular filtration rate (GFR) and GH clearance was observed in healthy humans (18). There are many data indicating that GFR is increased in obese subjects (19). Thus increased glomerular filtration of GH might play a role in its enhanced plasma clearance in obese subjects. However, it should be kept in mind that renal clearance of GH is dependent on both GFR and its glomerular sieving coefficient, reflecting fractional GH extraction by the glomerulotubular system. It is unknown whether the GH sieving coefficient is altered in obese people.

Changes in liver metabolism may play a role in the pathogenesis of increased GH clearance in obese individuals. Studies in patients with chronic renal failure reveal that ~50% of GH is cleared by extrarenal
tissues, i.e., the liver (18). Hepatic GH metabolism takes place through receptor-ligand internalization and lysosomal degradation (10, 23). Because obesity is characterized by changes in hepatic drug disposition (9), it is conceivable that hepatic hormone clearance is also altered in obese people. Thus changes in liver metabolism may contribute to increased GH clearance in obese individuals. Within this framework, it seems important to note that the dose of SMS we employed in this study was shown to leave liver blood flow unaffected (39).

It is conceivable that GH processing by excessive body fat stores contributes significantly to enhanced GH clearance in obese people. Adipocytes are classic target cells for GH. Studies in isolated rat adipocytes have revealed typical hormone-receptor internalization and subsequent processing of GH by these cells. Seventy-five percent of internalized GH is degraded while the remaining 25% reenters the circulation through receptor-ligand cycling (35). The strong correlation that we observed between body fat mass and clearance (r = 0.56) is in keeping with the notion that adipose tissue takes part in GH metabolism.

The increased metabolic clearance of GH that we observed in obese women cannot be due to an increase of binding capacity in their serum. Two GHBP that circulate in blood have been identified (5, 6, 20). A large part of a single, symmetric GH pulse is bound to GHBP soon after its distribution in serum (42). Binding of GH profoundly affects its biological behavior. Complexed GH is cleared at a much slower rate than free GH, probably because the GH/GHBP complex is largely confined to the circulation, whereas free GH is distributed into the intercellular space (2). Thus increased serum GH binding capacity (i.e., GHBP concentration) would lead to prolonged GH half-life and decreased metabolic clearance rate (42). We found GHBP to be slightly (but not significantly) increased in obese compared with NW women, which is in accordance with some (21, 41) but not all studies on GHBP in obesity (17, 27, 29, 32). It follows from previously discussed data that the increased metabolic clearance of GH we observed in obese women cannot be due to an increase of binding capacity in their serum. Moreover, obesity does not appear to be associated with changes in affinity of GHBP that could account for increased metabolic clearance rates (4, 21).

This study shows that exogenous 22-kDa hGH is cleared from the circulation at a higher rate in obese than in normal weight humans. Despite the fact that the distribution of excess body fat has profound effects on metabolism in general, it does not appear to affect GH clearance.

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