Effects of 2 wk of GH administration on 24-h indirect calorimetry in young, healthy, lean men

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Hansen, Mette, Rikke Morthorst, Benny Larsson, Allan Flyvbjerg, Michael Højby Rasmussen, Hans Ørskov, Arne Astrup, Michael Kjaer, and Kai Henrik Wiborg Lange. Effects of 2 wk of GH administration on 24-h indirect calorimetry in young, healthy, lean men. Am J Physiol Endocrinol Metab 289: E1030–E1038, 2005. First published July 26, 2005; doi:10.1152/ajpendo.00124.2005.—The present study was designed as a randomized, double-blind placebo (Plc)-controlled study to determine the effect of 2 wk of growth hormone administration (GH-adm.) on energy expenditure (EE) and substrate oxidation in healthy humans. Sixteen young healthy men were divided into two groups. The study consisted of two 24-h measurements (indirect calorimetry), separated by 2 wk of either Plc or GH injections (6 IU/day). At baseline, no significant differences were observed between the two groups in any of the measured anthropometric, hormonal, or metabolic parameters, neither did the parameters change over time in the Plc group. GH-adm. resulted in a 4.4% increase in 24-h EE (P < 0.05) and an increase in fat oxidation by 29% (P < 0.05). However, a decrease in the respiratory quotient was only observed in the postabsorptive phase after an overnight fast (0.84 ± 0.1 to 0.79 ± 0.1, P < 0.05). Furthermore, lean body mass (LBM) was increased by GH-adm. only [62.8 ± 2.5 kg (baseline) vs. 64.7 ± 2.4 kg (after), P < 0.001]. In conclusion, GH-adm. increases 24-h EE, which may be partly explained by increased LBM. Furthermore, GH-adm. stimulates fat combustion, especially in the postabsorptive state.

RMR (35, 37). In contrast, another study by the same authors showed a significant increase in RMR and the relative fat oxidation after 2 wk of GH-adm. (36). Importantly, the majority of these earlier studies are based on short-term (≤1 h) measurements by ventilated hoods (34–37). The main purpose of the present study was to clarify whether GH-adm. in supraphysiological doses has a stimulating effect on EE measured over a 24-h period in healthy, lean young men. Especially, we aimed to test whether GH causes an increase in relative fat oxidation, either directly or indirectly, by an increase in EE in young healthy subjects. GH or placebo was administrated daily for 14 days, and indirect calorimetry was performed in a respiration chamber before and after the intervention period.

METHODS

Subjects. Sixteen healthy trained young males participated in the study. None of the subjects was taking any medication and were all certified as being in good health on the basis of a screening blood test and medical examination. Maximal oxygen uptake was measured by a 5-min all-out bike test through a mouthpiece coupled to an AMIS 2001 automated metabolic cart (INNOVISION, Odense, Denmark; see Ref. 30). Subjects with a body mass index >25 kg/m², previous or present cancer disease, or metabolic disorders were excluded. Baseline characteristics of the subjects are shown in Table 1. Subjects were recruited by advertisements at local educational institutions. Informed written and oral consent was obtained according to the Helsinki Declaration, and the study protocol was approved by the Ethics Committee for Medical Research in Copenhagen (journal no. 01–004/04) and by the Danish National Board of Health (journal-no. 2612–1592).

Study protocol. Subjects were randomly assigned in a double-blinded fashion to receive either GH (n = 8) or placebo (Plc; n = 8) once daily for 2 wk. The study included J two 24-h stays in a respiration chamber with measurements of EE and substrate oxidation [kcal/min and respiratory quotient (RQ)] before and after 2 wk of intervention and 2 measurements of body weight (BW) and body composition before, immediately after the second stay in the respiration chamber, and 1 wk after the last injection of either GH or Plc. The blood samples in the postabsorptive phase were taken the morning before each respiration chamber stay.

Recombinant human GH, 6 IU/day, 2 mg/day, or Plc was injected subcutaneously, anterior at the midthigh level at 10:00 PM daily (Norditropin, SimpleXx, or placebo for SimpleXx; Novo Nordisk, Bagsvaerd, Denmark). Both drug and injection devices were similar for Plc and GH. After detailed instructions, the subjects were able to perform the injections by themselves at home. Subjects entered the...
GH ADMINISTRATION AND 24-H INDIRECT CALORIMETRY

Table 1. Baseline characteristics of subjects

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>GH Group (n = 8)</th>
<th>Pcl Group (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24±1</td>
<td>25±1</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72.6±3</td>
<td>71.9±3</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.81±0.02</td>
<td>1.84±0.03</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.2±0.5</td>
<td>21.4±0.4</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>9.0±1.1</td>
<td>8.5±1.4</td>
</tr>
<tr>
<td>EE, MJ/day</td>
<td>9.1±0.3</td>
<td>9.3±0.2</td>
</tr>
<tr>
<td>VO2 peak, ml·min⁻¹·kg⁻¹</td>
<td>60.1±2.4</td>
<td>57.8±1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of men. GH, growth hormone; Pcl, placebo; BMI, body mass index, calculated as the body wt (kg) divided by height² (m²); EE, energy requirement, estimated EE during the stay in the respiratory chamber, based on the estimated body composition measured by electrical bioimpedance; VO2 peak, maximal oxygen uptake per kg body wt measured during a 5-min all-out bike test. There were no significant differences in baseline characteristics between the two groups.

The subjects participated in a standardized activity program while in the chamber. The program included eating three meals (9:00 AM, 1:00 PM, and 7:00 PM), two periods of bicycling at 100 W (10:00–10:15 AM and 4:00–4:15 PM), and two periods of walking 25 times forward and backward in the chamber (at 11:30 AM and at 2:30 PM) and laying down/sleeping (11:00 PM-8:00 AM). The rest of the day, the subjects were sitting up reading or watching television.

The calculation of the energy content of the standardized diet served during the chamber stay (24-h EI) takes into account the activity outlined in the standardized activity program and the subjects individual height, weight, height, and body composition (FFM and FM) estimated by bioelectric impedance measurement (HTS-engineering, Odense, Denmark) (21). The equation used to estimate the energy content of the diet while in the chamber is based on earlier measurements in our chambers as follows: energy content (kJ/day) = −1,034 + (122 × FFM) + (29.1 × FM) + [5,783 × SPA (%estimated)/100] + (64 × min bicycling) (27). The diet during both chamber stays provided 9.3 MJ (Pcl groups, range 8.5–10.5 MJ) and 9.1 MJ (GH group, range 8.5–10.5 MJ; between groups, P = 0.74), and the macronutrient composition in all diets was 55.6% energy (%E) from carbohydrate, 27.2% E% from fat, and 16.4% E% from protein. The level of precision for weighing out the food ingredients of the standardized diet was ±1 g. Food left by the subjects was reweighed, item for item, and subtracted in the calculation of actual intake.

The habitual diet macronutrient composition was estimated by 7-day weighed food records (±1 g) collected before the first chamber stay. Additional food records were collected from the last week of the intervention period before the last chamber stay to monitor changes in habitual food intake. Dietary energy content and nutrient composition of both the standardized and habitual diet were calculated by Dankost dietary assessment software (version 3000; Subborg, Denmark). In addition, the food quotients, the theoretical RQ produced by the diets, was calculated (57).

Analytical methods. All of the blood samples were taken from a cubital vein in sealed vials in the postabsorptive state immediately before initiation of the 24-h measurements, as previously described. The vials used for determination of glucose, NEFA, contained heparin. The vials used for glucose determination contained sodium fluoride and EDTA. After separation by centrifugation, serum or plasma was stored at −80°C before analysis.

Serum GH was determined by a time-resolved immunofluorometric assay (TR-IFMA; Wallac Oy, Turku, Finland). Serum IGF-1 was measured in acid ethanol serum extracts using an in-house monoclonal antibody-based TR-IFMA as previously described (19). Serum insulin was determined by a TR-IFMA (Wallac Oy).

The concentration of plasma glucose was assessed spectrophotometrically (COMAS MIRA PLUS; Roche) by an enzymatic method (Glucose oxidase/H2O2/Foehr).

Serum NEFA were determined by a colorimetric method (Wako Chemicals, Neuss, Germany). Plasma glycerol was measured spectrophotometrically by a Hitachi 912 (Roche Diagnostics, Hvidovre, Denmark) using a glycerol kit (catalog no. 148270; Boehringer Mannheim).

Statistical analysis. Data are presented as means ± SE [95% confidence interval (CI)], except the results for ΔEE, which are shown as median, 95% CI, and range. The results from the DEXA scan and the respiratory measurements were analyzed for interaction between time point for the daily injections in a diary, and syringes and injection technique were reviewed at the end of the injection period to ensure compliance. The last injection was performed at 10:00 PM in the respiration chamber during the second day.

All of the anthropometric variables stated in this article originate from measurements in the postabsorptive state. To detect potential changes in body composition, due to the treatment, a dual-energy X-ray absorbmetry (DEXA) scan was performed a total of three times (model DPX-IQ; Lunar Radiation, Madison, WI) as follows: 1) immediately after the baseline respiration chamber stay, 2) after the second stay in the chamber after the injection period, and 3) 1 wk after the last injection of either GH or Pcl. The coefficients of variation (%) for the measurements from DEXA of FM, LBM, and bone mineral content (BMC) were determined to be 0.9, 4.7, and 1.5%, respectively.

Twenty-four-hour EE was measured in open-circuit respiration chambers as described in detail elsewhere (3). The chambers work independently, each having a floor area of 6.5 m² and a volume of 14.7 m³. The 24-h measurement in the chamber started at 9:00 AM until 9:00 AM the next day. To investigate a possible drug effect during particular time periods of the day, the respiration measurements were further divided into the following specific intervals: day EE (9:00 AM-10:00 PM), night EE (10:00 PM-9:00 AM) and basal metabolic rate (BMR; 8:00 AM-9:00 AM).

As part of an adaptation to the chamber, the subjects stayed overnight in the chamber before the 24-h measurements. The following morning, the subjects were woken at 8:00 AM, and body temperature and BW were measured. In addition, blood samples in the postabsorptive state were taken for further analyses of serum GH, serum insulin-like growth factor-I (IGF-I), serum insulin, plasma glucose, plasma glycerol, and plasma nonesterified fatty acids (NEFA). Electrodes (Blue Sensor VL-50-L; Medicotest, Oelstykke, Denmark) were placed on the chest for continuous monitoring of heart rate and electrocardiogram by a telemetry system (Dialogue, 2000; Danica Electronics, Copenhagen, Denmark) during the stay in the chamber. Afterward, the door of the chamber was locked, and the 24-h measurements were started at 9:00 AM. Spontaneous physical activity (SPA) during the stay in the chambers was assessed by two micro-wave radars (Sisor Mini Radar; Static Input System, Lausanne, Switzerland). The SPA measurements indicate the percentage of time the subjects are active to a detectable degree.

The gas exchange of the subjects was calculated from measurements of oxygen (Magno 4 G; Hartmann and Braun analyzers, Frankfurt, Germany) and carbon dioxide concentrations (Uras 3 G, Hartmann and Braun analyzers, Frankfurt, Germany) at the outlet of the chamber, and of airflow through the chambers (72,000 l/24 h). The room temperature was maintained constant at 24°C in the daytime and 18°C at night. The protein oxidation was calculated from nitrogen excreted in the urine, assuming combustion of 6.25 g protein/g N excreted. Nonprotein RQ (=CO2/O2) was calculated to estimate changes in the relative use of carbohydrate and fat as a fuel in the oxidative metabolism. A reduction in RQ indicates a decrease in carbohydrate oxidation and a corresponding increase in fat oxidation. Further descriptions of the used constants and equations for EE and substrate oxidation are described by Elia and Livesey (17). A calibration of the measuring of the gas analyzers found a coefficient of variation of 0.5% during 24-h measurements (3).
time and treatment by a two-way ANOVA with repeated measurements (SPSS version 10.0 for Windows). A univariate ANOVA with baseline values as covariates was used to test for between-groups differences in energy and substrate combustion, body composition, and concentrations of hormones and metabolites in the blood compared with baseline (Δ, after – baseline). When significant changes were observed, regression analyses were performed to identify potential explaining factors. Analyses of correlation were performed in the same context. Paired t-tests were used to detect significant within-group treatment effects in EE, RQ, substrate oxidation (kJ/min), body composition (DEXA), diet records, and blood parameters. Unpaired t-tests were used in the analyses of significant between-group differences in diet composition and baseline characteristics and values. For all the analyses, \( P < 0.05 \) (2-tailed) was considered statistically significant.

RESULTS

All sixteen males completed the study without reporting any side effects. Compliance was high (100%) based on the returned injection protocols and empty ampoules obtained from each subject.

Hormones and metabolites. After the last injection (10 h), serum GH in the GH group was still augmented compared with the Plc group (interaction between time and treatment, \( P < 0.0001 \); Fig. 1). Serum IGF-I was increased significantly after the GH-adm. period to a level above the normal range for serum samples in a postabsorptive phase (Fig. 1). The interaction between time and treatment for the changes in plasma glycerol and plasma NEFA were also significant (both \( P < 0.01 \); Fig. 1). After the GH injection period, the level of plasma glycerol was increased significantly by 166 ± 29% in the postabsorptive phase after overnight fasting compared with the baseline level, whereas a nonsignificant decrease in plasma glycerol concentration was observed in the Plc group (−18 ± 65%, \( P < 0.09 \)). Likewise, plasma NEFA was enhanced significantly after GH-adm. However, GH-adm. for 2 wk did not change the level of plasma glucose in the postabsorptive phase.

![Fig. 1. Postabsorptive concentrations of serum growth hormone (GH), serum IGF-I, plasma glycerol, plasma nonesterified fatty acid (NEFA), plasma glucose, and serum insulin before (baseline) and after injections of either GH (filled bars) or placebo (Plc, open bars). Values are means ± SE for each group of 8 subjects. GH group (after) higher than GH group (baseline): *\( P < 0.05 \), **\( P < 0.01 \), and ***\( P < 0.001 \). GH group (after) higher than Plc group (after), (#)\( P = 0.07 \), #\( P < 0.05 \), ##\( P < 0.01 \), and ###\( P < 0.001 \). No significant differences between groups were detected at baseline.](image-url)
phase (interaction between time and treatment, \( P = 0.18 \); Fig. 1) or the concentration of lactate (data not shown). In contrast, serum insulin was increased significantly in the GH group 10 h after the last injection compared with baseline and Plc (\( P < 0.01 \) and \( P < 0.05 \), respectively).

**Body composition.** Administration of GH led to a significant increase in LBM (2.3 ± 0.7 kg) after 2 wk (interaction between time and treatment, \( P < 0.005 \); Table 2). The eight subjects in the GH group all experienced an increase in LBM (2.3%) after the last injection compared with baseline and Plc (\( P < 0.067 \); Table 2). Six out of eight subjects in the GH-group experienced a decrease in FM. The two remaining subjects had an initial FM of only 3.3 kg (FM\% 4) and 6.0 kg (FM\% 9). No significant interaction between time and treatment was observed for the changes in BW (\( P = 0.363 \)), but a significant increase from baseline was observed in the GH group only (from 72.6 ± 2.8 to 73.7 ± 2.7 kg, \( P < 0.05 \)). After the last injection of GH (1 wk), the increase in BW was still significantly preserved in the GH group. Furthermore, 1 wk after the last injection of Plc an unintended increase in BW was also observed in the Plc group compared with at baseline.

**EE.** Figures 2 and 3 show the average and individual changes in EE, respectively. The interaction effect between time and treatment for the change in EE was significant for the whole 24-h period (\( P < 0.05 \)), and when the results for the night period were analyzed separately (\( P < 0.05 \)). The increase in EE in the GH group was small but significant compared with the changes in the Plc group, both during the whole 24-h period (4.4%, 95% CI 1.0 and 7.9, \( P < 0.05 \)) and when data from the period at night were analyzed separately (5.3%, 95% CI 1.0 and 9.6, \( P < 0.05 \)). Individual data showed an increase in 24-h EE in six out of eight subjects in the GH group compared with only two out of eight subjects in the Plc group. Data for daytime EE showed a strong tendency toward a significant interaction between time and treatment (\( P = 0.051 \)), and an analysis of the delta values showed an increase in daytime EE by 3.9% (95% CI 0.1 and 7.8). The results for the measurements of BMR showed an increase by 8.3% (95% CI 0.1 and 16.7) in EE after the GH-adm. period and a strong tendency toward a significant interaction between time and treatment (\( P = 0.06 \)).

After adjustment for the changes in LBM, the differences between the two groups in 24-h EE, BMR, and during the night period were no longer significant. Regression analyses indicate that the change in LBM can explain 21.6% (\( r^2 = 0.216 \)) of the

**Table 2. Effect of GH administration and time and treatment interaction**

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>Posttreatment</th>
<th>1 Week</th>
<th>Time and Treatment Effect* (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>72.6 ± 2.8</td>
<td>73.7 ± 2.7*</td>
<td>73.6 ± 2.8</td>
<td>0.363</td>
</tr>
<tr>
<td>Plc</td>
<td>71.9 ± 3.0</td>
<td>72.1 ± 3.1</td>
<td>73.0 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>6.5 ± 0.9</td>
<td>5.9 ± 0.7§</td>
<td>6.1 ± 0.7</td>
<td>0.184</td>
</tr>
<tr>
<td>GH</td>
<td>6.5 ± 0.9</td>
<td>5.9 ± 0.7§</td>
<td>6.1 ± 0.7</td>
<td>0.184</td>
</tr>
<tr>
<td>Plc</td>
<td>6.3 ± 1.3</td>
<td>6.9 ± 1.5</td>
<td>6.7 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>LBM, kg</td>
<td>62.8 ± 2.5</td>
<td>64.7 ± 2.4§</td>
<td>64.3 ± 2.3*</td>
<td>0.004</td>
</tr>
<tr>
<td>GH</td>
<td>62.3 ± 2.2</td>
<td>61.8 ± 1.9</td>
<td>62.9 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Plc</td>
<td>6.3 ± 1.3</td>
<td>6.9 ± 1.5</td>
<td>6.7 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>BMC, kg</td>
<td>3.2 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>0.842</td>
</tr>
<tr>
<td>GH</td>
<td>3.2 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>0.842</td>
</tr>
<tr>
<td>Plc</td>
<td>3.3 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE for each group of 8 subjects. BMC, bone mineral content; LBM, lean body mass excluding BMC. Body composition before, after, and 1 wk after administration of either GH or Plc. *Significantly different from baseline. † \( P = 0.051 \); ‡ \( P = 0.05 \); § \( P = 0.067 \); §§ \( P = 0.064 \); †† \( P = 0.08 \); ‡‡ \( P = 0.053 \).
change in EE during the 24-h period, and 32.5% ($r^2 = 0.325$) of the change in EE at night.

Energy balance and substrate oxidation. The content of energy and nutrients in the habitual diet was not significantly different between the two groups or between the two food record periods (before and during the intervention period). The macronutrient composition of the diet served during the stay in the respiratory chamber was similar to the mean composition of the habitual diet of the subjects (%energy from protein, carbohydrate, and fat). The energy content of the diet in the prechamber periods (14 –15 MJ/day) was higher than the energy content of the served diet consumed during the stay in the chamber (9 ± 0 MJ/day; $P < 0.001$), which was adjusted to the lower physical activity level in the chamber. The increased EE caused by treatment resulted in differences in energy balance between the two groups, since the energy content of the standardized diet was the same during the stay in the chamber pre- and postmeasurement (interaction between time and treatment, $P < 0.05$). The increase in EE resulted in a negative energy balance in the GH group during the stay in the chamber after the treatment period (−1.2 ± 0.1 MJ/24 h, $P < 0.001$), whereas the EE in the Plc group was not significantly different from the energy intake (−0.4 ± 0.2 MJ/24 h).

No significant interaction between time and treatment was found in RQ during the whole 24-h period or at night (interaction between time and treatment, 24-h and night, $P = 0.09$; daytime, $P = 0.11$). However, in the postabsorptive period, 10 h after the last injection of GH, a significant decrease in RQ was observed in the GH group compared with the Plc group (interaction between time and treatment, $P < 0.05$).

GH-adm. had a stimulating effect on the fat oxidation rate (kJ/min; interaction between time and treatment, all periods, $P < 0.05$; Fig. 4). The observed increase in fat oxidation (kJ/min) was mainly prominent in the postabsorptive phase, whereas calculated over the whole 24-h period the effect of treatment only tended to be significant ($P = 0.074$). At night (10:00 PM–9:00 AM), the treatment led to a 26 ± 6% increase in fat oxidation compared with the changes in the Plc group ($P < 0.01$). During the BMR measurements, the increase in fat oxidation after GH-adm. was even more pronounced (GH group: +40.9 ± 9.6%; Plc group: −10.9 ± 12.3%, $P < 0.01$ between groups). However, after adjustment for the changes in BMR the significant difference between the two groups in fat oxidation (8:00–9:00 AM; kJ/min) disappeared ($P = 0.26$).

The interaction between time and treatment for the absolute oxidation of protein (data not shown) and carbohydrate was not significant in any of the time periods (Fig. 4). However, a tendency toward a decrease in carbohydrate oxidation during the measurement of BMR was observed after GH-adm. (interaction between time and treatment, $P = 0.073$), and change in
carbohydrate oxidation from baseline was significantly lower after GH-adm. ($P < 0.05$).

**DISCUSSION**

The major new finding in the present study is a significant increase in EE over 24-h after 2 wk of GH-adm. to healthy lean men. Furthermore, GH-adm. resulted in a significant increase in the relative fat oxidation during the chamber stay in the postabsorptive phase, but not when calculated over the whole 24-h period. These findings observed during standardized conditions were accompanied by an increase in LBM, which seemed to play an important role in the effect of GH-adm. on EE and fat oxidation. Furthermore, a tendency toward a reduction in FM over the 2 wk of GH-adm. was observed.

**EE.** The observed increase in EE over a 24-h period after GH-adm. was small (4.4%) but significant. Six of eight subjects experienced an increase in EE. The observed stimulating effect of GH on EE confirms what has been observed in the literature after long-term GH-adm. to healthy individuals when measured over short time periods (<1 h) after an overnight fast. Increases of 10–15% in RMR in young healthy subjects (4, 36), 20% in obese subjects (26), and 12–31% in elderly subjects (28, 29) have previously been observed. Under the same circumstances, we observed a strong tendency toward an increase of 8% ($P = 0.053$) in BMR in the present study. The smaller effect of GH on EE in our study may be related to higher doses in the earlier studies and the fact that aged and obese subjects are characterized by having a relative somatopenia compared with young healthy subjects (42, 56). Furthermore, the stimulating effect of GH in the present study would most likely have been more pronounced if the GH group had not been in negative energy balance during the stay in the chamber after the intervention period. Earlier findings indicate that the effects of GH-adm. seem to be blunted by a hypocaloric diet (25).

In two studies by Møller and colleagues (35, 37) RMR did not change, but, in contrast to our and the above-mentioned studies, the subjects only had a single acute injection of GH (37) or infusion of GH over 4 h (35). This indicates that the stimulating effect of GH-adm. on EE is probably explained by an accumulating effect of repeated injections of GH. A likely explaining factor, measured in the present study, is an increase in metabolic active tissue (LBM). This fits together with the fact that GH is known to stimulate the energy consuming protein synthesis (18). A cross-sectional study ($n = 313$), from our laboratory, showed that ~80% of the variation in 24-h EE (indirect calorimetry) in healthy individuals could be explained by variations in FFM (2, 27). Furthermore, FFM explained ~80% of the variance in RMR after administration of GH for 6 mo to GH-deficient patients (53). In line with this, the difference between the two groups in 24-h EE disappeared after adjustment for change in LBM. This strongly supports that the increase in metabolic active tissue has a marked influence on the increase in EE observed after GH-adm. Furthermore, it can explain the disparity in the literature between acute and long-term effect of GH-adm. on EE (35–37).

The finding that GH both increased LBM and 24-h EE, and that these changes were highly correlated, makes it very likely that GH increased 24-h EE through the increased LBM. It is well known that the size of LBM is the major determinant of 24-h EE (3, 43, 58), and, after we adjusted 24-h EE for differences in LBM, the GH effect was no longer significant. However, we cannot exclude the possibility that other factors that covariates with both LBM and 24-h EE are also responsible for both effects.

In a study by Wolthers et al. (60), a significant increase in RMR with no detectable increase in LBM was observed after only 4 days of GH-adm. to healthy men. This underlines that other mediators, related to GH-adm., have a stimulating effect on EE. Earlier studies have confirmed that an increase in serum IGF-I (24, 45, 53), triiodothyronine (26, 34), and uncoupling proteins (UCP), especially UCP3 (41) seems to play a role in the enhancing effect of GH-adm. on EE. We observed a significant increase in both IGF-I and insulin, which may play an additional explaining role for the increase in EE. Furthermore, the increase in EE could potentially also be due to an increase in SPA (43). In the present study, a positive correlation between the changes in 24-h EE and SPA ($r^2 = 0.299$) was also observed; however, the nonsignificant changes in SPA were not related to the treatment ($P > 0.7$). Finally, GH-adm. may increase the energy-consuming futile cycling of substrates, indicated by both the marked lipolytic effect shown in the present study and increases in the level of ketone bodies (35, 36) as well by an increase in nonoxidative disposal of glucose observed by others (26). To sum up, the increase in EE is probably not primarily caused by a direct effect of GH but may be mediated indirectly by changes in LBM and hormonal changes related to the GH-adm. Additionally, the main effect of GH-adm. on EE may also be mediated by energy-costly futile cycles, such as relatively high rates of protein synthesis and degradation associated with a greater LBM, the later supported by the lack of difference in change in EE when data were averaged for changes in LBM.

**LBM and FM.** A 3% increase in LBM after GH-adm. was observed in the present study. GH is known to increase the amount of extracellular fluid in a dose-dependent manner by a direct effect on the renin-angiotensin system (22). However, after cessation of GH-adm., a known prompt diuretic effect is observed (64) that can be related to a short half-life of GH (6–20 min for the free fraction of GH; see Ref. 5). Because of this knowledge, an additional DEXA scan was performed 1 wk after the last injection in the present study. The additional DEXA scan confirmed the increase in LBM related to GH-adm. We strongly believe that the change in LBM seen with GH-adm. is not only a result of water retention but of true tissue changes.

The observed increase of 3% in LBM is comparable with earlier studies where increases of 3–6% have been observed after GH-adm. for 4 wk to 12 mo to GH-deficient patients (12, 15, 53) and healthy elderly (28, 29, 55) and obese (46) subjects. The increase in LBM does not seem to be accumulated over a longer period (12, 53). The number of studies where GH has been administered for prolonged periods to healthy young subjects is few, and the results are conflicting (14, 16, 61). In agreement with our results, increases in LBM have been observed after six and 12 wk of GH-adm. (14, 61). In contrast, Dyessig et al. (16) did not find any change in body composition after 6 wk of GH-adm. to strength athletes. The latter negative finding is probably explained by the baseline characteristics of subjects (BW 122% of normal, fat 10%) and their less precise way of determining LBM by skinfold measurements.
The increase in LBM indicates a positive nitrogen (N) balance induced by either an increase in the intake of protein in the diet or a decrease in excretion of N during the intervention period. No differences in the nutrient intake between groups were detected during the food registration periods. The intake of protein in the GH group was 1.7 g·kg⁻¹·day⁻¹. This is far higher than the recommended daily intake of protein (0.75 g·kg⁻¹·day⁻¹; see Ref. 51), and plenty of substrate should thereby be available to increase the rate of protein synthesis. Increases in whole body protein synthesis (10, 61) and muscle protein synthesis (10) have been observed by others. As an indicator of protein degradation, 24-h urine-N was collected and analyzed during the chamber stay. No change was observed. In the literature, a decrease in amino acid oxidation (9, 13) and reduction in N excretion (33) have been detected after acute GH-adm. In the present study, the negative energy balance in the GH group during the second stay in the chamber probably may have increased the degradation of proteins and thereby confounded the outcome of the N excretion rate calculations as a measurement of the general degradation rate. However, the negative energy balance was small, and after 2 wk of GH-adm. the protein turnover is more likely to have returned to a new steady-state level, and thus nitrogen balance may have been reestablished, however, at a higher LBM.

To establish an increase in LBM, it is essential that amino acids are available as substrate for the protein synthesis, and a stimulus to increase protein turnover is introduced (for example, by GH-adm.). It is known, that GH-adm. will increase protein synthesis both in whole body and in skeletal muscle (39). However, it has to be acknowledged that the LBM increase is an energy costly process. Nevertheless, the observed increase in LBM after 2 wk of GH-adm. indicates that the energy state in the lean tissue has been sufficient to build up new tissue. The food records did not confirm any change in energy intake during the intervention period. However, it is well known that diet recall is inaccurate to a certain degree (7, 20). Thus the subjects must have underreported their energy intake. Nevertheless, it has to be underlined that the treatment by time interaction was nonsignificant for the change in BW (P = 0.363). In addition, the reduction in energy-dense FM, even though nonsignificant, may have contributed with energy to the protein synthesis. This led us to suggest that a change in BW is not the main effect of GH-adm. to healthy lean individuals, whereas the results showing a change in body composition are more convincing.

Administration of GH invariably leads to promotion of lipolysis, stimulation of IGF-I production, and hyperinsulinemia (16, 28, 36, 53, 55). All of these secondary hormonal and metabolic changes, which were observed in the present study, will promote protein accretion (50, 54, 59). Especially of note, IGF-I administration has been associated with a stimulating effect on cell differentiation and proliferation, increase in the protein synthesis (10, 50), and a reduction in protein oxidation (24).

FM tended to decrease after 2 wk of GH-adm. Earlier studies have observed favorable decreases in FM by 3–21% when GH has been administrated for prolonged periods of time (>1 mo) to elderly (23, 29, 31, 40), obese (46) subjects and especially pronounced when administered to GH-deficient patients (47, 53). A short administration period was chosen in the present study. This may explain that FM only tended to decrease after treatment, which, however, is still impressive since the subjects were already very lean and not GH deficient. In this context, it also has to be emphasized that the subjects were instructed to eat a habitual non-energy-restricted diet during the administration period.

The upregulated EE may have led to a mobilization of fatty acids and thereby energy for building up new LBM. In addition, after GH-adm., an enhanced sensitivity to catecholamines and thereby stimulating effect on the activity of hormone sensitive lipase has been observed in the adipocytes (32). Furthermore, the mobilization of fat may be indirectly mediated by GH-adm. via inhibition of the action of antilipolytic compounds (e.g., adenosine, prostaglandins, and insulin (45) and/or a tissue-specific inhibition of the activity of lipoprotein lipase in the adipose tissue, and thereby reduction in the net uptake of fatty acids (44). In the present study, we observed a higher level of plasma glycerol after GH-adm., which supports the lipolytic effect of GH.

Substrate oxidation. To our knowledge, this study is the first study that has measured the effect of GH-adm. to healthy subjects on the substrate oxidation pattern over a 24-h period, characterized by both postabsorptive and postprandial phases. Our results in the postabsorptive state are in line with the results in the literature. An increase in absolute and relative fat oxidation has been the universal finding after both acute (4, 35, 37) and long-term (26) GH-adm. to GH-deficient and healthy subjects (4, 26, 34–36). In contradiction to the findings in the postabsorptive state, no significant change in RQ was observed during the 24-h period in the present study. A plausible explanation for the disparity could be that the intake of carbohydrates during the day has overwhelmed the effect of GH on the substrate oxidation pattern during the stay in the chamber. It is well known that consumption of carbohydrates has inhibitory effects on the mobilization, intracellular transport, and oxidation of fat, both directly and indirectly by stimulating secretion of insulin (11). The availability of glucose in the fed state may thereby have counteracted the GH-related increase in fat oxidation. In contrast, the effect of GH-adm. on substrate selection is found to be significantly affected in the postabsorptive state, supported by our findings during the BMR measurements. Additionally, as the results indicate, the accumulated negative energy balance during the stay in the chamber can probably explain a main part of the more pronounced stimulating effect on fat utilization and inhibiting effect on glucose oxidation during the BMR measurement at the end of the 24-h period compared with when calculated for the whole 24-h period. This statement is supported by the fact that the difference in fat oxidation between groups disappeared when the results were adjusted for difference in energy balance. In contrast to this explanation, it could be argued that the results in essence are dependent on the time and dosing of injections and would have been entirely different if another mode of GH-adm. had been applied (e.g., continuous infusion, 2 daily injections, higher dose, etc.). It cannot be ruled out that the insignificant effect on the substrate utilization pattern during the daytime may be related to a lower GH concentration during the daytime compared with the hours following directly after the last injection in the evening.

After 2 wk of GH-adm., a significantly higher serum insulin concentration was observed in the postabsorptive phase, indicating an increased insulin resistance. This supports earlier
findings where GH-adm. has been coupled to increased insulin resistance, when administered to GH-deficient patients (48) and healthy subjects (26, 36). The effect is mainly because of the lipolytic effect of GH (8, 52) but also because of a direct effect on skeletal muscle (4, 35). The fact that insulin concentration was increased after relatively short-term GH-adm. in this group of very lean individuals underlines the potent acute effect that GH-adm. in similar doses will have on groups predisposed to be glucose impaired before the treatment, such as elderly or obese individuals. The negative effect of GH-adm. on glucose tolerance has been confirmed in GH-deficient patients. Despite a significant increase in LBM and a reduction in FM, GH-adm. for 30 mo induced a significant deterioration in glucose metabolism. However, recent findings indicate that if GH is administered in very low doses to obese (1, 25, 63) and healthy subjects (62), GH-adm. may have less negative impact on glucose tolerance or even to some extent enhance insulin sensitivity in obese type II diabetic patients if combined with an energy-restricted diet, probably because of the combined positive effect on abdominal obesity (38). Nevertheless, before GH-adm. can be recommended as part of a treatment to change the body composition of obese individuals, further long-term studies are needed to confirm these new findings in light of ours and earlier findings showing an increased insulin resistance after GH-adm. and potential other side effects (15).

In conclusion, 2 wk of GH-adm. to young healthy, moderately trained, lean men resulted in a significant increase in 24-h EE. Part of the increase in EE and fat oxidation seems to be explained by an increase in LBM. Furthermore, GH-adm. had a stimulating effect on fat oxidation, which was especially pronounced in the postabsorptive state. These treatment effects sound as an attractive way of handling the obesity epidemiological trends in many countries, but further studies are needed in this area, and caution has to be taken because of potential side effects.

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