Growth hormone-induced insulin resistance is associated with increased intramyocellular triglyceride content but unaltered VLDL-triglyceride kinetics

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Am J Physiol Endocrinol Metab 292: E920–E927, 2007. First published November 28, 2006; doi:10.1152/ajpendo.00374.2006.—The ability of growth hormone (GH) to stimulate lipolysis and cause insulin resistance in skeletal muscle may be causally linked, but the mechanisms remain obscure. We investigated the impact of GH on the turnover of FFA and VLDL-TG, intramuscular triglyceride content (IMTG), and insulin sensitivity (euglycemic clamp) in nine healthy men in a randomized double-blind placebo-controlled crossover study after 8 days treatment with (A) Placebo + Placebo, (B) GH (2 mg daily) + Placebo, and (C) GH (2 mg daily) + Acipimox (250 mg × 3 daily). In the basal state, GH (B) increased FFA levels (P < 0.05), palmitate turnover (P < 0.05), and lipid oxidation (P = 0.05), but VLDL-TG kinetics were unaffected. Administration of acipimox (C) suppressed basal lipolysis but did not influence VLDL-TG kinetics. In the basal state, IMTG content increased after GH (B: P = 0.03). Insulin resistance was induced by GH irrespective of concomitant acipimox (P < 0.001). The turnover of FFA and VLDL-TG was suppressed by hyperinsulinemia during placebo and GH, whereas coadministration of acipimox induced a rebound increase FFA turnover and VLDL-TG clearance. We conclude that these results show that GH-induced insulin resistance is associated with increased IMTG and unaltered VLDL-TG kinetics; we hypothesize that fat oxidation in muscle tissue is an important primary effect of GH and that circulating FFA rather than VLDL-TG constitute the major source for this process; and the role of IMTG in the development of GH-induced insulin resistance merits future research.

acipimox; lipolysis; very-low-density lipoprotein-triglyceride kinetics; hyperinsulinemic euglycemic clamp; insulin sensitivity

A REPRODUCIBLE EFFECT OF GROWTH HORMONE (GH) is mobilization of body fat and stimulation of lipid oxidation. Administration of a physiological GH bolus in the postabsorptive state increases circulating levels of free fatty acids (FFA) after a lag phase of 2 h, which is accompanied by suppression of the uptake and oxidation of glucose in skeletal muscle (35, 36). Long-term GH administration translates into a significant reduction in fat mass, especially from the central compartments, and microdialysis studies demonstrate lipolysis directly in adipose tissue in vivo (10).

The mechanisms underlying these effects of GH are not fully understood, but there is evidence to support that activation of the hormone-sensitive lipase is involved (9), and we (37) and others (41) have observed that coadministration of acipimox, a long-acting nicotinic acid analog, abrogates the GH-induced rise in FFA. Moreover, we have observed (38) that long-term administration of acipimox in GH-treated adult patients with GH deficiency did not affect the rate of lipid oxidation despite a more than 50% reduction in the circulating FFA levels. This could indicate that GH may utilize alternative sources of lipid fuels for oxidation such as circulating very-low-density lipoprotein-triglyceride (VLDL-TG) or intramyocellular lipids. Another possibility could be that the turnover of circulating FFA was proportionally increased to meet the demand for oxidation.

Prolonged GH exposure is also associated with insulin resistance and in particular by inhibition of insulin-stimulated glucose uptake in skeletal muscle. Muscle biopsies obtained after systemic GH exposure to normal subjects and GH-deficient patients have shown that the suppression of glucose uptake, as assessed by the euglycemic clamp technique, is accompanied by unaltered insulin binding (2), increased levels of intracellular glucose (7), reduced glycogen synthase activity (2, 7), and reduced glucose oxidation (2). Numerous studies in healthy subjects and patients with obesity and type 2 diabetes have demonstrated that elevated FFA levels are involved in the pathogenesis of insulin resistance (3, 5), which makes it obvious to suggest a causal link between the lipolytic and insulin-anTagonistic effects of GH. In support of this, we (37) have observed that short-term acipimox administration in GH-deficient patients, which resulted in reduced serum FFA levels, significantly reduced the suppressive effects of GH on insulin-stimulated glucose uptake in muscle. In the present study, our aim was to study the impact of prolonged GH exposure on the turnover of circulating FFA and VLDL-TG, intramuscular TG (IMTG) content, and the sensitivity to insulin-stimulated glucose disposal.

SUBJECTS AND METHODS

Subjects and design. Nine healthy male volunteers participated in this study. All study participants gave informed consent according to the second Declaration of Helsinki. The Ethical Scientific Committee of Aarhus County approved the study. The study was monitored by the Good Clinical Practice Unit of Aarhus University Hospital. The participants were 23.2 ± 0.6 yr old (means ± SE), and BMI was 23.1 ± 0.5 kg/m², total cholesterol 4.2 ± 0.3 mmol/l, triglyceride 1.1 ± 0.2 mmol/l, LDL 2.2 (1.5, 4.5) mmol/l [median (range)], and Hb A1c 5.1 ± 0.1%. One subject had mild hypercholesterolemia. None of the participants received any regular medication, and all were nonsmokers.

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Each participant underwent three study periods of 8 days in a randomized, double-blind, placebo-controlled manner: (A) placebo tablets and placebo injections, (B) daily GH treatment (Norditropin SimplexX; Novo Nordisk, Copenaghen, Denmark; 2 mg sc at 2200, last injection on day 7) plus placebo tablets, and (C) GH (same as in study B) plus oral acipimox; Pfizer, New York, NY; 250 mg thrice daily at 0700, 1400, and 2100, last tablet on day 8 at 0700). The three study periods were separated by a washout period of 1–3 wk. Compliance was assessed by returned vials and tablets.

Before the study, the participants were instructed by a clinical dietician to consume a weight-maintaining diet containing 50–60% carbohydrates, maximum 30% fat, and protein at 10–15% of total energy intake. The participants were admitted to the Clinical Research Center on day 7 in the evening. The investigations were carried out after an overnight fast. Only ingestion of tap water was allowed. The studies were conducted in the supine position during thermoneutral conditions.

Substrate metabolism was investigated for 6.5 h on day 8 (0800–1430; Fig. 1). Before 0800, an intravenous catheter (Viggo, Helsingborg, Sweden) was placed in an antecubital vein for infusions. For blood sampling, a wrist vein of the contralateral hand was cannulated and kept in a heated box with an air temperature of 65°C to provide arterialized blood. The subjects were studied in the basal state for 210 min (0800–1330) followed by a hyperinsulinenic euglycemic clamp (1130–1430).

After drawing of baseline blood samples (0800), ex vivo labeled [1-14C]triolein VLDL-TG (10 μCi) was administered as a bolus injection over 10 min. At t = 0, 60, 120, 180, 210, 240, 300, 360, and 390 min, blood samples were drawn and analyzed for concentration and specific activity (SA) of labeled VLDL-TG and total TG concentration.

At 0800, a primed (20 μCi) continuous (12 μCi/h) infusion of [3-3H]glucose (Laegemiddelstyrelsen, Copenhagen, Denmark) was started and maintained for 6.5 h. After allowance of time for tracer equilibration, endogenous glucose production in the basal state was assessed at 210 min (0800–1330; Fig. 1). Before 0800, an intravenous catheter (Viggo, Helsingborg, Sweden) was placed in an antecubital vein for infusions. For blood sampling, a wrist vein of the contralateral hand was cannulated and kept in a heated box with an air temperature of 65°C to provide arterialized blood. The subjects were studied in the basal state for 210 min (0800–1330) followed by a hyperinsulinenic euglycemic clamp (1130–1430).

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At 0900 and 1330, a constant infusion of [9,10-3H]palmitate (0.3 μCi/min; Endocrine Research Unit, Rochester, MN) was started and maintained for 1 h. Palmitate turnover was assessed during the last 30 min of infusion.

At t = 150 min, a muscle biopsy was taken from the vastus lateralis muscle with a Bergström biopsy needle preceded by local anesthesia (1% lidocaine) at t = 120 min. A total amount of ~100 mg of muscle was aspirated, and biopsies were cleaned for blood (within 15 s) and snap-frozen in liquid nitrogen.

The infusion rate of insulin (Actrapid; Novo Nordisk, Gentofte, Denmark) during the clamp was 0.6 mU·kg⁻¹·min⁻¹. Plasma glucose levels were clamped at ~5 mmol/l by adjusting the rate of infusion of 20% glucose according to measurements every 10 min (8). Insulin sensitivity was calculated as the mean glucose infusion rate during the last 30 min of the clamp.

**Assays.** Plasma glucose was measured in duplicate immediately after sampling on a glucose analyzer (Beckman Coulter, Palo Alto, CA). A double monoclonal immunofluorometric assay (Delfia, Wallac, Turku, Finland) was used to measure serum GH. Plasma glucagon and serum total insulin-like growth factor I (IGF-I) were measured by Delfia in-house assay (16). Serum insulin was determined by commercial enzyme-linked immunosorbent assay (DAKO, Glostrup, Denmark). Serum total FFA was determined by a colorimetric method employing a commercial kit (WAKO Chemicals, Neuss, Germany). Glycerol and 3-hydroxybutyrate (BOH) were analyzed by autofluorometric enzymatic methods (23). Catecholamines were measured by liquid chromatography (12) (8 subjects were included in the data analysis). Plasma TG concentration was analyzed using a COBAS Fara II.

Endogenous glucose production in the basal state (t = 100–120 min) was calculated by dividing the [3-3H]glucose infusion rate by the steady-state plateau of [3-3H]glucose SA in plasma.

Systemic palmitate flux was measured with an isotope dilution technique and steady-state equations. Blood samples for measurements of palmitate concentration and SA were drawn before the infusion and after 30, 40, 50, and 60 min of the infusion period. Plasma palmitate concentration was determined by HPLC (34) using [1H₁]palmitate as internal standard (28). Plasma palmitate SA was determined by liquid scintillation counter. Systemic palmitate flux was calculated using the [9,10-3H]palmitate infusion rate divided by the steady-state palmitate SA (8 subjects were included in the data analysis).

Plasma palmitate oxidation during the basal steady-state period was determined as reported earlier by using the slope of the increase in 3H₂O in total body water to calculate the 3H₂O production rate for each participant (22). The plasma 3H₂O concentrations were measured as previously described (25). In brief, plasma proteins were precipitated by adding 0.5 ml of 1% perchloric acid to duplicate samples of 0.5 ml of plasma. After centrifugation, 0.7 ml of the supernatant was transferred to duplicate scintillation vials. Scintillation cocktail was added to one vial (wet), and the duplicate vial from each time point was dried down before adding 0.7 ml of deoxygenated water and scintillation cocktail (dry). The difference in ³H activity between the wet and the dry vials was taken as the ³H₂O radioactivity, which was then corrected to 1 ml of plasma water. The rate of whole body ³H₂O production (dpm/min) was determined by linear regression analysis of the concentration of ³H₂O in body water vs. time. The rate of ³H₂O production was divided by the average plasma [³H]palmitate SA.
during the same period to calculate plasma palmitate oxidation rates during that period (6 subjects were included in the data analysis).

Indirect calorimetry (Deltatrac; Datex Instruments, Helsinki, Finland) was performed for 30 min at $t = 90 – 120$ min (basal state) and $t = 360 – 390$ min (clamp state). From measurement of gas exchange, energy expenditure (EE) and respiratory quotient (RQ) were calculated. The initial 5 min of calorimetry were used for acclimatization, and calculations were based on 1-min measurements of the remaining period. Rates of lipid and glucose oxidation were calculated after correction for protein oxidation, which was estimated from the urinary excretion of urea (15).

All results of circulating hormones and metabolites are the means of measurements in the basal state $t = 100 – 120$ min and in the clamp state $t = 370 – 390$ min.

**VLDL-tracer.** $[1^{-13}C] $triolein-labeled VLDL-TG was prepared as previously described (18, 19). Briefly, the method relied on ex vivo incorporation of $[1^{-13}C] $triolein in each subject’s native VLDL particle with subsequent reinfusion during study day 8. VLDL-TG turnover was calculated from plasma disappearance rate of VLDL-TG SA (dpm/min) after a bolus injection of radiolabeled VLDL-TG at time 0, as previously described (18). In brief, the fractional catabolic rate (FCR) (pools/life) of the VLDL-TG pool was calculated from the slope of the log SA (dpm/μmol VLDL-TG) vs. time curve under basal ($t = 120 – 210$ min) and clamp ($t = 240 – 390$ min) conditions. This time point was chosen because control experiments performed in our laboratory have shown that the contribution of any free $[1^{-13}C] $triolein injected at $t = 0$ to the decay curve of $[1^{-13}C] $VLDL-TG is nominal after 2 h (18).

VLDL-TG kinetics were calculated as follows (19):

\[
\text{VLDL-TG production rate (μmol/min) = FCR/60 × CVLDL-TG × PV}
\]

\[
\text{VLDL-TG secretion rate (μmol/l plasma × min) = FCR/60 × CVLDL-TG}
\]

\[
\text{VLDL-TG clearance (ml/min) = VLDL-TG production rate ÷ CVLDL-TG}
\]

Plasma volume was calculated on the basis of the participants’ body weight (45)

\[
\text{PV} = 0.045 × \text{total body mass}
\]

**IMTG content and SA.** The samples were first washed in saline, and excess water was removed on a paper towel before the samples were weighed on a balance. Total muscle lipids were extracted by the excess water was removed on a paper towel before the samples were clamped.

**Statistics.** Normality of the data was tested by distributional diagnostic plots and Shapiro-Wilks test for normal data. Data from the participants were used only if data from all three treatments were available. Equality among the three treatments was assessed by simple ANOVA for repeated measurements. If data were not normally distributed, they were either log-transformed or Friedman’s test was applied. Between-treatment and clamp-basal differences were assessed by paired t-test or Wilcoxon matched-pairs signed ranks test. All calculations were performed using STATA for Windows version 8.2 (StataCorp, College Station, TX). P values <0.05 were considered significant. Parametric data are presented as means ± SE and non-parametric data as medians (range). There was no significant carry-over or period effect.

### RESULTS

#### Body weight. Total body weight increased significantly after GH treatment irrespective of concomitant acipimox treatment (in kg: A, 77.4 ± 2.0; B, 78.9 ± 1.9; C, 78.7 ± 2.1; P < 0.02).

#### Circulating hormones. Basal serum total IGF-I levels were significantly increased following GH treatment irrespective of concomitant acipimox administration, and these levels were not changed during the clamp (Table 1). Basal serum GH tended to differ among the three treatments, but there was no difference during hyperinsulinemia. Plasma levels of catecholamines in the basal state did not differ among the three treatments; during hyperinsulinemia, a significant increase in plasma norepinephrine levels was recorded in the studies involving GH administration (P < 0.01). Basal state plasma glucagon levels were comparable in the three treatments, whereas a significant decline in glucagon concentration during hyperinsulinemia was detected after treatment with placebo and GH + acipimox (P < 0.05). Serum insulin level in the basal state was significantly lower in the placebo treatment (P < 0.05) (Table 1), but at the end of hyperinsulinemia period insulin concentrations were elevated to the same level in all three treatments.

#### Circulating metabolites. Serum FFA levels in the basal state were significantly elevated in the GH condition (P < 0.04). In the GH + acipimox condition, serum FFA concentrations measured ~3 h after the last dose of acipimox were reduced (P < 0.003) compared with placebo (Fig. 2A). Serum FFA concentrations during hyperinsulinemia were suppressed in both the placebo (P < 0.0001) and GH (P < 0.0001) condition compared with basal conditions. In contrast, no suppression of

### Table 1. Circulating hormones

<table>
<thead>
<tr>
<th>Condition</th>
<th>Placebo</th>
<th>GH</th>
<th>GH + Acipimox</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I, μg/l</td>
<td>0 min 194 (174; 274)</td>
<td>384 (283; 766)</td>
<td>427 (315; 655)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>120 min 204 (164; 275)</td>
<td>420 (365; 693)</td>
<td>460 (325; 678)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>390 min 211 ± 14</td>
<td>457 ± 27</td>
<td>474 ± 37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GH, ng/ml</td>
<td>Basal 0.21 (0.04; 6.05)</td>
<td>2.15 (0.5; 4.07)</td>
<td>1.92 (0.92; 4.26)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Clamp 0.27 (0.07; 3.96)</td>
<td>0.94 (0.18; 4.02)</td>
<td>0.88 (0.34; 3.95)</td>
<td>0.11</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>Basal 25.5 (15.5; 54)</td>
<td>52 (41; 138)</td>
<td>38.5 (13; 153.5)</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>Clamp 242 ± 11</td>
<td>239 ± 15</td>
<td>219 ± 15</td>
<td>0.33</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>Basal 42 ± 4</td>
<td>39 ± 2.5</td>
<td>44 ± 3.5</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Clamp 42 (21; 51)</td>
<td>38 (22; 42)</td>
<td>33 (16; 64)</td>
<td>0.11</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>Basal 142 ± 22</td>
<td>144 ± 15</td>
<td>153 ± 16</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Clamp 155 ± 16</td>
<td>206 ± 18</td>
<td>200 ± 23</td>
<td>0.07</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>Basal 25 (25; 62.1)</td>
<td>25 (25; 44.9)</td>
<td>25 (25; 69.85)</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Clamp 25 (25; 169.5)</td>
<td>31.2 (25; 38.8)</td>
<td>28.8 (25; 69.5)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Data are means ± SE or medians (range). GH, growth hormone. *P < 0.05 vs. placebo; †P < 0.01 vs. placebo; ‡P < 0.001 vs. placebo; ††P < 0.05 vs. basal; ‡‡P < 0.01 vs. basal; †††P < 0.001 vs. basal.
serum FFA during the clamp state was observed in the GH + acipimox condition (P = 0.68 vs. basal concentrations). The same pattern was evident regarding the levels of glycerol and BOH, except that plasma glycerol concentrations were greater (P = 0.01) during the clamp state in the GH + acipimox condition (Table 2). Basal concentrations of total plasma TG and VLDL-TG were slightly greater after GH treatment and slightly lower after GH + acipimox compared with placebo. During hyperinsulinemia, total TG and VLDL-TG were suppressed in both the placebo and GH conditions (P < 0.002) but not during GH + acipimox (Table 2).

**Indirect calorimetry.** Basal EE was greater with GH treatment alone (P = 0.03) but not after GH + acipimox (P = 0.14) compared with placebo (Table 3). The increase in EE after GH was associated with a trend toward increased lipid oxidation (P = 0.08). Basal RQ was lower (P = 0.04) with GH treatment compared with GH + acipimox, which translated into decreased glucose oxidation (P < 0.05) and increased lipid oxidation (P = 0.06) (Table 3). During the hyperinsulinemic clamp, lipid oxidation was greater in the GH + acipimox condition than in the placebo condition. Protein oxidation was comparable among the three treatments.

**Glucose metabolism.** Basal plasma glucose levels increased following GH (P = 0.005) and GH + acipimox treatment (P = 0.05) compared with placebo (Fig. 2B). Basal endogenous glucose production [mg·kg⁻¹·min⁻¹; median (range)] was comparable among treatments: [2.04 (0.01–2.3) (placebo) vs. 2.28 (1.93–2.79) (GH) vs. 2.10 (1.66–2.26) (GH + acipimox), P = 0.31]. Insulin sensitivity, as measured by the glucose infusion rate required to maintain euglycemia, was significantly reduced by GH, irrespective of concomitant acipimox administration, compared with placebo [6.2 ± 0.7 (placebo) vs. 4.1 ± 0.6 (GH) vs. 3.6 ± 0.8 (GH + acipimox); Fig. 3].

**FFA and VLDL-TG kinetics.** Basal palmitate flux (μmol/min) was significantly increased during GH treatment (P = 0.04) and reduced during GH + acipimox (P = 0.007) compared with placebo [148 ± 15 (placebo) vs. 213 ± 24 (GH) vs. 90 ± 17 (GH + acipimox); Fig. 4]. During the insulin clamp, palmitate flux was suppressed after placebo (P = 0.0001) and GH treatment (P = 0.0002), whereas no difference between basal and clamp was noted after GH + acipimox (P = 0.22). The basal rate of palmitate oxidation (μmol·kg⁻¹·min⁻¹) tended to be elevated during GH treatment [3.8 ± 1.7 (placebo) vs. 8.6 ± 1.9 (GH) vs. 3.1 ± 0.9 (GH + acipimox), P < 0.09]. No difference was observed in basal VLDL-TG kinetics among the three treatment arms (Table 4). During the insulin clamp, the turnover of VLDL-TG was significantly suppressed after placebo and GH treatment (P < 0.03), whereas this effect of

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Table 2. **Circulating metabolites**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Condition</th>
<th>Placebo</th>
<th>GH</th>
<th>GH + Acipimox</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG, mmol/l</td>
<td>Basal</td>
<td>0.84 (0.46; 1.88)</td>
<td>0.96 (0.56; 2.74)</td>
<td>0.72 (0.3; 2.31)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>0.77 (0.35; 1.46)</td>
<td>0.75 (0.38; 2.32)</td>
<td>0.62 (0.25; 2.43)</td>
<td>0.37</td>
</tr>
<tr>
<td>VLDL-TG, mmol/l</td>
<td>Basal</td>
<td>0.40 (0.17; 0.86)</td>
<td>0.51 (0.17; 1.40)</td>
<td>0.31 (0.07; 1.28)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>0.30 (0.12; 0.74)</td>
<td>0.41 (0.11; 1.21)</td>
<td>0.25 (0.06; 1.44)</td>
<td>0.37</td>
</tr>
<tr>
<td>BOH, μM</td>
<td>Basal</td>
<td>32.5 (10; 155)</td>
<td>70 (15; 210)</td>
<td>17.5 (7.5; 122.5)</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>5 (5; 5)</td>
<td>5 (5; 7.5)</td>
<td>10 (5; 130)$^a$</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Glycerol, μM</td>
<td>Basal</td>
<td>31.4 ± 5.3</td>
<td>38.6 ± 4.1</td>
<td>21.7 ± 4.2$^g$</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>11.1 ± 1.0</td>
<td>12.8 ± 2.0$^h$</td>
<td>39.2 ± 6.9$^f$</td>
<td>&lt;0.003</td>
</tr>
</tbody>
</table>

Data are means ± SE or medians (range). TG, triglyceride; BOH, 3-hydroxybutyrate. Basal condition (100–120 min): ~3 h after last dose of acipimox 250 mg, ~12 h after last dose of GH 2 mg. Clamp condition (370–390 min): ~3 h after start of hyperinsulinemic (0.6mU/kg/min) euglycemic (~5 mmol/l) clamp, ~7.5 h after last dose of acipimox 250 mg, ~16 h after last dose of GH 2 mg. *P < 0.05 vs. placebo; †P < 0.01 vs. placebo; ‡P < 0.001 vs. placebo; §P < 0.05 vs. GH; $P < 0.05 vs. basal; $P < 0.01 vs. basal; ＄P < 0.001 vs. basal; ＄P < 0.01 vs. GH.
insulin was less when acipimox was administered together with GH (Table 4).

**IMTG content.** IMTG content (μmol/g wet wt) in muscle was different among the three treatments [median (range): 3.98 (2.54–6.61, A) vs. 5.37 (3.35–8.11, B) vs. 6.88 (3.20–13.68, C), P < 0.05; Fig. 5]. Post hoc analysis showed elevated IMTG after GH alone (B, P = 0.03) and after GH + acipimox (C, P = 0.05). The incorporation of [14C]oleate (from VLDL-TG) and [3H]palmitate (from palmitate) into IMTG in the basal state did not differ significantly among treatments (data not shown). A simple linear regression analysis from each treatment, with IMTG as the dependent variable and insulin sensitivity as the independent variable, showed no relationship [placebo: r = 0.24, P = 0.53; GH: r = –0.24, P = 0.54; GH + Acipimox: r = –0.22, P = 0.57].

**DISCUSSION**

This study was undertaken to further investigate the effects of GH on lipid metabolism and insulin sensitivity. This included assessment of basal and insulin-stimulated metabolism of circulating FFA and VLDL-TG, as well as fasting IMTG content, following GH administration in healthy subjects. In addition, acipimox was administered as model for selective suppression of circulating FFA levels. Our main finding is that the GH-induced increase in FFA turnover and oxidation is accompanied by increased IMTG and unaltered VLDL-TG kinetics. In addition, we observed that administration of acipimox together with GH suppressed lipolysis only in the basal state, whereas at the end of the subsequent clamp period a paradoxical increase in FFA turnover and VLDL-TG clearance was recorded. We used a relatively high dose of GH (2 mg daily) to achieve distinctly elevated serum GH levels compared with placebo. The dose of acipimox (250 mg thrice daily) was identical to that of a previous study in GH-deficient patients (38), in whom lipolysis became significantly suppressed.

A GH-induced stimulation of palmitate flux has previously been reported in the basal state, during fasting, and after exercise (10, 31, 39), and human in vivo data have shown that GH stimulates regional adipocyte lipolysis (10, 36) as well as muscle uptake of FFA (36) and lipid oxidation at the whole body level. We did not find a significant increase in isotopically measured palmitate oxidation among treatments, although GH treatment more than doubled palmitate oxidation compared with placebo and GH + acipimox.

The kinetics of VLDL-TG have not previously been studied in conjunction with GH. Hepatic fatty acid availability and chronic hyperinsulinemia are considered major determinants of VLDL-TG production (1, 24), and the majority of fatty acids in VLDL-TG are assumed to derive from circulating FFA (47).

Despite elevated insulin and FFA levels, we observed only
minor, nonsignificant increments in basal VLDL-TG production after GH and GH + acipimox exposure compared with placebo. A preferential direction of FFA toward oxidation and IMTG accumulation after GH may partly explain the lack of significance, although the small sample size should also be considered. Acute hyperinsulinemia, however, suppresses VLDL-TG production, irrespective of ambient FFA levels (33). Moreover, during the clamp, the suppression of VLDL-TG production was comparable between GH and placebo, even though the suppression was less pronounced during GH + acipimox treatment, probably caused by insufficient suppression of lipolysis in that group, as discussed below.

The observation that GH administration increased IMTG is, to our knowledge, also new, but it fits with previous reports of a relationship between muscle fat content and insulin resistance (5). An inverse correlation between IMTG assessed by NMR spectroscopy and insulin sensitivity has been reported in cross-sectional studies of healthy as well as insulin-resistant human subjects (27, 32), and a temporal association between accumulation of IMTG and subsequent development of insulin resistance (35). Thus, initiation of GH-induced insulin resistance does not depend on circulating FFAs, and it is likely that it may develop prior to accumulation of IMTG. It has also been reported that IMTG increases following exercise in fit individuals without compromising insulin sensitivity (17). This raises the question whether increased IMTG primarily is a consequence of increased FFA turnover and as such not obligatorily linked to insulin resistance. The mechanism responsible for the observed GH-induced increase in IMTG is also unresolved, but our data indicate that the source derives from circulating FFA rather than VLDL-TG. In support of this we (44) have previously observed unaltered activity of lipoprotein lipase (LPL) activity in muscle biopsies after GH administration in obese women during dietary restriction, as well as in GH-deficient adults (unpublished data). By contrast, GH has been shown to inhibit adipose tissue LPL activity in humans (43).

Table 4. VLDL-TG kinetics

<table>
<thead>
<tr>
<th>Condition</th>
<th>Placebo</th>
<th>GH</th>
<th>GH + Acip</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-TG FCR, pools/h</td>
<td>Basal</td>
<td>0.21 ± 0.04</td>
<td>0.19 ± 0.05</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>0.09 ± 0.01*</td>
<td>0.07 ± 0.02*</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>VLDL-TG production, μmol/min</td>
<td>Basal</td>
<td>4.04 ± 0.74</td>
<td>5.19 ± 1.29</td>
<td>4.65 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>1.60 (0.03; 2.99)*</td>
<td>0.57 (0.08; 6.49)*</td>
<td>2.10 (0; 11.49)</td>
</tr>
<tr>
<td>VLDL-TG secretion, μmol·min⁻¹·l⁻¹</td>
<td>Basal</td>
<td>1.18 ± 0.22</td>
<td>1.47 ± 0.37</td>
<td>1.31 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>0.46 (0.01; 0.87)*</td>
<td>0.16 (0.02; 1.77)*</td>
<td>0.63 (0; 3.26)</td>
</tr>
<tr>
<td>VLDL-TG clearance, ml/min</td>
<td>Basal</td>
<td>12.2 ± 2.5</td>
<td>11.4 ± 3.1</td>
<td>17.3 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>5.0 ± 0.9*</td>
<td>4.2 ± 1.2*</td>
<td>8.5 ± 1.4*</td>
</tr>
</tbody>
</table>

Data are means ± SE or medians (range). FCR, fractional catabolic rate. *P < 0.05 vs. placebo; †P < 0.01 vs. GH; ‡P < 0.05 vs. basal; §P < 0.01 vs. basal.

The question of whether accumulation of IMTG comprises the first step in GH-induced insulin resistance is akin to the issues raised when insulin resistance develops during acute infusion of lipids (5). It is not possible to answer this question using our data, because our subjects were studied after 8 days of GH treatment. Previous data involving acute GH infusion in combination with a glucose clamp have demonstrated a lag phase of 2 h before onset of insulin resistance (14). Møller et al. (36), on the other hand, recorded an almost acute suppression of glucose uptake across the forearm in the basal state during a GH infusion in healthy subjects, and another study involving short-term GH infusion and glucose clamp recorded a decrease in forearm glucose uptake and glucose infusion rate and an increase in hepatic glucose production before there was any detectable increase in circulating levels and forearm uptake of lipid intermediates (35). Thus, initiation of GH-induced insulin resistance does not depend on circulating FFAs, and it is likely that it may develop prior to accumulation of IMTG. It has also been reported that IMTG increases following exercise in fit individuals without compromising insulin sensitivity (17). That GH-induced insulin resistance to some extent is causally linked to stimulation of lipolysis is supported by a study in GH-deficient adults involving administration of GH and acipimox in a 2 × 2 factorial design (37). During combined treatment with GH and acipimox, insulin resistance was significantly reduced compared with GH alone. This effect of acipimox has been confirmed by others (46), but the study by Nielsen et al. (37) also indicated a residual insulin-antagonistic effect of GH during antilipolysis, i.e., when comparing insulin sensitivity after acipimox alone with GH plus acipimox. At any rate, the present data clearly demonstrate that the GH-induced increase in FFA flux is accompanied by accumulation of IMTG and insulin resistance.

In the present study, GH + acipimox treatment suppressed basal lipolysis, as judged by significantly reduced palmitate...
turnover and levels of glycerol and BOH and a trend toward reduced lipid oxidation. Basal FFA levels, on the other hand, were initially elevated but became suppressed at $t = 100$ min (i.e. $\approx -3$ h after ingestion of acipimox). The latter may reflect that complete and sustained circadian suppression of FFA release is not obtained by thrice daily administration of acipimox. This notion is further supported by our observation of a paradoxical elevation in FFA turnover and lipid oxidation and induction of insulin resistance during the clamp in the GH + acipimox arm, since the last dose of acipimox on each study day was given $\approx -4.5$ h prior to the start of the clamp. A rebound increase in lipolysis during acipimox treatment has previously been reported in normal humans and patients with type 2 diabetes mellitus (6, 40, 48). In the latter studies, this could theoretically have been induced by an increase in GH secretion, but that mechanism is obviously not plausible in the present experiment. In addition, acipimox in combination with GH caused an increase in VLDL-TG turnover during the clamp, and this combination was also associated with elevated basal IMTG levels compared with placebo. Although the mechanism subserving the rebound effect of acipimox in our study remains uncertain, the observation indirectly supports the existence of a close association between FFA metabolism and insulin sensitivity.

The cytosolic mechanisms linking FFA to insulin resistance remain unclear, but the original hypothesis by Randle et al. (42), that substrate competition between FFA and pyruvate is responsible via feedback inhibition of glucoytic enzymes, has been challenged by newer data (4, 20). Several studies have demonstrated that FFA interfere with insulin signaling and in particular may blunt insulin receptor substrate-1-associated phosphatidylinositol (PI) 3-kinase activity (11), which is considered a key enzyme for activation of glucose transporter 4. But so far it has not been possible to detect a significant effect of GH on either basal (30) or insulin-stimulated PI 3-kinase activity (29) despite concomitant stimulation of lipolysis (29, 30) and insulin resistance (29). Whether the discrepant results reflect methodological or biological differences remains to be further studied.

In summary, the data from the present study demonstrate that the lipolytic effects of GH involve a stimulation of the turnover and oxidation of FFA together with unaltered VLDL-TG kinetics and accumulation of IMTG. This was associated with insulin resistance during a hyperinsulinemic euglycemic clamp. We suggest the hypothesis that fat oxidation in muscle tissue could be a central effect of GH and that circulating FFA rather than VLDL-TG constitute the major source for this process. Our results also underscore the association between FFA metabolism and insulin resistance in skeletal muscle, which has implications beyond the pharmacodynamics of GH.

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DISCLOSURES

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