Endurance training and GH administration in elderly women: effects on abdominal adipose tissue lipolysis

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Endurance training and GH administration in elderly women: effects on abdominal adipose tissue lipolysis. Am J Physiol Endocrinol Metab 280: E886–E897, 2001.—In the present study, the effect of endurance training alone and endurance training combined with recombinant human growth hormone (rhGH) administration on subcutaneous abdominal adipose tissue lipolysis was investigated. Sixteen healthy women [age 75 ± 2 yr (mean ± SE)] underwent a 12-wk endurance training program on a cycle ergometer. rhGH was administered in a randomized, double-blinded, placebo-controlled design in addition to the training program. Subcutaneous abdominal adipose tissue lipolysis was estimated by means of microdialysis combined with measurements of subcutaneous abdominal adipose tissue blood flow (ATBF; 133Xe washout). Whole body fat oxidation was estimated simultaneously by indirect calorimetry. Before and after completion of the training program, measurements were performed both at rest and during 60 min of continuous cycling at a workload corresponding to 60% of pretraining peak oxygen uptake. Endurance training alone did not affect subcutaneous abdominal adipose tissue lipolysis either at rest or during exercise, as reflected by identical levels of interstitial adipose tissue glycerol, subcutaneous abdominal ATBF, and plasma nonesterified fatty acids before and after completion of the training program. Similarly, no effect on subcutaneous abdominal adipose tissue lipolysis was observed when combining endurance training with rhGH administration. However, in both the placebo and the GH groups, fat oxidation was significantly increased during exercise performed at the same absolute workload after completion of the training program. We conclude that the changed lipid metabolism during exercise observed after endurance training alone or after endurance training combined with rhGH administration is not due to alterations in subcutaneous abdominal adipose tissue metabolism in elderly women.

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ADVANCING AGE is accompanied by increased fat mass (FM) and decreased lean body mass (LBM) (21, 37). Evidently, this may be attributed to a concomitant decline in physical activity (12), but aging per se undoubtedly also plays a significant role. Impaired adipose tissue lipolysis and decreased ability to mobilize nonesterified fatty acids (NEFA) from adipose tissue in the elderly may be part of the age-related factors contributing to the increased FM. This is supported by findings in isolated adipocytes (26) but is not evident from in vivo measurements using whole body techniques (19, 38). In addition, differences in body composition and aerobic fitness are always potential confounders in studies that compare young and elderly individuals.

It is well established that, also in older age, endurance training causes significant improvements in peak oxygen uptake (VO2 peak) (20, 36), skeletal muscle enzyme activities (6), and whole body fat oxidation (39). Whether endurance training has any impact on adipose tissue lipolysis is, however, questionable. Longitudinal studies in young subjects have not been able to show any increase in either whole body (13, 27, 31) or regional lipolysis (13) as a result of endurance training. Only one study has, to our knowledge, addressed the impact of endurance training on lipid kinetics in the elderly (39). In that study, no effect was found on either whole body glycerol rate of appearance (Rα) or NEFA Ra either at rest or during exercise at the same absolute workload.

Growth hormone (GH) is a lipolytic hormone (32) with a substantial potential for reducing FM when administered exogenously (17, 33, 34). An intravenous bolus of recombinant human (rh)GH results in increased adipose tissue glycerol concentration, indicating increased adipose tissue lipolysis (11). With advancing age, there is a substantial decrease in GH

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secretion in the elderly (41). This decrease is due partly to increased FM and reduced physical fitness, but aging per se does also play a role (42).

With this background, the present study aimed at investigating whether endurance training alone or endurance training combined with rhGH administration in doses that rejuvenate serum insulin-like growth factor (IGF) I levels would increase adipose tissue lipolysis in elderly women. Subcutaneous abdominal adipose tissue lipolysis was estimated by means of microdialysis combined with determinations of subcutaneous adipose tissue blood flow (ATBF; $^{133}$Xe washout) before and after completion of a 12-wk supervised endurance training program. Measurements were performed both at rest and during continuous cycling at a workload corresponding to 60% of pretraining VO$_2$ peak.

METHODS

Subjects

Seventeen healthy elderly women, age 75 ± 1 (70–82) yr (mean ± SE [range]), height 160 ± 1 (148–170) cm, body weight 66.2 ± 2.5 (49.4–89.0) kg, and body mass index 25.8 ± 1.0 (19.5–32.7) kg/m$^2$ were initially recruited for the study. Informed consent was obtained according to the Declaration of Helsinki 2, and the study protocol was approved by the Ethics Committee for Medical Research in Copenhagen (KF 02–130/97) and by the Danish National Board of Health (journal number 5312-181-1997). Before inclusion, each subject underwent a comprehensive medical evaluation including history, physical examination, routine blood tests, and an exercise electrocardiogram (ECG). Exclusion criteria were diabetes, cardiac, malignant disease, anemia, and medication known to interfere with fat and/or skeletal muscle metabolism. Specifically, hormone replacement therapy was not allowed, nor were $\beta$-blockers, $\beta$-blockers, or any antidepressive medication.

Experimental Protocol

After inclusion, subjects were randomized in blocks of four to receive either a placebo or rhGH, and the subjects then underwent a 12-wk, closely supervised endurance training program on cycle ergometers. Measurements were performed at baseline and after 12 wk.

Administration of rhGH

rhGH (Norditropin; Novo Nordisk, Denmark) was administered subcutaneously in the thigh one time daily in a randomized, double-blinded, placebo-controlled design. After thorough instruction, subjects were able to perform the injections themselves at home in the evening before bedtime. Syringes were checked ≥1 time/wk and injection technique was monitored several times during the course of the study. To avoid side effects, the dose was increased over 3 wk. During the 1st wk, the dose was 0.5 IU/m$^2$, during the 2nd wk 1.0 IU/m$^2$, and for the remainder of the study period 1.5 IU/m$^2$ (12 $\mu$g·kg$^{-1}$·day$^{-1}$). Side effects were controlled for by weighing, checking for edema, and questioning the subjects 1 time/wk. If side effects occurred, the dose was cut in half until they disappeared or were tolerable to both the subject and the investigators. If a subject abandoned the study before completion, another subject who received the same randomization number replaced her. Blinding of both investigators and subjects was maintained until data acquisition was completed for the whole study.

Main Experiment Day

The subjects were examined in the morning after an overnight fast. Body composition was determined by dual-energy X-ray absorptiometry (DEXA) scanning. A catheter was then inserted into a radial artery, and two microdialysis catheters were placed in the subcutaneous adipose tissue in the right hypogastric region, allowing for double determinations of interstitial glycerol concentrations. To measure local ATBF, a small depot of $^{133}$Xe was injected into the subcutaneous adipose tissue on the contratralateral side, and a scintillation detector was strapped to the skin above the depot. Resting measurements were commenced ≥45 min after insertion of the microdialysis catheters. The subjects rested supine and dressed at a room temperature of 22–23°C. Immediately after the resting period, the subjects started to exercise on an electromagnetically braked cycle ergometer (Ergometrics ER 900L, Ergoline, Bitz, Germany) in a semisupine position for 60 min. The load was adjusted by 5-W steps throughout the experiment to correspond to 60% of previously determined individual VO$_2$ peak. Although the power output of the cycling ergometer was independent of pedaling frequency over a wide range, the frequency was adjusted individually between 60 and 70 rpm and kept constant throughout the exercise period by use of a metronome. This was done to avoid fluctuations in whole body oxygen consumption (VO$_2$) due to body movements caused by shifts in pedaling frequency. ECG and heart rate (HR) were registered continuously via an Athena (S & W, Copenhagen, Denmark) interfaced to the Oxycon Champion (software version 3.12, Jaeger, Würzburg, Germany). After the exercise period, the experimental day was completed by the taking of a fat biopsy from the subcutaneous abdominal adipose tissue. The subjects then underwent a 12-wk endurance training program, after which the same measurements were repeated under the same conditions and ~40–48 h after the last training session. Measurements during exercise were thus made at the same absolute workload before and after the training period.

Training Program

The subjects trained on a cycle ergometer (Monark, model 818 E, Varberg, Sweden) 3 times/wk for 12 wk. Each subject followed an interval program of 60 min in each training session. An interval program was chosen for psychological reasons to obtain a high degree of compliance. The subjects were equipped with a wireless heart rate monitor (HRM; Polar Vantage, Polar Electro OY, Kempele, Finland), which enabled them to monitor HR and exercise duration continuously. After a 10-min warm-up period, the subjects performed seven intervals of 2, 4, 6, 8, 6, 4, and 2 min long, respectively, each interval being separated by 2 min from the previous interval. During the intervals, the subjects adjusted the workload so that HR, within 5 beats/min, was kept at a level corresponding to 75% of VO$_2$ peak. In the period between the intervals, the subjects were allowed to decrease the load to 50% of the interval load. The program was completed by a 5-min cool-down period. HR was monitored and registered continuously, and an investigator supervised each training session. During a typical training session of 60 min, HR averaged a value corresponding to a VO$_2$ of 65–70% of VO$_2$ peak. By use of an HRM as an indicator of workload, it was ensured that the subjects trained at the same relative level of VO$_2$ peak during the 12-wk training period. The subjects were carefully instructed to continue their normal daily...
life without any other change than following the training program. They were specifically told not to change their eating habits.

**DEXA**

Body composition was determined by DEXA scan. Subjects were scanned in the morning after an overnight fast by means of a Lunar DPX-IQ scanner (software version 4.6 C; Lunar, Madison, WI) before and after the 12-wk training period. Either a fast (12 min)- or a medium (25 min)-mode scanning procedure was used depending on the distance between the beam collector and the skin of the abdomen. The same two trained technicians performed all scans, and the same investigator analyzed all scans with the extended research analysis software provided by Lunar. In the analysis, total body scans were divided into three regions: arms, legs, and trunk. Total and regional scans were further divided into three compartments: fat-free mass (FFM), FM, and bone mineral content (BMC).

**Determination of VO₂ peak and Indirect Calorimetry**

On a separate day, individual VO₂ peak was determined on an electromagnetically braked cycling ergometer. A protocol was used starting at 15 W and increasing with 15 W every 2 min until exhaustion. VO₂ and CO₂ production (VCO₂) were measured on an Oxycron Champion with a face mask and an external volume sensor in the open-system approach giving breath-by-breath data. The accuracy of the system was checked by combustion of 99.6% ethanol, and the flowmeter was checked by nitrogen infusion. Before each protocol, the gas analyzers were calibrated using gas mixtures of known composition, and the external volume sensor was calibrated using an external syringe of known volume. ECG and HR were registered continuously. VO₂ peak was chosen as the highest VO₂ attained during the test. From the correlation between VO₂ and work output during the test, the workload corresponding to an energy requirement of 60% of VO₂ peak was obtained and used as the initial load in the experimental protocol. After completion of the 12-wk training program, the test was repeated to determine the effect of the training protocol on VO₂ peak.

In the submaximal experimental protocol, VO₂ and VCO₂ were measured at the time intervals described previously using the same system and identical setup as in the VO₂ peak test. According to the measurements of VO₂, the workload was adjusted by steps of 5 W during the exercise period. This ensured that a VO₂ corresponding to 60% of individual pre-training VO₂ peak was continuously attained. Resting measurements were obtained twice during 5-min periods in the resting period, and exercise measurements were obtained three times during 5-min periods after 15, 30, and 55 min of exercise.

**Arterial Catheterization**

During local analgesia with lidocaine (1%), a catheter (Arterial Cannula with FloSwitch, 20 gauge/1.0 × 45 mm, Ohmeda, Swindon, UK) was inserted into a radial artery. The catheter was kept patent by regular flushing with isotonic sodium chloride containing heparin (10 U/ml).

**Blood Flow Measurements**

Subcutaneous ATBF was measured on the contralateral side of the microdialysis catheters by the local 133Xe washout method (24). One megabequerel of 133Xe dissolved in 0.1 ml of sterile, isotonic sodium chloride was slowly injected into the middle layer of the subcutaneous adipose tissue with a 25-gauge cannula, taking care not to inject any air bubbles. The needle was kept in place for 30 s before withdrawal to avoid any leak. The γ-radiation of the 133Xe in the adipose tissue was registered by a portable ScI scintillation detector, which was strapped to the skin above the 133Xe depot and connected to a multichannel analyzer system (Oakfield Instruments, Oxford, UK). The distance from the detector to the 133Xe depot was adjusted so that the initial counting rate was ~1,500 cps, which was well below the maximal counting rate limit of the system. Counts were collected in 30-s periods starting at least 30 min after the injection. ATBF was calculated as $-k \times \lambda \times 100$ (ml·100 g⁻¹·min⁻¹), where $k$ is the rate constant of the monoexponential washout curve, and $\lambda$ is the tissue-to-blood partition coefficient for 133Xe. Plasma flow was calculated as blood flow × (1 – hematocrit). Plasma water flow was calculated by multiplying plasma flow by 0.94 (30).

**Microdialysis**

Microdialysis catheters (CMA 60; Carnegie Medicine/Microdialysis, Solna, Sweden; 20-kDa molecular cutoff, OD 0.5 mm; length 30 mm) were inserted in parallel into the periumbilical subcutaneous fat tissue in the right hypogastric region during local epidermal analgesia. Individual catheters were placed with a vertical spacing of 1.5 cm between catheters. The catheters were perfused with a perfusate containing 3 mM glucose and 1 mM lactate in isotonic sodium chloride at 3 μl/min, each with a high-precision syringe pump (CMA 100; Carnegie Medicine). By use of the internal reference technique (35), the in vivo recovery of glycerol was determined by adding 5 nM [U-14C]glycerol (specific activity: 7,400 GBq/mmol; New England Nuclear, Boston, MA) to the perfusate. Five microliters of perfusate (duplicate) and dialysate (single) were counted in a liquid scintillation counter with appropriate settings for the 14C channel.

**Fat Biopsy**

During local epidermal analgesia with 1% lidocaine, an open fat biopsy was obtained through a skin incision after each experiment to determine individual λ-values (3). The biopsy was taken from the subcutaneous adipose tissue corresponding to the area where the 133Xe depot had been injected previously.

**Sampling**

**Blood.** Arterial blood was sampled at 30 and 60 min in the resting period and at 5, 15, 30, 45, and 60 min in the exercising period. Blood was collected into vials at 4°C for measurements of metabolites [glycerol, NEFA, triacylglycerol (TAG), glucose, lactate] and hormones [insulin, catecholamines, cortisol, GH, IGF-I, IGF-II, IGF-binding protein 3 (IGFBP-3), and acid-labile subunit (ALS)]. Whole blood was immediately deproteinized by being mixed with 0.6 N perchloric acid or the plasma being separated by centrifugation at 4°C. Blood for determination of catecholamines was stabilized by adding 20 μl of a solution containing EGTA, reduced glutathione, and NaOH per milliliter of blood (EGTA-glutathione solution: 950 mg EGTA, 600 mg reduced glutathione, 1,100 μl 6 M NaOH, 10 ml distilled H₂O; pH 6.5).

**Dialysate.** Dialysate for determination of glycerol was sampled continuously into sealed vials (Microvials, P000001, CMA/Microdialysis), and the vials were collected at the same time intervals that the blood sampling was performed. All blood and dialysate samples were stored at −80°C until analysis.
Analytical Methods

Glycerol was measured in duplicate in neutralized, deproteinized extracts of whole blood with a Monarch Plus 750 (Instrumentation Laboratory, Lexington, KY), and NEFA and TAG were measured in plasma as described by Coppack et al. (7). Plasma glucose and lactate were determined on the YSI 2300 glucose/l-lactate analyzer by means of the glucose oxidase method and the l-lactate oxidase reaction (Yellow Springs Instruments, Yellow Springs, OH). Plasma catecholamines were determined by a single-isotope radioenzymatic method (5). Plasma insulin was determined by ELISA (DAKO, Denmark). Plasma cortisol was determined by RIA (Dia Sorin, Stillwater, MN). Serum GH was determined by time-resolved immunofluorescence assay (Delfia, Wallac Oy, Turku, Finland). Serum total IGF-I, IGF-II, IGFBP-3 and ALS were determined as previously described (22). Hematocrit was determined by the microhematocrit method. Dialysate glycerol was determined spectrophotometrically in duplicate by use of the CMA 600 Microdialysis Analyzer.

Calculations

Interstitial concentrations (C_i) were calculated using the internal reference calibration method (35). On the basis of in vitro experiments, it is assumed that the relative recovery (RR) from the interstitial fluid to the perfusate of unlabeled metabolite equates relative loss (RL) from the perfusate to the interstitial fluid of labeled metabolite. RR was calculated for each individual sample as RR = RL = (C_p - C_a)/C_p, where C_p is disintegrations/min (dpm) in the perfusate, and C_a is dpm in the dialysate. C_a was calculated as C_a = C_d/RR, where C_d is dialysate concentration, and the average C_d determined from simultaneous measurements in the two microdialysis fibers was used as an estimate of C_a.

Adipose venous concentrations of glycerol were calculated using Fick's law of diffusion for a thin membrane: \[ d = -PS(\text{C}_1 - \text{C}_2), \]
where \( J \) is the substrate flux, \( P \) the membrane permeability of the substrate, \( S \) the membrane surface area, and \( \text{C}_1 \) and \( \text{C}_2 \) the concentrations on the two sides of the membrane, \( \text{C}_1 \) being higher than \( \text{C}_2 \). Integrating this equation over the entire length of a capillary gives, according to Intaglietta and Johnson (15)

\[ (\text{C}_v - \text{C}_1)/(\text{C}_v - \text{C}_2) = e^{-PSQ} \]
giving

\[ C_{v,\text{calc}} = [1/(\text{C}_v - \text{C}_1) - (1 - e^{-PSQ})] + \text{C}_a \]
for the tissue output situation. \( C_{v,\text{calc}} \) denotes calculated venous plasma water concentration of glycerol, \( \text{C}_a \) arterial plasma water concentration, \( Q \) plasma water flow, and \( PS \) the permeability \( \times \) surface area product. The PS product was set to 3 ml·100 g⁻¹·min⁻¹ for glycerol, because molecules similar in size are known to have these values (25). It was assumed that the PS product remained constant within the range of blood flows recorded (30). Conversions of whole blood concentrations of glycerol to plasma water concentrations and vice versa were accomplished by use of the hematocrit and by the assumption of a distribution volume of 0.70 for glycerol in the erythrocyte (40).

Net fluxes of glycerol across the subcutaneous abdominal adipose tissue were calculated by multiplication of the appropriate blood flow by the arteriovenous concentration difference. Whole body lipid and carbohydrate oxidation rates were calculated using the equations given by Frayn (8), and the nitrogen excretion rate was set to 135 µg·kg⁻¹·min⁻¹ (4).

Statistical Analysis

All data are presented as means ± SE. Repeated-measures one-way ANOVA and repeated-measures two-way ANOVA with post hoc Bonferroni tests were used to detect significant changes in metabolite and hormone concentrations as well as respiratory exchange ratio values with time, training, or training + GH administration when appropriate. Student’s paired and unpaired t-tests were used to detect significant differences between data before and after the training period when appropriate. \( P < 0.05 \) (two tailed) was considered significant.

RESULTS

Subjects, Body Composition, \( \text{V}_{\text{O}_2 \text{ peak}} \), and HR

Sixteen of the seventeen recruited subjects completed the study. One subject left after 3 wk, because she felt the training program was too demanding. Subject characteristics for the 16 remaining subjects, divided into a placebo group (\( n = 8 \)) and a GH group (\( n = 8 \)), are presented in Table 1. At baseline, body weight was significantly greater in the GH group compared with the placebo group, and body fat (but not body fat percentage) also tended to be larger (\( P < 0.062 \)) in the GH group compared with the placebo group. The groups were similar with respect to all other subject characteristics (Table 1). Training alone did not cause any change either in total body weight or in total or regional body composition (Table 1). However, when training was combined with rhGH administration, there was a significant decrease in body fat, which was counterbalanced by a significant increase in FFM, the net result being no change in total body weight (Table 1). The changes in FM and FFM occurred in all three body regions in the GH group, and fat loss was not related to FM at baseline (data not shown). BMC remained the same in both groups (Table 1).

There were no differences in \( \text{V}_{\text{O}_2 \text{ peak}} \) either at baseline or after 12 wk between the two groups expressed in absolute terms, relative to body weight, or relative to FFM (Table 1). \( \text{V}_{\text{O}_2 \text{ peak}} \) (ml·min⁻¹·kg⁻¹) increased by 19 ± 5% in the placebo group and by 17 ± 2% in the GH group (both \( P < 0.02 \)) as a result of endurance training + placebo/rhGH. When expressed relative to FFM (i.e., as ml·min⁻¹·kg LB⁻¹), \( \text{V}_{\text{O}_2 \text{ peak}} \) increased by 16 ± 4% in the placebo group and by 9 ± 3% in the GH group (both \( P < 0.02 \); Table 1).

rhGH Administration

Of the eight subjects receiving rhGH, five experienced side effects that necessitated a reduction in the prescheduled rhGH dose. With no side effects a typical end dose was 1.5–2.0 IU/day (7 µg·kg⁻¹·day⁻¹). Side effects appeared from 4 to 10 wk after GH start and were mainly ankle edema (\( n = 5 \)), swollen fingers (\( n = 2 \)), carpal tunnel syndrome (\( n = 2 \)), and headache during the night and early morning (\( n = 1 \)). Eventually, all side effects disappeared after termination of rhGH administration. Some side effects persisted until 3 wk after termination of rhGH administration (ankle edema and carpal tunnel syndrome).
Table 1. Age, body composition, and V̇O₂peak at baseline and after 12-wk endurance training ± placebo/rhGH

<table>
<thead>
<tr>
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<th>Placebo Group</th>
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<th>GH Group</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>12 wk</td>
<td>Baseline</td>
<td>12 wk</td>
</tr>
<tr>
<td>Age, yr</td>
<td>75 ± 2(70–82)</td>
<td>60 ± 2(49.2–75.3)</td>
<td>73 ± 2(59.9–90.9)</td>
<td>66 ± 2(57.1–90.9)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>61.2 ± 2.7(49.4–72.5)</td>
<td>60.8 ± 2.9(49.2–75.3)</td>
<td>71.3 ± 3.6(59.9–89.0)</td>
<td>71.6 ± 3.7(59.9–90.8)</td>
</tr>
<tr>
<td>Body fat, kg</td>
<td>23.1 ± 2.0(14.7–30.9)</td>
<td>22.5 ± 2.3(13.5–34.5)</td>
<td>30.0 ± 2.7(18.1–40.8)</td>
<td>27.2 ± 3.0(14.4–40.3)</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>37.4 ± 2.3(28.4–47.5)</td>
<td>36.4 ± 2.4(25.6–45.7)</td>
<td>41.6 ± 2.1(29.7–49.0)</td>
<td>37.3 ± 2.6(23.9–49.0)</td>
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<tr>
<td>FFM, kg</td>
<td>36.2 ± 2.3(23.9–41.5)</td>
<td>36.4 ± 2.1(30.3–40.9)</td>
<td>39.1 ± 1.5(31.8–45.8)</td>
<td>42.1 ± 1.7(35.9–50.5)</td>
</tr>
<tr>
<td>FFM, %</td>
<td>59.5 ± 2.2(49.9–68.2)</td>
<td>60.4 ± 2.4(51.0–71.1)</td>
<td>55.2 ± 1.9(48.6–66.1)</td>
<td>59.4 ± 2.5(48.4–71.8)</td>
</tr>
<tr>
<td>BMC, kg</td>
<td>1.92 ± 0.13(1.29–2.37)</td>
<td>1.94 ± 0.14(1.33–2.45)</td>
<td>2.28 ± 0.06(2.02–2.60)</td>
<td>2.30 ± 0.26(2.13–2.60)</td>
</tr>
<tr>
<td>V̇O₂peak, l/min</td>
<td>1.36 ± 0.09(0.92–1.70)</td>
<td>1.59 ± 0.08(1.24–1.88)</td>
<td>1.43 ± 0.08(1.15–1.76)</td>
<td>1.68 ± 0.06(1.45–1.95)</td>
</tr>
<tr>
<td>ALS, mg/l</td>
<td>22.2 ± 0.8(18.6–25.6)</td>
<td>26.3 ± 1.4(22.1–34.2)</td>
<td>20.4 ± 1.3(15.8–26.3)</td>
<td>23.8 ± 1.3(18.7–29.0)</td>
</tr>
<tr>
<td>ALS, LBM -1, min-1</td>
<td>37.8 ± 2.2(27.5–46.7)</td>
<td>43.7 ± 1.8(36.8–48.9)</td>
<td>36.8 ± 1.7(29.8–43.2)</td>
<td>40.1 ± 1.6(30.7–44.9)</td>
</tr>
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</table>

Values are means ± SE with ranges in parentheses; n = 8 subjects in each group. FFM, fat-free mass; BMC, bone mineral content; V̇O₂peak, peak oxygen uptake; LBM, lean body mass. *Significant change within a group vs. baseline value; #significant difference between groups, (P < 0.05). See RESULTS for further details.

rhGH-Related Serum Markers

Resting arterial serum IGF-I, IGF-II, IGFBP-3, and ALS were similar in both groups at baseline (Table 2). Endurance training alone did not influence these parameters, whereas endurance training combined with rhGH caused all parameters to increase significantly (Table 2).

Relative Recovery

Relative recovery for glycerol was significantly lower at rest than during exercise (0.24 ± 0.01 vs. 0.28 ± 0.01; P < 0.0007).

Blood Glycerol, Plasma NEFA, and Interstitial Glycerol Concentrations and Regional Glycerol Mobilization

In both groups, blood glycerol increased significantly from rest to exercise, reaching a plateau after 30 min of exercise before and after the training period (all P < 0.0001). Overall, blood glycerol was slightly, but significantly, lower after the training period in the placebo group (P = 0.004), whereas no effect of endurance training combined with rhGH was observed in the GH group (Fig. 1A). In both groups, arterial plasma NEFA concentrations decreased significantly during the first 5 min of exercise (all P < 0.05) and then returned to a level slightly above resting levels before and after the training period (all P < 0.05; Fig. 1B). There was no effect of training alone on arterial plasma NEFA concentrations (P < 0.77), whereas overall, training combined with rhGH caused a reduction in arterial plasma NEFA concentrations (P < 0.0028). Interstitial glycerol concentration in the subcutaneous abdominal adipose tissue increased significantly from rest to exercise before and after the training period in both groups (both P < 0.0001), but there was no effect of endurance training alone (P < 0.97) or endurance training combined with rhGH (P < 0.86; Fig. 1C). Similarly, glycerol mobilization from the subcutaneous abdominal adipose tissue increased significantly from rest to exercise in both groups before and after the training period (placebo group, P < 0.0001; GH group, P < 0.0022), but no effect of either endurance training alone or endurance training combined with rhGH could be detected (placebo group, P < 0.47; GH group, P < 0.18).

Plasma and Serum Hormone Concentrations

Arterial plasma epinephrine and norepinephrine concentrations increased significantly from rest to exercise in both groups before and after the training period (all P < 0.0001; Figs. 2A and B). Resting values were not different between groups at any time point and were not affected by endurance training alone or by endurance training combined with rhGH. However, the training period caused an equal and significant decrease in catecholamine values obtained during exercise in both groups (all P < 0.0001). Arterial serum GH concentrations were similar in both groups at all time points at baseline and increased significantly from rest to exercise at baseline (placebo group, P < 0.007; GH group, P < 0.003; Fig. 2C). Endurance training alone resulted in a small, but significant, augmen-

Table 2. GH-related serum markers at baseline and after 12-wk endurance training ± placebo/rhGH

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<th>Placebo Group</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>12 wk</td>
<td>Baseline</td>
<td>12 wk</td>
</tr>
<tr>
<td>IGF-II, µg/l</td>
<td>738 ± 52(515–923)</td>
<td>801 ± 59(509–1015)</td>
<td>750 ± 44(508–923)</td>
<td>898 ± 67(616–1262)</td>
</tr>
<tr>
<td>ALS, mg/l</td>
<td>18.50 ± 2.20(9.44–27.55)</td>
<td>18.15 ± 1.77(10.34–25.29)</td>
<td>16.98 ± 1.38(8.46–20.55)</td>
<td>26.68 ± 3.27(8.93–41.80)</td>
</tr>
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</table>

Values are means ± SE with ranges in parentheses; n = 8 subjects in each group. IGF, insulin-like growth factor; IGFBP-3, IGF binding protein 3; ALS, acid-labile subunit. *Significant change within a group vs. baseline (P < 0.05). See RESULTS for further details.
Fig. 1. A: arterial whole blood glycerol; B: arterial plasma nonesterified fatty acids (NEFA); C: interstitial glycerol; D: subcutaneous abdominal adipose tissue glycerol mobilization. Measurements were made at rest and during 60 min of continuous cycling at a workload eliciting 60% of pretraining peak oxygen uptake ($V_{\text{O}_2\text{peak}}$). Initial measurements were made at baseline and were repeated after a 12-wk endurance training program + placebo or recombinant human growth hormone (rhGH). Symbols and error bars represent means ± SE, respectively; $n = 8$ subjects in each group. See RESULTS for further details.

Fig. 2. A: arterial plasma epinephrine; B: arterial plasma norepinephrine; C: arterial serum GH; D: arterial plasma insulin; E: arterial plasma cortisol. Measurements were made as described in Fig. 1. Symbols and error bars represent means ± SE, respectively; $n = 8$ in each group. See RESULTS for further details.
tation in the serum GH response to exercise ($P < 0.023$). In the GH group, serum GH values were significantly greater overall after the training period combined with rhGH ($P < 0.004$) and, in contrast to the placebo group, no increase in serum GH could be demonstrated from rest to exercise in the GH group ($P < 0.24$). Arterial plasma insulin concentrations were similar between groups at all time points both before and after the 12-wk training period (Fig. 2D). In both groups, plasma insulin concentrations decreased significantly during the first 5 min of exercise ($P < 0.01$), but neither training alone nor training combined with rhGH had any effect on plasma insulin concentrations (Fig. 2D). Arterial plasma cortisol concentrations were significantly lower overall in the placebo group compared with values in the GH group at baseline ($P < 0.045$; Fig. 2E). In both groups, plasma cortisol concentrations were decreased overall after the training period (both $P < 0.0001$). However, this decrease was significantly larger in the GH group ($P < 0.0001$), resulting in lower serum cortisol concentrations in the GH group compared with the placebo group after the training period ($P < 0.0008$; Fig. 2E).

### Plasma Lactate, Glucose, and Arterial Hematocrit

Arterial plasma lactate concentrations increased from rest to exercise in both groups before and after the training period (both: $P < 0.0001$; Fig. 3A). Endurance training alone and endurance training combined with rhGH caused a significant reduction in this increase at all time points during exercise (all $P < 0.0001$). Additionally, in both groups, arterial plasma lactate remained at a constant level during exercise after the training period, whereas it decreased significantly throughout the exercise period before the training period (placebo group, $P < 0.01$; GH group, $P < 0.006$; Fig. 3A). In the placebo group, arterial plasma glucose decreased significantly over time both before and after the training period ($P < 0.0001$ and $P < 0.03$, respectively), and a similar pattern was observed in the GH group (Fig. 3B). Endurance training alone had no effect on arterial plasma glucose, whereas endurance training combined with rhGH caused an overall increase in arterial plasma glucose concentrations ($P < 0.0001$; Fig. 3B). Arterial hematocrit increased significantly from rest to exercise in both groups (all $P < 0.0001$) and was, overall, significantly lower in both groups after the training period (both $P < 0.0001$; Fig. 3C).

### Indirect Calorimetry and HR During Exercise

RER values obtained through the facial mask in the resting period were inaccurate due to hyperventilation. Therefore, only RER and V̇O₂ data obtained during exercise were found acceptable for analysis. V̇O₂ during exercise was slightly lower after the training period in both groups (placebo group, 8.0 ± 0.3%; $P < 0.0002$; GH group, 4.9 ± 0.5%, $P < 0.027$; Fig. 4A). Overall, RER values obtained during exercise at baseline

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Fig. 3. A: arterial plasma lactate; B: arterial plasma glucose; C: arterial hematocrit. Measurements were made as described in Fig. 1. Symbols and error bars represent means ± SE, respectively; $n = 8$ in each group. See RESULTS for further details.
tended to be lower in the GH group compared with the placebo group \((P < 0.12)\) and were significantly lower in the GH group compared with the placebo group after the 12-wk training period \((P < 0.02; \text{Fig. 4B})\). In both groups, RER values obtained during exercise were significantly lower after the 12-wk endurance training period compared with baseline \((P < 0.0001; \text{Fig. 4B})\), but there was no difference in the magnitude of this decrease between the two groups \((P < 0.85)\). Fat oxidation \((\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})\) during exercise was similar in both groups at baseline and after the 12-wk training period \((\text{Fig. 4C})\). Overall, fat oxidation during exercise increased to a similar extent in both groups as a result of the interventions \((P < 0.006; \text{placebo group}; P < 0.004; \text{GH group}; P < 0.94; \text{Fig. 4C})\). HR during exercise was significantly lower after the training period in both groups \((P < 0.0001; \text{Fig. 4D})\).

**Xe Tissue-to-Blood Partition Coefficient**

In three subjects, the Xe tissue-to-blood partition coefficient \(\lambda\) was determined from the obtained fat biopsy \((\text{due to technical circumstances, the fat biopsies obtained from the other subjects could not be analyzed})\). Mean \(\lambda\) was 10.0 in those subjects \((11.1, 10.7, \text{and } 8.4, \text{respectively})\); accordingly, \(\lambda\) was set to 10 in all subjects.

**Subcutaneous Abdominal ATBF**

In the placebo group, mean subcutaneous abdominal ATBF \((\text{ml} \cdot \text{100 g}^{-1} \cdot \text{min}^{-1})\) increased significantly from rest to exercise both before \((1.2 \pm 0.2; \text{rest}, 1.0 \pm 0.2; \text{exercise}, 2.4 \pm 0.3; P < 0.001)\) and after the 12-wk \((\text{rest}, 1.2 \pm 0.2; \text{exercise}, 2.2 \pm 0.3; P < 0.005)\) and after the 12-wk (rest, 1.0 ± 0.2; exercise, 2.4 ± 0.3; \(P < 0.001)\) training program \((\text{Fig. 5A})\). In the GH group, mean subcutaneous abdominal ATBF did not change from rest to exercise either at baseline \((\text{rest}, 2.4 \pm 0.4; \text{exercise}, 2.4 \pm 0.3; P < 0.94)\) or after the 12-wk endurance training + rhGH \((\text{rest}, 2.6 \pm 0.4; \text{exercise}, 2.8 \pm 0.3; P < 0.69; \text{Fig. 5B})\). Mean subcutaneous abdominal ATBF at rest as well as during exercise did not change as a result of endurance training alone or endurance training combined with rhGH \((\text{Fig. 5, A and B})\).

**DISCUSSION**

In the present study, no measurable effect of endurance training alone on subcutaneous abdominal adipose tissue lipolysis could be demonstrated in elderly women. This was evidenced by identical levels of subcutaneous abdominal adipose tissue interstitial glycerol concentration and by unchanged subcutaneous ATBF before and after completion of a 12-wk supervised endurance training program \((\text{Figs. 1 and 5})\). Additionally, plasma NEFA concentrations during exercise were slightly decreased after completion of the training program, which indirectly supports that whole body lipolysis during exercise was not increased after endurance training alone. When endurance training was combined with rhGH administration, serum IGF-I levels were augmented to juvenile levels. This resulted in a tendency for subcutaneous adipose tissue glycerol mobilization to be higher at rest, but not during continuous exercise, after endurance training. However,
similar to the observations in the placebo group, neither interstitial nor subcutaneous abdominal ATBF was changed in the GH group, and plasma NEFA concentrations were also not affected by endurance training combined with rhGH administration. Nevertheless, despite the lack of a demonstrable increase in regional lipolysis, there was a significant increase in the amount of fat oxidized during exercise at the same absolute level in both groups after completion of the training program.

Previously published papers comprising a longitudinal design are uniform with respect to the lack of effect of endurance training on whole body glycerol Ra, which, in young subjects, has been shown to be unaltered (13), decreased (27, 31), and increased (9). Only one published paper has, to our knowledge, addressed this issue in older subjects, and that study showed that endurance training did not affect NEFA Ra (39). Evidently, a major limitation of whole body techniques is that regional contributions from adipose tissue and skeletal muscle cannot be distinguished from one another. To address the effect of endurance training specifically on subcutaneous abdominal adipose tissue lipolysis, we used the microdialysis technique. We also chose elderly women, because aging might be associated with impaired lipolysis, at least in vitro; therefore, a greater response to endurance training might be anticipated a priori in the elderly. Microdialysis is well suited for measuring the concentrations of small, water-soluble compounds (e.g., glycerol) in the interstitial space. To calculate net fluxes of glycerol, the interstitial concentrations must be recalculated to venous concentrations. It should be emphasized that, with the experimental approach used in the present experiments, glycerol mobilization rates are calculated by multiplying an arteriovenous difference (where the venous concentration is calculated from the measured interstitial concentration) with an average blood flow value that does not necessarily represent the blood flow value in the vicinity of the microdialysis probe. Obviously, this may introduce errors, especially when the number of subjects is low. However, on the basis of a sample size of eight, a paired, one-sided t-test with a significance level of 5% and defining the relative (relevant) difference to be 25%, it can be calculated that the power of detecting a significant increase in interstitial glycerol concentration (from 800 to 1,000 mmol/l, SD = 200) is ~88%. Similarly, it can be calculated that the power of detecting a significant increase in ATBF (from 2.3 to 2.9 ml·min⁻¹·100 g⁻¹, SD = 0.7) is ~78%. The data from the present study therefore support the conclusion that endurance training has little, if any, effect on subcutaneous abdominal adipose tissue lipolysis. This conclusion is also in agreement with the findings of Horowitz et al. (13) in a recent endurance training study performed in young women by use of the microdialysis technique.

In the present study, we evaluated the training effects on subcutaneous abdominal adipose tissue lipolysis at the same absolute level of pretraining V̇O₂ peak. It would have been desirable also to measure the training effect at the same relative level of V̇O₂ peak, because it cannot be excluded that the observed reduction in sympathetic nervous system activity (Fig. 2, A and B) may have occulted a stimulation of adipose tissue lipolysis induced by endurance training. However, this argument is based on the assumption that there is a close relationship between sympathetic nervous system activity and activation/degree of adipose tissue lipolysis in the range of 50–60% of V̇O₂ peak, and such a close relationship in this specific range is not evident from the available literature.
Subcutaneous abdominal adipose tissue lipolysis was not augmented by endurance training, whereas fat oxidation was significantly increased during exercise performed at the same relative level of pretraining \( V_{\text{O}_2\text{peak}} \). Theoretically, the additional amount of oxidized fat may be derived from increased NEFA clearance resulting from decreased adipose tissue NEFA reesterification induced by endurance exercise training. However, with the methodology used in the present study, i.e., microdialysis, this question cannot be addressed. Another explanation is that the extra amount of oxidized fat may be derived from tissues other than the subcutaneous abdominal adipose tissue, and several possibilities exist. In vitro measurements have demonstrated significant regional differences in adipose tissue with respect to quantity and quality of adrenoceptors and lipolytic activity (1, 43). Furthermore, in vitro studies suggest that intra-abdominal adipose tissue has the highest, subcutaneous abdominal adipose tissue an intermediate, and subcutaneous femoral adipose tissue the lowest lipolytic activity of these three depots (23). Whether this is true also in vivo remains to be determined, but in vivo measurements of perirenal ATBF have shown significantly higher values during prolonged exercise and after exercise compared with similar values obtained from subcutaneous abdominal adipose tissue (2). The increased amount of fat oxidized after endurance training may, therefore, originate from visceral fat depots. However indirect, this view is not supported by the uniform findings of unchanged whole body glyceral \( R_g \) after endurance training (9, 13, 31, 39). Alternatively, endurance training may increase intramuscular fat depots and also induce a more ready utilization of intramuscular fat during exercise. This has been suggested by Hurley et al. (14) and indirectly by Martin et al. (27). However, there are major methodological problems in determining muscle triglyceride content, and several other studies have not been able to confirm this effect of endurance training on muscle triglyceride content and utilization (18). In addition, intramuscular lipid content has been reported to be positively correlated with the degree of obesity (10).

It is well known that an intravenous GH bolus acutely increases plasma NEFA concentrations in a dose-dependent manner (29), and it has also been recently shown that an intravenous GH bolus causes a significant increase in subcutaneous abdominal adipose tissue interstitial glycerol concentration (11). However, the present study was designed to investigate the effect of endurance training combined with long-term rhGH administration. Therefore, measurements were performed \(-11 \text{h} \) after subcutaneous rhGH administration, when the acute effects of rhGH administration on lipolysis were anticipated to have subsided (16, 28). We did not find increased interstitial glycerol levels in the subcutaneous abdominal adipose tissue after endurance training combined with rhGH administration either at rest or during exercise. One explanation is that long-term rhGH administration results in increased adipose tissue lipolysis only of relatively short duration after the injection (<11 h). It may also be a reflection of the body composition in the present study population and the associated elevated plasma NEFA levels exerting negative feedback on adipose tissue lipolysis, at least during resting conditions. During exercise, adipose tissue lipolysis is maximally activated most likely through increased sympathoadrenergic activity, and rhGH administration may not have any additive effect.

An interesting finding in the present study is that, overall, serum GH was increased during exercise after endurance training in the placebo group (Fig. 3C). Previous studies in young subjects have generally not shown any effect of training on the GH axis. However, Weltman et al. (44) found that 1 yr of endurance training at intensities above lactate threshold increases the pulsatile release of GH, whereas the GH response to acute exercise actually decreases if exercise is performed at the same absolute intensity (45). The present data suggest that the GH response to exercise may be augmented by exercise training in the elderly.

As expected, there was an overall increase in serum GH after endurance training combined with GH administration (Fig. 3C). However, whereas serum GH increased significantly from rest to exercise before the training program, no increase was observed after completion of the training program combined with GH administration. This indicates that the present GH administration schedule causes negative feedback regulation on pituitary GH release during exercise. Furthermore, GH administration caused an overall increase in plasma glucose concentrations in this group despite significant improvements in fitness level and a significant reduction in body fat.

The present body composition data showed significant changes in the GH group, whereas no changes could be detected in the placebo group. In agreement with findings in other studies, there was a substantial decrease in FM accompanied by a similar increase in FFM with GH administration. However, as we discussed in a previous paper (22), we believe that a substantial proportion of the increase in FFM is due to accumulation of extracellular water, not to increased muscle mass.

In conclusion, the present study demonstrates that endurance training alone does not affect subcutaneous abdominal adipose tissue lipolysis in elderly women either at rest or during continuous cycling at a level corresponding to 60% of pretraining \( V_{\text{O}_2\text{peak}} \). Additionally, combining endurance training with rejuvenating serum IGF-I levels by rhGH administration does not have any measurable effect on subcutaneous abdominal adipose tissue lipolysis in the present experimental design. This was evidenced by similar adipose tissue interstitial glycerol concentrations and subcutaneous abdominal ATBF, both at rest and during exercise, before and after completion of a 12-wk endurance training program combined with rhGH administration. In contrast, whole body fat oxidation during exercise was significantly higher both after endurance training alone and after endurance training combined with
rhGH. These findings indicate that the changed lipid metabolism during exercise observed after endurance training with and without rhGH administration is not due to alterations in subcutaneous abdominal adipose tissue metabolism in elderly women.

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