Physiologically, growth hormone (GH) is secreted in pulses with episodic bursts shortly after the onset of sleep and postprandially. Such pulses increase circulating levels of free fatty acids and glycerol. We tested whether small GH pulses have detectable effects on intercellular glycerol concentrations in adipose tissue, and whether there would be regional differences between femoral and abdominal subcutaneous fat, by employing microdialysis for 6 h after administration of GH (200 µg) or saline intravenously. Subcutaneous adipose tissue blood flow (ATBF) was measured by the local Xenon washout method. Baseline of interstitial glycerol was higher in adipose tissue than in blood (220 ± 12 (abdominal) vs. 38 ± 2 (blood) µmol/l, P < 0.0005; 149 ± 9 (femoral) vs. 38 ± 2 (blood) µmol/l, P < 0.0005) and higher in abdominal adipose tissue compared with femoral adipose tissue (P < 0.0005). Administration of GH induced an increase in interstitial glycerol in both abdominal and femoral adipose tissue (ANOVA: abdominal, P = 0.04; femoral, P = 0.03). There was no overall difference in the response to GH in the two regions during the study period as a whole (ANOVA: P = 0.5), but during peak stimulation of lipolysis abdominal adipose tissue was, in absolute but not in relative terms, stimulated more markedly than femoral adipose tissue (ANOVA: P = 0.03 from 45 to 225 min). Peak interstitial glycerol values of 253 ± 37 and 336 ± 74 µmol/l were seen after 135 and 165 min in femoral and abdominal adipose tissue, respectively. ATBF was not statistically different in the two situations (ANOVA: P = 0.7). In conclusion, we have shown that a physiological GH pulse induces a dose-dependent stimulation of lipid oxidation and increase circulating levels of FFA and glycerol (33, 34). It has also been reported that pulsatile GH exposure increases whole body palmitate flux (8).

Patients with GH deficiency (GHD) are at increased risk of atherosclerosis and coronary artery disease (7, 32, 38) and have increased amounts of abdominal fat, which has been associated with cardiovascular disease (7, 14); GH treatment has been reported to specifically reduce the amount of visceral fat in both GHD patients and normal adults (7, 24) and to reduce abdominal subcutaneous fat mass selectively in children (39).

The current study was designed to test the hypotheses that small physiologically meaningful GH pulses have detectable effects on intercellular glycerol concentrations in adipose tissue and that these effects may be most prominent in central subcutaneous fat of the abdomen. To pursue this, we examined two different regions of subcutaneous adipose tissue, abdominal and femoral, after insertion of microdialysis fibers thus enabling assessment of regional changes in glycerol content after exposure to either GH or saline.

MATERIALS AND METHODS

Subjects. Eight healthy males gave their written informed consent after receiving oral and written information concerning the study according to the Declaration of Helsinki II. The study was approved by the Aarhus County Ethical Scientific Committee.

Experimental protocol. Subjects were admitted to the Clinical Research Center in the morning after an overnight fast (10–12 h) without any caffeine consumption or cigarette smoking; only ingestion of tap water was allowed. Participants were asked not to perform major physical exercise, to consume a weight-maintaining carbohydrate-rich diet for the last 3 days before examination, and to refrain from alcohol intake on the day before investigation. The average age, weight, and body mass index of the subjects was 25.5 ± 1.3 (23–33) [mean ± SE (range)] yr, 78.9 ± 2.7 (68–90) kg, and 23.6 ± 0.6 (21.1–26.0) kg/m², respectively. Participants were placed in the supine position in a bed in light clothes at room temperature ~22–24°C and remained there throughout the study. One intravenous catheter (Vigo, Helsingborg, Sweden) was placed in an antecubital vein for infusion and another in a vein draining a hand that was warmed in a box with an air temperature ~65°C to provide arterialized blood. Each subject was studied two times in a randomized manner with at least 2-wk intervals. After 1 h of calibration with perfusion of the microdialysis catheters (see below), either

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Effects of growth hormone in adipose tissue

GH (200 µg dissolved in NaCl) or NaCl was given intravenously over 25 min. Arterialized blood samples were drawn every 15 min for 7 h starting 60 min before infusion of GH or NaCl. Indirect calorimetry was performed in the basal period after 45 min of rest and 4 h after infusion of either GH or NaCl. No untoward clinical events occurred.

Microdialysis and calculations. Microdialysis fibers (CMA 60 microdialysis catheter; CMA, Stockholm, Sweden) were placed in abdominal and femoral subcutaneous adipose tissue after anesthetization of the skin with 0.05 ml lidocaine at the sites of perforation of the skin. The microdialysis catheters have a molecular cutoff of 20 kDa. Immediately after placement, perfusion of the fibers was started at a rate of 2 µl/min with a Ringer-acetate solution containing glycerol in a concentration of 25 mmol/l. At a perfusion rate of 2 µl/min, the exchange over the fiber did not reach 100% equilibrium, and subsequently, the internal reference calibration technique was used to calculate the relative recovery (RR; see Refs. 31 and 42). To the perfusate, a small amount of [3H]glycerol was added, and in the perfusate and in each dialysate sample the specific activity of [3H]glycerol was measured. The RR was calculated for each dialysate sample as

\[
\frac{(C_p^* - C_d^*)}{C_p^*} = \frac{(C_d - C_p)}{RR + C_p}
\]

where \(C_p^*\) is disintegrations per minute in the perfusate and \(C_d^*\) is disintegration per minute in the dialysate. It is assumed that the RR of the unlabeled glycerol in the dialysate equals the relative loss of the labeled glycerol from the perfusate. The mean RR was 39 ± 1%, and the coefficient of variation (CV) of the RRs determined for each individual was 10 ± 1%. The overall mean of the RRs for each individual was used for further calculations.

Interstitial concentration was calculated as

\[
\frac{(C_d - C_p)}{RR + C_p}
\]

where \(C_d\) is dialysate concentration and \(C_p\) is perfusate concentration. The RR was constant throughout the experiments, and there was no difference in recovery between the two study regions.

Blood flow measurements. In six subjects, the subcutaneous adipose tissue blood flow (ATBF) in the abdominal region in which dialysis was performed was measured by the local \(^{133}\)Xe washout method (27). In short, 3.7 MBq (0.1 ml) \(^{133}\)Xe were injected in the subcutaneous area of interest, equivalent to a whole body radiation dose of 0.5 mSv. Disappearance of \(^{133}\)Xe was monitored with a 2 x 2-in. NaI detector (model 905) coupled to a photomultiplier base (model 276; EG&G Ortec, Wokingham, Berks, UK) covered by a cylindrical copper collimator and coupled to a multichannel AceMate (model 925) amplifier (EG&G Ortec). The system was connected to a computer for simultaneous sampling. Counts were collected every minute and were plotted on a semilogarithmic diagram as a function of time. ATBF was calculated as

\[
\text{ATBF} = k \times \lambda \times 100 \text{ (ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1})
\]

where \(k\) is the rate constant of the washout curve and \(\lambda\) is the tissue-to-blood partition coefficient for \(^{133}\)Xe at equilibrium; we used a value of 10 for \(\lambda\) (27). It was anticipated that changes in ATBF were comparable in both regions of interest (21, 43).

Indirect calorimetry. Indirect calorimetry (DeltaTrac Metabolic Monitor; Datex, Helsinki, Finland) with a ventilated hood at 40 l/min was performed; energy expenditure, respiratory quotient, and 24-h excretion of urea were measured from the excretion rate of urea in urine collected during the entire study period, and glucose, protein, and lipid oxidation were calculated (10). Calibration of the system was done by combustion of a known amount of 99.6% ethanol.

Assays. Plasma glucose was measured immediately after sampling in duplicate on an autoanalyzer (Beckman Instruments, Palo Alto, CA) by the glucose oxidase method. GH was measured with a double monodonal immunofluorometric assay (DELFIA; Wallac Oy, Turku, Finland). The interassay CV in samples varied between 1.7 and 2.4%, the intra-assay CV varied between 1.9 and 3.0% for GH concentrations of 12.08 and 0.27 ng/ml, and the detection limit was 0.01 ng/ml. Serum insulin was measured by enzyme-linked immunosorbent assay, employing a two-site immunosassay. The assay does not detect proinsulin, split-(32—33) and des-(31—32)-proinsulin, whereas split-(65—66) and des-(64—65)-proinsulin cross-react 30 and 63%, respectively (1). The intra-assay CV was 2.0% (n = 75) at a serum level of 200 PM. Serum FFA was determined by a colorimetric method employing a commercial kit (Wako Chemicals, Neuss, Germany). Blood samples for determination of alanine, glycerol, 3-hydroxybutyrate (3-OHB), and lactate were deproteinized with perchloric acid and were assayed by an automated fluorometric method (29). Plasma glucagon was measured by an RIA (35). Dialysate glycerol was measured by an automated spectrophotometric kinetic enzymatic analyzer (CMA 600; CMA).

Statistical analysis. All statistical calculations were done with SPSS for Windows version 8.0 (SPSS, Chicago, IL). Data were examined by Student's two-tailed unpaired and paired t-tests. Repeated-measures ANOVA (GLM) was used to test for differences with time between the GH situation and the placebo situation, i.e., to examine whether more glycerol was released in the GH situation compared with the placebo situation (the interaction between time and treatment), and for comparison between changes in the femoral and abdominal region based on \(\Delta\) values (i.e., GH – control). Results are expressed as means ± SE. Significance levels under 5% were considered significant.

RESULTS

Circulating hormones. In Fig. 1, the pertinent hormones are illustrated. Administration of GH increased GH to a peak of 17.7 ± 2.2 µg/l from baseline values of 0.2 ± 0.1 µg/l, whereas in the control situation serum GH was constant (ANOVA: \(P = 0.0005\)). Serum insulin and glucagon stayed constant around 20 ± 1 pmol/l and 54 ± 2 ng/l, respectively, throughout the study during both treatments (ANOVA: insulin, \(P = 0.7\); glucagon, \(P = 0.8\)), although there was a time-treatment interaction in the profile of insulin (ANOVA: \(P = 0.04\)).

Lipid intermediates in blood. Baseline values were comparable with regard to all three intermediates (FFA: 0.44 ± 0.05 (GH) vs. 0.43 ± 0.04 (NaCl) mmol/l; 3-OHB: 74 ± 22 (GH) vs. 95 ± 24 (NaCl) mmol/l; glycerol: 34 ± 3 (GH) vs. 42 ± 3 (NaCl) mmol/l; \(P = 0.8\), not significant; Fig. 2).

The administration of GH induced an increase in all three lipid intermediates, with a peak in glycerol of 79 ± 7 mmol/l after 135 min, FFA of 0.90 ± 0.08 mmol/l after 165 min, and 3-OHB of 269 ± 90 mmol/l after 195 min (ANOVA: FFA, \(P = 0.0005\); 3-OHB, \(P = 0.0005\); glycerol, \(P = 0.002\)). In the placebo situation, there was a slight significant increase in FFA with time and no change in glycerol and 3-OHB.
Interstitial glycerol in abdominal and femoral adipose tissue: GH vs. placebo. Administration of GH induced changes in interstitial glycerol comparable with the changes observed in blood (Fig. 3). Baseline values were higher in adipose tissue than in blood \[220 \pm 12 \text{ (abdominal)} \text{ vs. } 149 \pm 9 \text{ (femoral)} \mu\text{mol/l, } P < 0.0005\], whereas baseline values of interstitial glycerol were comparable in the two treatments \[230 \pm 15 \text{ (GH) vs. } 208 \pm 21 \text{ (NaCl)} \mu\text{mol/l, } P = 0.4; \text{ femoral adipose tissue: } 148 \pm 11 \text{ (GH) vs. } 151 \pm 14 \text{ (NaCl)} \mu\text{mol/l, } P = 0.9\]. Administration of GH induced an increase in interstitial glycerol compared with basal levels in both abdominal and femoral adipose tissue (ANOVA: abdominal, \(P = 0.04\); femoral, \(P = 0.03\)). There was no effect of saline administration in interstitial glycerol with time in either abdominal or femoral adipose tissue (ANOVA: abdominal, \(P = 0.7\); femoral, \(P = 0.3\)).

Interstitial glycerol in abdominal vs. femoral adipose tissue. The interstitial glycerol level was higher in abdominal adipose tissue compared with femoral adipose tissue \[220 \pm 12 \text{ (abdominal)} \text{ vs. } 149 \pm 9 \text{ (femoral)} \mu\text{mol/l, } P < 0.0005\]. There was no overall difference in the response to GH in the two regions during the study period as a whole (ANOVA: \(P = 0.5\)). However, during the period with the most marked increments in glycerol concentrations in blood and subcutaneous tissue, the absolute increment was more pronounced in abdominal adipose tissue than the femoral adipose tissue (ANOVA: \(P = 0.03\), from time = 45–225 min; Fig. 4). Peak interstitial glycerol values of 253 \pm 37 and 336 \pm 74 \mu\text{mol/l were seen after 135 and 165 min in femoral and abdominal adipose tissue, respectively. However, due to the difference in basal glycerol values in abdominal and femoral adipose tissue, the relative increase over basal was similar. At the time of maximal stimulation of lipolysis, there was a 1.5-fold increase in the release of glycerol in the abdominal adipose tissue (time = 165 min) and a 1.3-fold increase in release of glycerol in the femoral adipose tissue (time = 135 min; \(P = 0.5\)). Thus, despite greater absolute increases in interstitial glycerol in abdominal adipose tissue, the relative increases were similar.

Glucose and gluconeogenic intermediates in blood. Plasma glucose stayed constant throughout the study during both treatments (ANOVA: \(P = 0.6\); Fig. 5). Alanine and lactate, however, declined with time during both treatments (ANOVA: \(P = 0.0005\)).

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Fig. 1. A: growth hormone (GH) vs. time during the study. B: insulin vs. time during the study. C: glucagon vs. time during the study. GH; ○, saline. Error bars indicate means ± SE, and \(P\) values indicate the interaction between time and treatment (i.e., GH or saline) analyzed by ANOVA.

Fig. 2. A: serum nonesterified fatty acid (NEFA) vs. time during the study. B: serum 3-hydroxybutyrate (3-OHB) vs. time during the study. C: serum glycerol vs. time during the study. GH; ○, saline. Error bars indicate means ± SE, and \(P\) values indicate the interaction between time and treatment (i.e., GH or saline) analyzed by ANOVA.
tion of GH caused an accentuated decline in alanine (ANOVA: \( P < 0.0005 \)), whereas lactate did not differ during the two treatments (ANOVA: \( P > 0.7 \); Fig. 5).

Abdominal ATBF. ATBF was not statistically different during the two treatments (2.8 ± 0.6 (saline) vs. 3.8 ± 0.9 (GH) ml·100 g\(^{-1}\)·min\(^{-1}\), \( P = 0.2, n = 6 \)) and stayed constant throughout the study period.

Energy expenditure. Energy expenditure was constant on the day of saline infusion (1,765 ± 46 (basal) vs. 1,760 ± 50 (infusion) kcal/24 h, \( P = 0.9 \)), whereas energy expenditure increased on the day of GH infusion (1,736 ± 42 (basal) vs. 1,839 ± 47 (infusion) kcal/24 h, \( P = 0.01 \)). There was a tendency toward a decrease in urinary nitrogen excretion during the day of GH infusion (12.0 ± 1.0 (GH) vs. 16.1 ± 2.4 (saline) g/24 h, \( P = 0.06, n = 7 \)). Basal measurements on the two study days were comparable (data not shown). The respiratory quotient decreased in both situations (saline: 0.83 ± 0.01 (basal) vs. 0.82 ± 0.01 (infusion), \( P = 0.01 \); GH: 0.84 ± 0.02 (basal) vs. 0.80 ± 0.01 (infusion), \( P = 0.02 \), with a tendency to a greater decrease during the day of GH infusion [\( \Delta \text{decrease: -0.02 ± 0.01 (saline) vs. -0.04 ± 0.01 (GH), } P = 0.1 \)]. Protein oxidation decreased [399 ± 34 (GH) vs. 534 ± 81 (saline) kcal/24 h, \( P = 0.06 \)], lipid oxidation increased [1,001 ± 67 (GH) vs. 745 ± 91 (saline) kcal/24 h, \( P = 0.01 \)], and glucose oxidation was unchanged [439 ± 22 (GH) vs. 464 ± 62 (saline) kcal/24 h, \( P = 0.7 \)].

DISCUSSION

The present study was undertaken to define the effects of GH on regional interstitial glycerol concentrations in abdominal and in femoral fat. Our main findings are that a single GH pulse increases interstitial glycerol concentrations in both femoral and abdominal adipose tissue, the absolute increase being more prominent in the abdomen. It should be noted that, with the present dosage of GH, we obtained a serum profile closely resembling a large spontaneous secretory burst with regard to duration (25 min) and magnitude (17.7 ± 2.2 µg/l; see Ref. 18).

It is well established that GH is a hormone with potent lipolytic and protein-sparing effects, and the impact of a single bolus of GH, imitating a physiological pulse, has been characterized in terms of circulating levels of lipid intermediates (20, 33, 34, 36). Here, by indirect calorimetry, we found an increase in lipid oxidation illustrating the effect of GH on whole body metabolism. We found that glycerol levels in the interstitial fluid of subcutaneous adipose tissue were significantly affected by a physiological pulse of GH in a time-dependent manner closely paralleling the picture seen in blood. The identical patterns of changes in glycerol and FFA in the circulation and glycerol in the interstitial fluid with peak values between 135 and 180 min probably reflect that both substances reach equilib-
rrium fast due to a rapid turnover. The present data fit well with previous observations linking nocturnal peaks of GH with the maximum level of FFA 120 min later (40). At present, the exact mode of the intracellular action of GH is not completely known. In vitro GH possesses both insulin-like and lipolytic actions (13), with the insulin-like action of GH probably being governed by intracellular Ca\(^{2+}\) levels (11). The lipolytic actions may be mediated through stimulation of gene expression after binding of the GH receptor with JAK2 tyrosine kinase and subsequent activation of the complex (2). The intracellular signaling process leading to stimulation of lipolysis is not known in detail but appears to involve activation of adenyl cyclase stimulating intracellular cAMP production (48), which initiates a chain of reactions, including RNA and protein synthesis, resulting in the activation of hormone-sensitive lipase. In vitro, this activation of lipolysis takes 1–2 h, which is entirely consistent with our in vivo findings.

It is not likely that the elevated glycerol concentrations may have been influenced by changes in circulating levels of insulin or glucagon; in accordance with previous studies (33, 34, 46), we observed virtually unchanged concentrations of the two hormones. Furthermore, because ATBF estimated by Xenon washout did not decrease after administration of GH, it appears by far most plausible that the increased glycerol concentration is a direct consequence of GH stimulation of lipolysis (4, 17, 22). Previously, it has been shown that small amounts of GH do not acutely affect blood flow in the forearm (33, 34), compatible with a lack of any effects on flow in adipose tissue.

Microdialysis allows continuous monitoring of changes of fluxes of a variety of compounds from interstitial fluid to the dialysate, and it has been utilized in a large number of tissues in the human body since it was first introduced (30). True equilibrium is not accomplished across the membrane, unless a very low flow rate is used, and RR of an internal standard may be used to correct the ensuing deficit (31, 42). Thus the changes in estimated interstitial glycerol concentration can be seen as an index of lipolysis (4, 17, 22). Calculated baseline values for glycerol obtained in the present study correspond closely to the ones available in the literature (16, 17, 37, 41). The observation that glycerol concentrations (and presumably the rate of lipolysis) in males are elevated in abdominal subcutaneous adipose tissue compared with peripheral tissue is also compatible with previous findings (21, 22). On this background, it is debatable whether GH has any preferential effects on abdominal fat; it could be argued that in relative terms the increments in glycerol concentrations in the abdomen are not different from the femoral changes. In both tissues, 1.3- to 1.5-fold increases of baseline values were seen, and, since the degree of increase over basal was the same in both tissues, the sensitivity to GH may be said to be the same. On the other hand, the absolute response was two-to threefold more pronounced in the abdomen; to the extent that this exaggerated glycerol response reflects an equally exaggerated stimulation of local lipolysis, these metabolic effects of GH will lead to preferential loss of abdominal subcutaneous fat. Thus the intracellular events that take place after binding to the GH receptor seem to be amplified in abdominal adipose tissue compared with peripheral adipose tissue. In this respect, GH resembles catecholamines, which have also been reported to have distinct lipolytic effects on the abdominal depots (5, 44, 45).

Recently, the decisive role of subcutaneous abdominal fat in determining insulin sensitivity and perhaps thereby the risk of future cardiovascular disease has been highlighted (14). The most potent endogenous activators of lipolysis in adipose tissue are catecholamines (5, 44, 45), and it is thus conceivable that the action of GH, like the action of catecholamines (6), is more pronounced in abdominal than in femoral subcutaneous adipose tissue. Consistent with this theory, it has been shown that substitution of GH in GH-deficient patients protects these patients from the otherwise increased risk of cardiovascular disease (38), by specifically decreasing upper body fat. It is not likely that GH exerts its action via catecholamines, since infusion of GH in humans does not affect the levels of circulating catecholamines (unpublished observations). Microdialysis in sheep has
previously shown that GH increases basal lipolysis and increases the maximum lipolytic rate in the presence of catecholamines both in vivo and in vitro (9). Consistent with these findings, the action of GH on cAMP production in adipocytes is increased by catecholamines (3), and, on the other hand, GH sensitizes adipocytes to catecholamines (47), suggesting a synergistic effect of GH and catecholamines on lipolysis.

It remains uncertain how GH affects lipolysis in the intra-abdominal visceral fat. Judging from studies involving computed tomography scans of the abdomen in GHD patients and normal adults before and after GH treatment, GH appears to have at least the same lipolytic potential viscerally as subcutaneously (7, 24, 26).

It is well known that circulating levels of FFA are elevated in poorly regulated diabetes (23, 25), and, recently, circadian lipolysis has been studied in normal and diabetic individuals; the results implicate that increased nocturnal levels of GH may cause the perturbed circadian rhythm of lipid mobilization in insulin-dependent diabetes mellitus, with increased levels of glycerol during the night (16). The current study underlines the lipolytic effect of GH and provides additional evidence that GH is a key component in the regulation of lipid metabolism. Ordinarily, GH is secreted in a pulsatile fashion (18), and this pattern may be disrupted in certain pathological conditions (12, 15, 19). Although one study has suggested that pulsatile exposure to GH is necessary for maximum lipolytic effect (8), other protocols have found evidence of strong lipolytic effects of GH after constant infusion (28), and it therefore remains to be determined whether pulsatile GH delivery per se augments the lipolytic properties of the hormone.

In summary, we have shown that a physiological pulse of GH increases interstitial glycerol, and presumably lipolysis, in adipose subcutaneous tissue, with the absolute response being more pronounced in abdominal than in femoral adipose tissue. The study underlines the potent lipolytic effect of a pulse of GH and places GH as a strong contender among hormones regulating the rate of lipolysis.

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