Regulation of Lipoprotein Lipase and Hormone-Sensitive Lipase Activity and Gene Expression in Adipose and Muscle Tissue by Growth Hormone Treatment During Weight Loss in Obese Patients

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It is well known that growth hormone (GH) treatment reduces fat mass (FM), which presumably is mediated through stimulation of triglyceride breakdown and inhibition of adipose tissue lipoprotein lipase activity (AT-LPL). However, it is unknown which of the 2 GH-regulated pathways are of most importance for the reduction in FM. We investigated the effect of weight loss together with GH treatment on the activity and gene expression of LPL and hormone-sensitive lipase (HSL) in AT and muscle tissue. A very–low-calorie diet ([VLCD] 740 kcal/d) was given to 18 obese women (body mass index [BMI] > 35 kg/m^2) and half of them were treated with GH (0.04 IU/kg) for 4 weeks in a randomized double-blind placebo-controlled study. Subcutaneous fat and muscle biopsies were taken before and after 4 weeks. Weight loss after 4 weeks was similar in the 2 groups, with a reduction of 4.5% (placebo) and 4.6% (GH) and a reduction of FM by 7.4% and 9.0% ([NS] nonsignificant). The weight loss resulted in a small and NS reduction of AT-LPL activity by 20% ± 12% in the placebo group, but in the GH group, AT-LPL was significantly reduced by 65% ± 8% ($P < .01$). Muscle LPL (M-LPL) activity was not affected by the weight loss alone, but a significant reduction was observed in the GH group (20.4% ± 10%, $P < .05$). AT-HSL activity was significantly enhanced after weight loss, but GH had no additional effect on this minor increment. This is in accordance with the finding that the increment in free fatty acid (FFA) after weight loss was similar in the 2 groups. GH treatment was associated with a significant reduction of high-density lipoprotein (HDL) cholesterol ($P < .05$). In conclusion, GH significantly inhibited AT-LPL activity but had no additional effect on the hypocaloric-induced loss of FM, indicating that under such circumstances, AT-LPL does not directly regulate adipose tissue mass. GH was not found to have opposite effects on the activity of LPL in adipose tissue and muscle, since GH treatment reduced them both (by 65% and 20%, respectively). The VLCD-induced weight loss was associated with a minor enhanced activity of AT-HSL with no independent effect of GH. Thus, concerning body weight, FM, and lipolytic activity, treatment with GH offers no extra benefits during a VLCD for 4 weeks.

LIPOPROTEIN LIPASE (LPL) is a glycoprotein produced by several extrahepatic tissues, and is most abundant in adipose tissue and muscle, where it is involved in the uptake of fat (free fatty acids [FFAs]) in these tissues. The activity of LPL is regulated in a tissue-specific manner[1] and may participate in energy-partitioning processes. The corresponding gene has been suggested as a candidate implicated in the etiology of obesity and type 2 diabetes.[2] The enzyme activity is modulated by several endocrine factors which are related to the state of energy balance of the organism.[3]

We have recently shown that growth hormone (GH) treatment of obese women for 5 weeks resulted in a 50% reduction of adipose tissue LPL (AT-LPL) activity,[4] and similar findings have been obtained by adding GH to human adipose tissue in vitro.[5] These findings indicate that GH may be of importance for the uptake of FFA in adipose tissue and for the expansion of fat stores. In addition, GH also has effects on adipose tissue triglyceride breakdown (lipolysis), since GH infusion in humans is associated with an increased concentration of FFAs and glycerol in the plasma after a lag period of about 2 hours.[6,7] However, the biochemical mechanisms involved in the lipolytic effect of GH remain to be determined.[7] However, several possibilities have been suggested, such as upregulation of the hormone-sensitive lipase (HSL),[8] upregulation of the β-adrenergic receptors,[9] inhibition of the G_i protein in adipocytes,[10] and antagonizing the antilipolytic effect of insulin.[11] Since GH treatment of GH-deficient adults and obese subjects is associated with a reduction of the fat mass (FM), we have suggested that this effect might be mediated, in part, by the GH-induced reduction of AT-LPL and by the concomitant stimulation of lipolysis.[4] However, it is still unknown which of the 2 processes is of most importance for the GH-induced reduction of FM in vivo.

The differential hormonal regulation of muscle LPL (M-LPL) activity and AT-LPL activity is complex and is not fully understood. Insulin is able to increase LPL activity in adipose tissue[12] but seems to reduce LPL activity in muscle tissue during short-term insulin infusion.[13,14] Epinephrine reduces LPL activity in adipose tissue but stimulates the activity in skeletal muscle.[15,16] Thus, both insulin and epinephrine exert opposite effects on LPL activity in adipose tissue and muscle, which may play a role in the rapid switch of substrate metabolism between adipose and muscle tissue, for example, after a meal where high insulin directs lipids to storage in the adipose tissue, and in association with exercise and stress where epinephrine directs lipids to muscle tissue for oxidation. However, it is unknown whether GH regulates M-LPL and whether GH has differential effects on LPL in adipose and muscle tissue. It has been shown that LPL activity, particularly in muscle tissue, is closely related to the regulation of plasma triglyceride and high-density lipoprotein (HDL) cholesterol

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levels,[14,17] and considering that GH affects lipoprotein levels,[18-20] it is of additional interest to investigate the effect of GH on M-LPL in humans.

In the present study, the effect of GH along with a very–low-calorie diet (VLCD) treatment was investigated on LPL and HSL activities and gene expression in both adipose and muscle tissue to determine the importance of LPL and HSL activity for changes in body weight and body composition, and to determine whether alterations in enzyme activities can be related to changes at the gene expression level.

SUBJECTS AND METHODS

Subjects

Eighteen obese women (33 kg/m^2 < body mass index [BMI] <45 kg/m^2) who were referred to the outpatient clinic at the hospital for weight reduction comprised the study group. Their body weight was stable for at least 2 months before the investigation. All were premenopausal and used no medication known to affect lipid and glucose metabolism such as oral contraceptives or antihypertensive medications. Before inclusion in the study, a physical examination was found to be normal. Moreover, after ingestion of 75 g glucose, all subjects had a normal 2-hour oral glucose tolerance curve. Clinical characteristics are outlined in Table 1. The study was approved by the local ethics committee.

Study Design

The obese women were all treated with a very–low-calorie diet ([VLCD] BliLet, Nycomed, Denmark) with 740 kcal/d for 4 weeks (containing 85 g protein, 73 g carbohydrate, 12 g fat, and vitamins and minerals). The investigation was performed as a double-blind placebo-controlled parallel study. The women were treated in a randomized order with GH (Norditropin; Novo Nordisk, Gentofte, Denmark) or placebo (saline) for 4 weeks. GH was administered subcutaneously once daily in the evening, and the dose was gradually increased during the 4 weeks: 0.03 IU/kg ideal body weight in the first week, 0.04 IU/kg in week 2, 0.06 IU/kg in week 3, and 0.08 IU/kg in week 4. However, the total daily dose never exceeded 6 IU. Body composition and anthropometric measurements and fat and muscle biopsies were obtained before and at the end of the 4-week treatment period.

Anthropometric Measurements

After an overnight fast, the height and body weight of the subjects were measured and the BMI (weight in kilograms divided by the square of the height in meters) was calculated. Waist and hip circumferences were measured in the supine position. The waist was measured midway between the lower costae and crista. The hip circumference was measured at the widest part of the hip region. The circumferences were measured twice and the mean value was used. The waist to hip ratio (WHR) was calculated and used as an index of abdominal fatness. The degree of abdominal adiposity was also determined by measuring the sagittal diameter. This diameter was measured with the patient in the supine position at the level of the umbilicus as previously described.[21]

Dual-Energy X-Ray Absorptiometry Scan

Body composition was determined by dual-energy x-ray absorptiometry (DEXA) scan using a Hologic QDR-1000/W scanner (Waltham, MA) as previously described.[22] From this scan, the amount of fat mass (FM), lean body mass (LBM), and bone mass (BM) can be determined in different parts of the body, as well as in the whole body. LBM comprises both muscle tissue and nonmuscle fat-free tissue (including the water space).[23] Body weight from the scan (DEXA weight) was determined by summation of the instrument-provided measurements of LBM, FM, and BM.

Biopsies

Blood samples and biopsies were obtained in the morning after an overnight fast. The last GH/placebo injection was administered in the evening on the day before the investigations (about 12 hours before). Adipose tissue was obtained from the subcutaneous abdominal region (periumbilically). The biopsy was obtained by needle aspiration (liposuction) after local anesthesia with lidocaine (1%). The adipose tissue was frozen in liquid nitrogen for later measurement of enzyme activities and RNA extraction.

Muscle biopsies (250 to 300 mg) were obtained from the vastus lateralis muscle with a Bergström biopsy needle as previously de-

Table 1. Anthropometric Measurements, Lipids, and Hormones Before and After 4 Weeks of Treatment With a VLCD With and Without GH Supplementation

Parameter	Placebo		GH-Treated	
	0 wk	4 wk	0 wk	4 wk
No. of subjects	10		8	
Age (yr)	34.6 ± 2.8		35.3 ± 3.8	
Body weight (kg)	117.2 ± 5.6	111.9 ± 5.8	110.4 ± 6.4	105.4 ± 5.6*
BMI (kg/m^2)	41.7 ± 2	39.8 ± 2.1	42.4 ± 2.5	40.4 ± 2.1*
FM (kg)	59.64 ± 4.9	55.29 ± 4.3	56.16 ± 4.8	51.12 ± 4.9*
FM%	50.2 ± 2.48	48.7 ± 2.35	50.6 ± 1.7	48.0 ± 2.1*
WHR	0.9 ± 0.03	0.88 ± 0.04	0.88 ± 0.03	0.89 ± 0.03
Waist (cm)	118.3 ± 4.1	110.1 ± 5	114.4 ± 4.6	110.4 ± 4.1*
Sagittal diameter (cm)	26.8 ± 1.4	24.9 ± 0.9	25.9 ± 1.3	23.2 ± 0.7*
FFA (nmol/L)	580 ± 75	818 ± 91	547 ± 78	896 ± 165*
Total cholesterol (mmol/L)	5.4 ± 0.28	4.48 ± 0.3	5.63 ± 0.4	4.5 ± 0.4*
HDL cholesterol (mmol/L)	1.14 ± 0.12	1.07 ± 0.09	1.23 ± 0.09	0.99 ± 0.84†
Triglyceride (mmol/L)	1.8 ± 0.36	1.57 ± 0.44	2.09 ± 0.55	1.62 ± 0.84
Insulin (pmol/L)	92 ± 13	81 ± 16	95 ± 18	108 ± 24
Leptin (ng/mL)	49 ± 6	31 ± 2	59 ± 8	29 ± 5*
IGF-I (ng/mL)	184 ± 24	169 ± 22	211 ± 24	415 ± 36†

NOTE. FM and FM% are the FM determined in absolute and relative terms from DEXA scan (mean ± SE).

*Significant elevation (FFA) or reduction (all others) after 4 weeks of VLCD in each group but no significant differences between the 2 groups.

†Significant alteration only in the GH group.

scribed.[22] Under local anesthesia (lidocaine), a small incision was made through the skin and muscle sheath 15 to 20 cm above the knee on the lateral aspect of the thigh.[22] The muscle biopsy was cleaned of blood and fat droplets and immediately frozen in liquid nitrogen and stored at −80°C until analysis. We were unable to obtain tissue biopsies from all participants either because of technical problems or because some of the participants refused to undergo the second biopsy.

AT-LPL Assay

LPL was extracted from the adipose tissue as previously described[24] with modifications. In brief, after weighing (about 500 mg in duplicate), the adipose tissue was homogenized (Potter-Elvehjem–type glass homogenizer, 25 strokes) and the homogenate was centrifuged (45 minutes at 14,000×*g* at 4°C). LPL activity was determined in the fat-free postmitochondrial infranatant. Enzyme activity was measured as hydrolyzed ^{14}C-triolein after incubation of 0.1 mL eluted enzyme with 0.1 mL substrate for 60 minutes at 37°C in a shaking water bath. The substrate was prepared with ^{14}C-triolein and unlabeled triolein (final triolein concentration, 3.5 mmol/L), 1α-lecithin and human fasted serum (as donor of ApoCII), all emulsified (Branson Sonifer) with 0.2 mol/L Tris hydrochloride buffer containing 1% fatty acid–poor human albumin. The reaction was stopped by adding chloroform:methanol: heptane 250:230:180. After addition of carbonate buffer (pH 10.5, 0.1 mol/L), aliquots of the upper phase were taken for liquid scintillation counting. LPL activity was expressed as nanomoles of FFA released per hour, and this value is expressed per milligram of protein. The intraassay coefficient of variation was 8%. All assays were performed on the same day to avoid day-to-day variation.

M-LPL Assay

The M-LPL assay was based on previously described methods[14,25] with minor modifications. About 30 mg of the muscle was cut (on ice) into small pieces (3 to 5 mg) and placed in incubation tubes. The tissue was incubated in a heparin-containing (200 IU · L^{-1}) medium with a serum-activated triolein emulsion with ^{14}C-triolein as tracer and albumin as acceptor of released fatty acids. The mixture was incubated for 30 minutes on an ice bath and then for a further 120 minutes at 37°C in a shaking water bath. LPL activity was measured on the same day to eliminate the influence of day-to-day variation.

AT-HSL Assay

HSL was measured as neutral cholesteryl esterase activity using minor modifications of methods previously described.[26] Briefly, substrate for the assay was prepared by adding 3.75 μCi cholesteryl-(1-^{14}C) oleate, 0.043 mmol phosphatidylcholine, and 0.011 mmol cholesterol oleate to 3 mL 100-mmol/L potassium phosphate buffer (pH 7.0) containing 5 mmol/L sodium taurocholate. The substrate was sonicated for 10 minutes (Branson Sonifer/Cell disrupter, Danbury, CT) with the output setting on 4. After homogenization, the homogenates were centrifuged at 4°C at 20,000×*g* for 45 minutes, the infranatants under the fat cake were carefully removed, and aliquots of 100 μL were analyzed in triplicate for neutral cholesteryl activity by adding 10 μL substrate at 37°C for 60 minutes. The results are presented as nanomoles of cholesteryl oleate hydrolyzed per milligram of protein per hour.

Reverse Transcription–PCR Assay for Detection of LPL and HSL mRNA in Adipose Tissue and Skeletal Muscle

Reverse transcription (RT) was performed using random hexamer primers as described by the manufacturer (GeneAmp RNA PCR Kit; Perkin Elmer Cetus, Norwalk, CT) at 23°C for 10 minutes and 42°C for 60 minutes and terminated by increasing the temperature to 95°C for 10 minutes. Then, the PCR mastermix containing the specific primers and AmpliTaq GOLD DNA polymerase was added.

To determine the linearity of DNA amplification, preliminary experiments were performed for HSL, LPL, and β-actin in adipose tissue and skeletal muscle separately. A linear increase in PCR product was observed when using RNA between 1 and 300 ng (data not shown); thus, all subsequent PCRs were performed using 25 ng RNA. A second set of PCRs using 25 ng RNA were designed to determine the appropriate number of cycles. Having determined these conditions, we further optimized the protocol to perform semiquantitative multiplex PCR with primer dropping.[27] Semiquantitative multiplex PCR estimated the relative amount of message RNA to a known housekeeping gene (β-actin) as an internal control to monitor the sample-to-sample variability of the RT step, as well as the PCR step. However, as the target message was present at a much lower copy number than the housekeeping gene, we added the housekeeping primers after a few cycles of amplification of the target DNA. By experiment, the number of necessary initial cycles before dropping the housekeeping primers was determined for HSL and LPL in both adipose tissue and skeletal muscle. For HSL in adipose tissue, β-actin was dropped after 9 cycles and the remaining 24 cycles were performed. For LPL in adipose tissue, β-actin primers and LPL primers were added together and the PCR was performed using 24 cycles. For HSL in skeletal muscle, β-actin primers were dropped after 15 cycles, with 24 remaining. Finally, for LPL in skeletal muscle, β-actin was dropped after 8 cycles, with 24 remaining. A similar set-up was used for negative controls except that the RT was omitted and no PCR products were detected under these conditions.

The PCR products were loaded on a 2% agarose gel, stained with ethidium bromide, and analyzed using the Bio-Rad Gel-Doc 1000 system (Richmond, CA), where the ratio between the target PCR product and the β-actin PCR product was calculated. The coefficient of variation for the LPL to β-actin ratio was 12.4% (n = 15). The following primer pairs were used: HSL, 5′-GAA GGC GGC ACG GAC GCC and 5′- GCT GGT GCG GCG GGA CAC (PCR product, 402 base pairs); LPL, 5′-GAG ATT TCT CTG TAT GGC ACC and 5′-CTG CAA ATG AGA CAC TTT CTC (PCR product, 277 base pairs); and β-actin, 5′-TGTGCCCATCTACGAGGGGTATGC-3′ and 5′-GGTACATGGTG-GTGCCGCCAGACA-3′ (PCR product, 521 base pairs). PCR was performed in a Perkin Elmer GeneAmp model 9700 with the following program: 95°C for 10 minutes and then each cycle consisting of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 60 seconds, and finally, product extension for 5 minutes at 72°C.

Other Assays

GH, insulin-like growth factor-I (IGF-I), and insulin levels were measured by radioimmunoassay.[21] NEFA was determined by a radiochemical method and leptin by RIA (Linco Research, St. Charles, MO).

Statistics

Data analyses were performed using the SPSS statistical package (SPSS, Chicago, IL). Student's paired (within-group comparison) and unpaired (between-group comparison) analysis and Pearson's correlation analysis were used. *P* values less than .05 were considered significant. Data in the text and tables are presented as the mean ± SE.

RESULTS

Effect on Body Weight and Anthropometry

Basal characteristics of the subjects are listed in Table 1. The effect of GH in the GH-treated group was demonstrated by a significant increase in IGF-I from 211 ± 24 to 415 ± 36 ng/mL ($P < .001$) as compared with a small and nonsignificant (NS) reduction in the placebo group. Body weight was reduced to a similar degree in the 2 groups after treatment for 4 weeks with a VLCD, with a reduction of 4.57% ± 3.5% for placebo and 4.64% ± 2.4% in GH-treated subjects (NS). Moreover, the FM determined by DEXA scan was reduced 7.4% ± 1.2% and 9.04% ± 1.9%, respectively (NS). Waist circumference and

Table 2. LPL and HSL Enzyme Activity in Adipose and Muscle Tissue

Parameter	Placebo		GH-Treated	
	0 wk	4 wk	0 wk	4 wk
AT-LPL				
Mean ± SE	212 ± 75	184 ± 92	289 ± 57	89 ± 20*§
Change (%)		−20 ± 12		−65 ± 8†§
M-LPL				
Mean ± SE	381 ± 44	390 ± 20	424 ± 56	316 ± 14
Change (%)		+8.2 ± 8		−21.4 ± 10†‡
AT-HSL				
Mean ± SE	4,191 ± 654	5,872 ± 554*‡	4,439 ± 384	5,985 ± 580*‡
Change (%)		+85.6 ± 52		+42.5 ± 19

NOTE. LPL and HSL activity were determined in adipose tissue and muscle biopsies before and after 4 weeks of treatment with a VLCD (−/+ GH treatment). AT-LPL is expressed as nmol FFA/h/mg protein, M-LPL as nmol FFA/h/g muscle tissue, and AT-HSL as nmol FFA/h/mg protein.

*Comparison of values before and after 4 weeks in each group.

†Comparison of values between the 2 groups.

‡$P \leq .05$.

§$P < .01$.

sagittal diameter were reduced to a similar extent in the 2 groups, with no change in the WHR (Table 1).

Effects of VLCD and GH on LPL and HSL

AT-LPL. The weight loss induced by the VLCD was associated with a NS reduction of LPL activity of about 20% in the placebo group (Table 2). However, in the GH group, a pronounced reduction of LPL activity of 65% ($P < .01$) was recorded, which was a significantly more pronounced reduction as compared with placebo ($P < .01$). The VLCD-induced weight loss was associated with a significantly reduced LPL mRNA expression of about 38% ($P < .05$) in both groups, with no observed independent effect of GH on the level of gene expression (Table 3).

M-LPL. The weight loss obtained after 4 weeks of VLCD treatment had no effect on LPL activity in muscle tissue in the placebo group, but a small but significant reduction (21%, $P < .05$) was observed in the GH group (Table 2). The gene expression of LPL in muscle tissue was not affected by the weight loss or by GH (Table 3).

AT-HSL. The variation in the determination of HSL activity in the present study was very high, and particularly the interindividual variation was high, which is the explanation for the seeming discrepancy between changes in the absolute values as compared with relative changes. A significant increment in HSL activity in adipose tissue was observed in association with the weight loss ($P < .05$) in both groups, with no specific independent effect of GH (Table 2). In contrast, the weight loss induced by VLCD resulted in a significant reduction of HSL gene expression in adipose tissue by 42% ($P < .005$) in the placebo group and 28% ($P < .05$) in the GH group (Table 3). However, this reduction in mRNA was not significantly different between the 2 groups.

M-HSL. Because of the shortage of muscle material, only the gene expression and not the activity of HSL was measured in muscle tissue, and a 30% reduction of gene expression was found after weight loss in both groups, which was not significant (Table 3).

Plasma Lipids and Hormones

The serum level of FFA as a marker of adipose tissue lipolysis increased rapidly after the start of the VLCD (data not shown), but the increment (about 2-fold) and the absolute level after 4 weeks of weight loss were similar in the 2 groups (Table 1). Total cholesterol was significantly reduced by the hypocaloric diet, but no independent effect of GH was observed. There was a trend for a reduction of triglycerides after the weight loss, but again GH had no effect. However, GH induced a significant decrease in HDL cholesterol ($P < .01$), with only a trend for a reduction in the control group. There was a trend for a decrease in the insulin level in the control group after the VLCD and a trend for an elevation of insulin with GH treatment, but none of

Table 3. LPL and HSL mRNA Expression in Adipose and Muscle Tissue

Parameter	Placebo			GH-Treated		
	No.	0 wk	4 wk	No.	0 wk	4 wk
AT-LPL	9	53.4 ± 8.7	32.7 ± 7.4*	7	57.8 ± 10.7	35.6 ± 5.0*
Change (%)			−38			−38.4
M-LPL	8	98.5 ± 24	95.8 ± 15.9	8	101.9 ± 26	122.6 ± 22.4
Change (%)			−2.7			+20.3
AT-HSL	9	360.2 ± 48.3	208.5 ± 31.3†	8	324.9 ± 37.6	232.4 ± 17*
Change (%)			−42			−28.4
M-HSL	8	200.6 ± 33.9	138.5 ± 23.6	8	192.0 ± 35.6	132.4 ± 25.5
Change (%)			−30.9			−31

NOTE. Specific mRNA was determined by RT-PCR before and after 4 weeks of VLCD treatment (±GH) in fat and muscle biopsies. The specific mRNA is expressed in arbitrary units.

*$P < .05$, †$P < .005$ *v* before VLCD ($t = 0$). There were no significant differences between the 2 groups.

these alterations reached statistical significance (Table 1). Leptin was significantly ($P < .01$) and similarly reduced after 4 weeks of weight loss in both groups, and this reduction in the leptin level was already detected after 1 week of VLCD treatment (data not shown).

Correlation Analysis

The decline in AT-LPL after 4 weeks of weight loss was not related to any measurement of the associated reduction in body weight or reduction in FM (data not shown). Moreover, the increment of AT-HSL activity after 4 weeks of weight loss was not correlated with any measurement of absolute or relative weight loss/change in FM in the present study. The initial level of insulin was correlated with the initial level of FFA ($r = .53$, $P < .02$), triglyceride ($r = .53$, $P < .02$), and HDL cholesterol ($r = -.44$, $P = .05$). The initial levels of both insulin and FFA were negatively correlated with the relative decrease of AT-LPL activity after the 4-week VLCD in both groups ($P = .05$ to $.08$), indicating that high initial levels of insulin/FFA were associated with a lesser reduction in AT-LPL activity after the VLCD.

DISCUSSION

Treatment of obese subjects with a VLCD resulted in a weight loss of 5 to 6 kg during 4 weeks, but weight loss together with changes in FM and anthropometric measurements were similar in the placebo group compared with the GH-treated group. These findings are in accordance with other investigations where GH treatment added to a hypocaloric diet has no additional effects on total weight loss or loss of FM,[28] but are in contrast to studies where GH treatment is combined with an isocaloric diet. Under the latter conditions, GH treatment generally induces a significant reduction in the FM of the body.[4,7,29] Thus, during hypocaloric conditions, the effect of GH on body composition seems minimalized. Finally, treatment for 4 weeks may be an insufficient time to observe the full effects of GH on body composition.

Obesity is characterized by enhanced activity of LPL in adipose tissue,[30] which may contribute to the expansion of the adipose tissue. In addition, obesity is characterized by low plasma levels of GH, and we have previously found that GH treatment of obese women is associated with a significant reduction of LPL activity (by 50%) in adipose tissue.[4] Thus, we proposed that this GH-induced reduction of AT-LPL might partly explain the concomitant reduction of the total FM in the subject.[4] In the present study, a pronounced reduction of AT-LPL activity was also found after GH treatment, but as already mentioned, this reduction in LPL was not followed by an additional reduction in FM, indicating, as recently reviewed by Fielding and Frayn,[3] that FFA uptake in adipose cells involves regulation by several factors other than the LPL pathway.

LPL gene expression was also determined in the present study, and it was found not to be related to AT-LPL activity, indicating that GH-induced alterations of LPL activity may be due to posttranscriptional changes. Similar conclusions concerning the effect of GH on LPL have been obtained from in vitro incubations with human adipose tissue.[5] The exact mechanism for this posttranscriptional effect of GH on AT-LPL still is not elucidated, but it has been suggested that GH may inactivate the active site of the enzyme in some way since the immunoreactive LPL enzyme is often unchanged after treatment with GH whereas LPL activity is reduced.[31]

In a recent study by Johannsson et al[32] in which abdominally obese subjects were treated with GH, the reduction of AT-LPL activity (29% reduction after 6 weeks) was generally less versus our own studies (reduction of LPL by about 50%). Moreover, after treatment for 9 months, Johannsson et al found an increment of 37% in AT-LPL activity, but the absolute level of AT-LPL was not significantly different from the level in the placebo group. These latter findings may suggest that the inhibitory effect of GH on AT-LPL activity may diminish or even revert after prolonged treatment periods with GH. However, in another study, we found that after 4 months' GH treatment of GH-deficient adults, GH still was associated with a pronounced reduction in AT-LPL activity (to be published).

GH treatment is generally associated with enhanced fatty acid oxidation, and this could be due to enhanced uptake of FFA in muscle tissue, perhaps mediated by enhanced M-LPL activity. However, in the present study, we found no indication of a stimulatory effect of GH on LPL activity or LPL gene expression in biopsies from skeletal muscle. On the contrary, a small reduction (20%) of M-LPL activity was found after GH treatment. Our group[14] and others[17] have previously found that high M-LPL activity is closely related to the level of triglyceride (low) and particularly HDL cholesterol (high). In the present study, HDL cholesterol was significantly reduced after GH treatment, which might be related to the lower M-LPL activity. Thus, in contrast to, for example, insulin and catecholamines, GH does not exert opposite effects on LPL activity in adipose and muscle tissue. Thus, the GH effects on substrate partitioning between muscle and adipose tissue seem not to involve the LPL pathway.

Infusion of GH is generally associated with enhanced triglyceride breakdown and enhanced plasma FFA after a lag period of about 2 hours.[7] The biochemical mechanisms involved in this lipolytic effect of GH have not yet been clarified, but several mechanisms have been suggested,[7] and among them is the finding that GH stimulates the expression of HSL in adipose cells.[8] HSL activity measured in the present study in adipose tissue was variable (Table 2). However, a significant increment of HSL activity after the VLCD was found in both groups in adipose tissue, indicating that weight loss is associated with higher HSL activity. However, GH treatment has no additional effect on HSL activity together with the VLCD. This is in accordance with a study in which rat adipocytes were incubated with GH and no effects on HSL mRNA were found[33] and another study in which lipolysis was shown to be similar in adipocytes from GH-treated subjects and placebo-treated subjects.[34] Comparing HSL activity with HSL mRNA in adipose tissue (Tables 2 and 3), some peculiar results arose whereby weight loss was associated with small increments of HSL activity and simultaneous decrements of HSL gene expression by 30% to 40%. These findings may suggest that the regulation of HSL enzyme activity may also partly occur at a posttranslational level.

The increment of FFA was similar after the VLCD in the 2 groups. As already mentioned, this is in contrast to infusion or treatment studies with GH along with an isocaloric diet where GH generally has clear lipolytic properties. The lack of a lipolytic effect of GH in the present study may be due to the

possibly maximally stimulated lipolysis induced by the hypocaloric diet.

In conclusion, GH significantly inhibited AT-LPL activity together with a VLCD-induced weight loss, but this reduced LPL activity was not related to any excess loss of FM. GH had no opposite effects on the activity of LPL in adipose tissue and muscle, since GH treatment reduced them both (by 65% and 21%). The VLCD-induced weight loss was associated with an enhanced expression and activity of AT-HSI, but GH had no effect on HSL along with the VLCD. VLCD treatment induced a pronounced lipolytic effect with high FFA levels, and in this situation GH had no additional lipolytic effect. Thus, concerning the effects on body weight, FM, and lipolytic activity, supplementary treatment with GH along with a VLCD offers no extra benefits during a 4-week study. However, the possible positive effects of GH in association with the VLCD in relation to the preservation of lean body mass, protein turnover, and bone turnover remain to be investigated.

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