Expression of insulin target genes in skeletal muscle and adipose tissue in adult patients with growth hormone deficiency: effect of one year recombinant human growth hormone therapy

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Abstract

Our aim was to investigate the effects of one year recombinant human growth hormone (rhGH) therapy on the regulation by insulin of gene expression in muscle and adipose tissue in adults with secondary GH deficiency (GHD). Six GHD subjects without upper-body obesity were submitted to a 3-h euglycemic hyperinsulinemic clamp before and after one year of rhGH therapy. Muscle and abdominal subcutaneous adipose tissue biopsies were taken before and at the end of each clamp. The mRNA levels of insulin receptor, p85 α -phosphatidylinositol-3 kinase (p85 α PI-3K), insulin dependent glucose transporter (Glut4), hexokinase II, glycogen synthase, lipoprotein lipase (LPL) in muscle and in adipose tissue, hormone sensitive lipase and peroxisome proliferator-activated receptor γ (PPAR γ) in adipose tissue were quantified by RT-competitive PCR. One year treatment with rhGH (1.25 IU/day) increased plasma IGF-I concentrations (54 ± 7 vs 154 ± 11 ng/ml, $P < 0.01$) but did not affect

insulin-stimulated glucose disposal rate measured during the hyperinsulinemic clamp (74 ± 9 vs 85 ± 5 μ mol/kg free fat mass/min). Insulin significantly increased p85 α PI-3K, hexokinase II and Glut4 mRNA levels in muscle both before and after rhGH treatment. One year of GH therapy increased LPL mRNA levels in muscle (38 ± 2 vs 70 ± 7 amol/ μ g total RNA, $P < 0.05$) and in adipose tissue (2490 ± 260 vs 4860 ± 880 amol/ μ g total RNA, $P < 0.05$), but did not change the expression of the other mRNAs. We conclude from this study that GH therapy did not alter whole body insulin sensitivity and the response of gene expression to insulin in skeletal muscle of adult GHD patients, but it did increase LPL expression in muscle and adipose tissue. This result could be related to the documented beneficial effect of GH therapy on lipid metabolism.

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Introduction

Insulin sensitivity has been reported to be impaired in some hypopituitary patients with growth hormone deficiency (GHD) who receive replacement therapy for other pituitary deficits (O'Neal *et al.* 1994, Hew *et al.* 1996, Hwu *et al.* 1997, Carroll *et al.* 1998, Christopher *et al.* 1998, Alford *et al.* 1999). Using the euglycemic hyperinsulinemic clamp methodology, some investigators have found a reduction in insulin-stimulated glucose utilization in GHD patients compared with matched control subjects (Hew *et al.* 1996, Christopher *et al.* 1998). However, it has also been reported that in adults with GHD, glucose turnover is normal when expressed per lean body mass (Salomon *et al.* 1994). It is also important to note that reduced insulin sensitivity was mainly observed

in obese GHD patients (Page *et al.* 1994), suggesting that obesity, rather than GHD *per se*, might be responsible for insulin resistance. In addition to obesity, other factors associated with GHD may contribute to alterations in insulin sensitivity such as increased visceral fat mass (Ho *et al.* 1996), sedentariness (Jorgensen *et al.* 1996) and the doses of thyroxine (Jap *et al.* 1989) and corticosteroids (Horber *et al.* 1991) used during replacement therapy.

The effect of growth hormone (GH) replacement on insulin sensitivity is also controversial. Several studies have shown that insulin sensitivity is either reduced (Beshyah *et al.* 1995, Weaver *et al.* 1995), or unchanged (Fowelin *et al.* 1993, Christopher *et al.* 1998, Bulow & Erfurth 1999) during recombinant human (rh) GH treatment. This reduction may be a transitory short-term phenomenon, as long-term treatment did not modify insulin

Table 1 Individual characteristics of GHD patients and effect of GH treatment on plasma IGF-I concentration

Patient no.	Replacement therapy	Sex	Age (yr)	Years after diagnosis	GH dose (IU/day)	IGF-I (µg/l)	
						– GH	+ GH
1	CS+GS	F	33	8	2	55	164
2	CS+GS+T	F	27	4	1	44	130
3	CS+GS+T	F	39	5	1	49	119
4	CS+GS+T	M	50	3	1.5	84	183
5	CS+GS	M	39	1	2	59	183
6	GS	M	45	14	0.5	32	146

CS, corticosteroids; GS, gonadal steroids; T, thyroxine; – GH, at baseline; +GH, after 12 months of GH treatment. IGF-I (µg/l): normal values for age (yr) (Kratzsch *et al.* 1993): 20–30=195; 31–40=153; 41–50=120.

sensitivity (Fowelin *et al.* 1993, Hew *et al.* 1996). In one study, however, a normalization of insulin sensitivity was observed after 1 year of rhGH treatment in insulin-resistant adult GHD subjects (Hwu *et al.* 1997). It has been proposed that this effect could be mainly related to the reduction of total body fat (Hwu *et al.* 1997).

Few studies have investigated the possible mechanisms of the proposed alteration in insulin sensitivity in GHD. It has recently been reported that in adult GHD patients with decreased insulin-mediated glucose utilization, a reduction of glycogen synthase activity in the muscle may be implicated in the impaired insulin action (Hew *et al.* 1996, Christopher *et al.* 1998). The same group also demonstrated that 1 year of GH replacement therapy did not restore this defect (Christopher *et al.* 1998).

In the present study, as part of a large multicenter rhGH trial in GHD (Borson-Chazot *et al.* 1999), we have determined the expression profile of eight genes coding for proteins involved in insulin action in skeletal muscle and adipose tissue and have studied the regulation of the expression of these genes by insulin using the euglycemic hyperinsulinemic clamp method (DeFronzo *et al.* 1979). This molecular approach has been performed to characterize GHD patients and the effect of GH replacement therapy on insulin sensitivity at the gene expression level. We have aimed to verify whether the regulation by insulin of gene expression may be altered in GHD patients in a similar way as has been reported in other groups of insulin-resistant subjects such as type 2 diabetic patients (Andersen *et al.* 1993, Andreelli *et al.* 1999).

Subjects and Methods

Subjects

Six patients (3 men and 3 women), participating in a multicentric therapeutic trial on rhGH (Pharmacia, Stockholm, Sweden), were enrolled in the present study

(Table 1). These patients were GH deficient because of pituitary disorders (pituitary adenoma in five patients and craniopharyngioma in one). All presented a multiple pituitary deficit and had been on stable replacement therapy for at least 2 years. None of these subjects had been previously treated with GH or for acromegaly. The criterion for the diagnosis of GHD was a GH peak below 3 µg/l during an insulin-induced hypoglycemia test (glycemia <2.2 mmol/l). In the six investigated patients the GH peak was below 1 µg/l. A second GH stimulating test (glucagon-propranolol or GH-releasing hormone) confirmed the diagnosis for each patient. Both tests were performed after the age of 20 years and within the 5 years preceding the inclusion in the study. The basal plasma insulin-like growth factor-I (IGF-I) concentrations of the subjects were lower than normal age-adjusted values (Table 1 and Kratzsch *et al.* 1993). None of the subjects had a personal history of diabetes or hypertension. Their ages ranged from 27 to 50 years (Table 1). Their mean body mass index (BMI) was 24.6 kg/m², with a waist-to-hip ratio lower than 0.80 for the women and 0.95 for the men (Table 1) indicating that none of the subjects was characterized by upper-body obesity.

Study design

The study was approved by the Ethical Committee of Hospices Civils de Lyon (France) and was performed in accordance with the Helsinki guidelines. The objective of the study was explained to the patients and their written consent was obtained. The subjects were treated by daily subcutaneous injection of rhGH (Genotonorm, Pharmacia) at a dose that was adapted on an individual basis so as to maintain IGF-I concentrations in the 95 percentile of the normal range using an age-adjusted reference (legend of Table 1 and Kratzsch *et al.* 1993). For each patient the GH dose that was used is indicated in Table 1 and never exceeded 2 IU/day (range 0.5–2 IU/day).

A euglycemic hyperinsulinemic clamp to assess insulin sensitivity, and tissue biopsies to study the regulation of specific mRNA expression, were performed before (– GH) and after (+GH) one year of rhGH treatment. In addition, body composition was estimated by bioelectric impedancemetry (BIA 101, Eugedia, Paris, France) before and after one year of rhGH treatment.

Euglycemic hyperinsulinemic clamp study

All studies were performed in the morning after an overnight fast and at least 10 h after the last GH injection. Basal glucose turnover rate was determined by dilution tracer methodology using a primed 6,6 [²H₂] glucose (Eurisotop, St Aubain, France) infusion (0.11 µmol/kg per min) for 2 h as described previously (Laville *et al.* 1996). Then a 3-h euglycemic hyperinsulinemic clamp was started by the infusion of insulin (Actrapid Novo, Copenhagen, Denmark) at a rate of 450 pmol/m² per min (Laville *et al.* 1996). Any decrease in blood glucose was prevented by adapted infusion of 20% glucose solution (Aguettant, Lyon, France).

Tissue biopsies

Muscle samples (43 ± 3 mg wet wt, *n*=24) were taken under local anesthesia by per cutaneous biopsies of the *vastus lateralis* muscle using Weil Blakesley pliers as previously described (Laville *et al.* 1996). Abdominal subcutaneous adipose tissue was aspirated from the periumbilical area through a 2.3 mm (13 gauge) needle under local anesthesia (Vidal *et al.* 1996). To study the regulation of gene expression by insulin (Laville *et al.* 1996, Andreelli *et al.* 1999), both muscle and adipose tissue biopsies were taken before the clamp and after the 3 hours of the euglycemic hyperinsulinemic clamp. Tissue samples were immediately frozen in liquid nitrogen and stored at – 80 °C until analysis. For one subject (#2), biopsies were obtained only in the basal state, both before and during GH therapy.

Indirect calorimetry

To estimate lipid and glucose oxidation rates, respiratory exchange measurements were carried out during the last 30 min of the basal period and during the last hour of the euglycemic hyperinsulinemic clamp. Measurements were made using a computerized follow-through canopy gas-analyzer system (Deltatrac Metabolic Monitor, Datex, Helsinki, Finland). Total carbohydrate and lipid oxidation rates were calculated according to the published equations (Ferrannini 1988).

Analytical procedures

For the determination of metabolite and hormone concentrations and of 6,6 [²H₂] glucose isotopic enrichment,

blood samples were drawn every 10 min during the last 30 min of the basal period and during the last hour of the hyperinsulinemic clamp. Plasma metabolites were measured using enzymatic methods and C-peptide and insulin concentrations using radioimmunoassays. Plasma isotopic enrichment of 6,6 [²H₂] glucose was determined by gas chromatography-mass spectrometry (5971 MSD, Hewlett-Packard, Paolo Alto, CA, USA) as described (Beylot *et al.* 1986). Basal glucose turnover rate was calculated using steady-state equations.

Total RNA preparation

Frozen muscle samples were powdered in liquid nitrogen, and total RNA was prepared using guanidium thiocyanate and phenol/chloroform extraction followed by alcohol precipitation (Chomczynski & Sacchi 1987). The preparation of total RNA from adipose tissue was performed using the RNeasy mini kit (Qiagen, Courtaboeuf, France). The mean size of fat tissue that was used for total RNA preparation was 90 ± 4 mg wet wt (*n*=24). Yields of total RNA were 35 ± 3 µg/100 mg wet wt muscle and 1.4 ± 0.1 µg/100 mg wet wt adipose tissue, and were similar to samples taken before or after the growth hormone therapy. The 260 nm to 280 nm absorption ratios were always between 1.8 and 2.1. Total RNA solutions were stored at – 80 °C.

Quantification of messenger RNAs

The mRNA concentrations of insulin receptor (IR), p85α-phosphatidylinositol-3 kinase (p85αPI-3K), insulin dependent glucose transporter (Glut4), hexokinase II (HKII), glycogen synthase (GS) and lipoprotein lipase (LPL) in skeletal muscle and in adipose tissue, hormone sensitive lipase (HSL) and peroxisome proliferator-activated receptor γ (PPARγ) in adipose tissue were quantified by reverse transcription followed by competitive polymerase chain reaction (RT-cPCR) (Auboeuf & Vidal 1997). The conditions of the assays, the sequences of the primers and the validation of the method have been described previously (Laville *et al.* 1996, Auboeuf *et al.* 1997, Andreelli *et al.* 2000). To accurately determine the effect of rhGH treatment on gene expression, total RNA of the four muscle or adipose tissue biopsies from the same individual were prepared simultaneously, and the assays of a given mRNA were made in parallel, in the same run of PCR with the same working solutions of competitors. The results are expressed in amol/µg total RNA.

Statistical analysis

Nonparametric Wilcoxon's test for paired values was used when comparing mRNA levels before and during rhGH

Table 2 Effect of GH treatment on body composition

	Weight (kg)		BMI (kg/m ²)		Body fat (%)		W/H ratio	
	– GH	+GH	– GH	+GH	– GH	+GH	– GH	+GH
Patient no.								
1	49	47	19.1	18.6	27	26	0.80	0.77
2	60	63	24.7	25.9	38	39	0.80	0.83
3	62	64	22.2	22.9	34	35	0.70	0.75
4	98	98	31.3	31.3	30	29	0.92	0.93
5	70	64	21.8	19.6	29	25	0.82	0.81
6	89	90	29.1	29.4	40	38	0.93	0.94
Mean ± S.E.M.	71.3 ± 7.6	69.3 ± 8.2	24.7 ± 1.9	24.6 ± 2.1	33 ± 2	32 ± 2	0.83 ± 0.04	0.84 ± 0.03

– GH, at baseline; +GH, after 12 months of GH treatment; W/H, waist-to-hip ratio.

treatment or before and during hyperinsulinemic clamp. The threshold for significance was set at $P < 0.05$. Data in text and figures are presented as means ± S.E.M.

Results

Effect of GH replacement on anthropometric, hormonal and metabolic parameters

As classically observed, the six GHD subjects were characterized by higher body fat mass (Table 2) than the theoretical values that can be calculated from their sex, age and BMI ($33 \pm 2\%$ vs $23 \pm 2\%$). Before rhGH treatment, the patients had normal plasma concentrations of glucose, insulin, non-esterified fatty acids (NEFA) and triglyceride (Table 3) when compared with our previously reported values for age-matched healthy subjects (Laville *et al.* 1996, Andreelli *et al.* 1999). Whole body glucose disposal rate both in the basal state and during the hyperinsulinemic clamp was normal when referred to the free fat mass (FFM) (Table 3) or the body surface area of the subjects (420 ± 30 and $2160 \pm 180 \mu\text{mol}/\text{m}^2$ per min, basal and during the clamp respectively). In independent studies with healthy lean subjects but using the same hyperinsulinemic clamp method (Andreelli *et al.* 1999, 2000), we have found values of $460 \pm 30 \mu\text{mol}/\text{m}^2$ per min for the basal period and $1900 \pm 150 \mu\text{mol}/\text{m}^2$ per min during the clamp. After one year of rhGH treatment, a threefold increase in plasma IGF-I concentrations was observed (54 ± 7 vs $154 \pm 11 \mu\text{g}/\text{l}$, $P < 0.01$, Table 1). There was no change in body weight, percentage of body fat or waist-to-hip ratio (Table 2) during treatment. After an overnight fast, glycemia, insulinemia, C-peptide (1.2 ± 0.5 vs $1.0 \pm 0.4 \mu\text{g}/\text{l}$, after vs during rhGH treatment), NEFA, triglyceride and β -hydroxybutyrate concentrations were unchanged by rhGH treatment (Table 3). Glucose turnover rates (Table 3), glucose oxidation rates (8.9 ± 2 vs $9.8 \pm 0.7 \mu\text{mol}/\text{kg}$ FFM/min, before vs during treatment), lipid oxidation rates (1.1 ± 0.2 vs $1.1 \pm 0.1 \mu\text{mol}/$

kg FFM/min, before vs during treatment) and protein oxidation rates (1.0 ± 0.1 vs $0.8 \pm 0.1 \mu\text{mol}/\text{kg}$ FFM/min, before vs during treatment) were also not modified. Resting metabolic rate was the same before and during rhGH treatment (1490 ± 130 vs 1500 ± 140 kcal/day, before vs after rhGH). Finally, one year of rhGH treatment did not significantly modify insulin-stimulated glucose disposal rates and the different metabolic parameters that were measured during the euglycemic hyperinsulinemic clamp (Table 3).

Table 3 Metabolic and hormonal variables before (Basal) and during the last 30 min of the euglycemic hyperinsulinemic clamp (Infusion), at baseline (– GH) and after 12 months of GH treatment (+GH). Glucose turnover rate was determined at basal and glucose infusion rate during euglycemic hyperinsulinemic clamp, both at – GH and +GH periods

	– GH	+GH
Insulinemia (pmol/l)		
Basal	31 ± 6	36 ± 9
Infusion	1412 ± 161*	1215 ± 131*
Glycemia (mmol/l)		
Basal	4.7 ± 0.3	4.9 ± 0.2
Infusion	4.7 ± 0.2	4.7 ± 0.4
Triglyceride (μmol/l)		
Basal	730 ± 140	780 ± 104
Infusion	540 ± 107	578 ± 66
NEFA (μmol/l)		
Basal	430 ± 32	380 ± 52
Infusion	43 ± 6*	36 ± 6*
β -Hydroxybutyrate (μmol/l)		
Basal	75 ± 16	94 ± 20
Infusion	25 ± 3*	28 ± 6*
Glucose clamp study		
Basal glucose turnover rate (μmol/kgFFM/min)	16 ± 1	17 ± 1
Glucose infusion rate (μmol/kgFFM/min)	85 ± 5	74 ± 9

* $P < 0.001$ infusion vs basal.

Figure 1 Expression profiles of the target mRNAs in tissues of GHD patients. Concentrations of the target mRNAs were determined by RT-cPCR in *vastus lateralis* muscle and in abdominal subcutaneous adipose tissue before (open bars) and after (closed bars) 12 months of rhGH treatment. Data are means $\pm$ S.E.M., $n=6$; * $P=0.046$ and ** $P=0.027$. Values of mRNA levels (mean $\pm$ S.D.) determined with the same methodology in tissues of 9 healthy lean control subjects (Lefebvre *et al.*, 1998, Andreelli *et al.*, 2000) were: 14 ± 5 amol/ μ g total RNA for IR; 10 ± 3 for Glut4; 6 ± 3 for p85 α PI-3K; 1 ± 0.7 for HKII; 333 ± 131 for GS; 44 ± 16 for LPL in muscle and 1370 ± 160 for LPL in adipose tissue; 460 ± 100 for HSL; and 42 ± 8 for PPAR γ .

Effect of rhGH replacement on skeletal muscle and adipose tissue gene expression

The absolute levels of the target mRNAs, determined by RT-cPCR, in total RNA preparations from *vastus lateralis* muscle and from abdominal subcutaneous adipose tissue are shown in Fig. 1. In the basal state, after an overnight fast, the expression profiles of the mRNAs were similar to those we have previously found in control subjects using the same RT-cPCR based assays (legend of Fig. 1 and Lefebvre *et al.* 1998, Andreelli *et al.* 2000). In skeletal muscle, glycogen synthase mRNA was expressed at the highest level whereas HKII mRNA level was low. The mRNA levels of p85 α PI-3K seemed lower in GHD patients (2.1 ± 0.7 amol/ μ g total RNA) than in healthy control subjects (6 ± 3 amol/ μ g total RNA) (Andreelli *et al.* 2000). In adipose tissue, LPL was expressed at the highest levels (Fig. 1). HSL mRNA levels seemed to be higher in GHD patients (914 ± 87 amol/ μ g total RNA) than in healthy control subjects (460 ± 100 amol/ μ g total RNA) (Lefebvre *et al.* 1998). After one year of rhGH

treatment, the expression levels of IR (8.4 ± 1.4 vs 12 ± 3 amol/ μ g total RNA), Glut4 (12 ± 2 vs 10 ± 1 amol/ μ g total RNA), p85 α PI-3K (2.1 ± 0.7 vs 1.6 ± 0.2 amol/ μ g total RNA), HKII (0.7 ± 0.2 vs 0.8 ± 0.2 amol/ μ g total RNA) and GS (397 ± 40 vs 340 ± 77 amol/ μ g total RNA) were not modified in skeletal muscle. In contrast, LPL gene expression was significantly increased by rhGH treatment (48 ± 11 vs 79 ± 15 amol/ μ g total RNA, $P=0.046$) (Fig. 1). In adipose tissue also, GH replacement produced a marked increase in LPL mRNA levels (2270 ± 216 vs 4712 ± 802 amol/ μ g total RNA, $P=0.027$) without affecting HSL (914 ± 87 vs 951 ± 159 amol/ μ g total RNA) and total PPAR γ (46 ± 8 vs 43 ± 7 amol/ μ g total RNA) expression (Fig. 1). The changes in LPL mRNA expression upon rhGH treatment were not associated with modification in plasma triglyceride or NEFA concentrations (data not shown).

Regulation of muscle gene expression by insulin in GHD patients before and during rhGH treatment

We and others have demonstrated that insulin upregulates the expression of p85 α PI-3K (Laville *et al.* 1996, Andreelli *et al.* 1999), Glut4 (Schalin-Jantti *et al.* 1994, Laville *et al.* 1996) and HKII (Pendergrass *et al.* 1998) in human skeletal muscle. We therefore investigated whether the response of these three genes to short-term insulin infusion was altered in GHD patients. Figure 2 clearly shows that insulin infusion increased the mRNA levels of p85 α PI-3K ($P=0.043$), Glut4 ($P=0.079$) and HKII ($P=0.043$) in the muscle of GHD patients. The mRNA levels of Glut4 clearly increased in four subjects and did not change in one. The response to insulin of the three mRNAs was also found after one year of rhGH treatment ($P=0.043$, Fig. 2).

Discussion

We have investigated the effect of one year rhGH replacement therapy on the regulation by insulin of the expression of specific key genes in muscle and adipose tissue in a group of adult GHD patients. Growth hormone deficiency has been associated with insulin resistance in some adult patients (O'Neal *et al.* 1994, Hew *et al.* 1996, Hwu *et al.* 1997, Carroll *et al.* 1998, Christopher *et al.* 1998, Alford *et al.* 1999). However, depending on the studied populations and also on the method used to estimate insulin sensitivity, the presence of insulin resistance in adult GHD is still a controversial issue. Indeed, some authors have found a reduction (O'Neal *et al.* 1994, Hew *et al.* 1996, Hwu *et al.* 1997, Christopher *et al.* 1998, Alford *et al.* 1999), while others have found a normal (Page *et al.* 1994, Salomon *et al.* 1994) insulin sensitivity in GHD patients. Obesity, increased truncal fat mass, hormonal replacement therapy and other confounding factors may influence insulin sensitivity, and also unravel a putative

Figure 2 Insulin-induced changes in the expression levels of p85 α PI-3K, Glut4 and HKII in skeletal muscle of GHD patients. The concentrations of the target mRNA was measured in the basal state (Basal) and at the end of a 3-h hyperinsulinemic euglycemic clamp (Infusion) both before (A) and during (B) rhGH treatment. After clamp biopsies were not available for one patient.

direct role of the deficiency in GH. Nevertheless, because insulin resistance is an important cardiovascular risk factor (Reaven 1988) and because GHD patients are at high risk for cardiovascular disease (Rosen & Bengtsson 1990), it is of great importance to define clearly whether insulin action is impaired in GHD patients and whether replacement therapy with GH affects insulin response *in vivo*.

We have investigated GHD subjects with waist-to-hip ratios lower than 0.80 for the women and 0.95 for the men to limit the influence of upper-body obesity as a confounding factor for insulin resistance (Krotkiewski *et al.* 1990, Seidell *et al.* 1990). In this group of patients, fasting plasma concentrations of insulin, C-peptide, glucose, NEFA and triglycerides were normal when compared with data obtained in age-, sex- and BMI-matched healthy subjects (Laville *et al.* 1996, Andreelli *et al.* 1999, 2000). In addition, using the gold standard for measuring insulin action, the euglycemic hyperinsulinemic clamp method, we found that these patients had a normal insulin sensitivity of whole-body glucose metabolism. This result

contrasted with most of the previous reports (O'Neal *et al.* 1994, Hew *et al.* 1996, Hwu *et al.* 1997, Christopher *et al.* 1998, Alford *et al.* 1999) that showed a reduced insulin sensitivity in different groups of adult GHD. One could thus suggest that upper-body obesity may be one of the causal parameters of insulin resistance in GHD (Page *et al.* 1994). Further studies are needed to verify this hypothesis by comparing insulin sensitivity in adult GHD patients with and without obesity.

One year of rhGH replacement therapy, at an average dose of 1.25 IU/day, significantly increased plasma IGF-I concentrations to normal levels. In a larger cohort, we have previously reported that the same rhGH treatment has beneficial effects on intima-media thickness and on plasma lipid and lipoprotein profile and was associated with a significant reduction in the fat mass of the subjects (Borson-Chazot *et al.* 1999). Here, the anthropometric parameters of the six GHD patients were not significantly affected after one year of rhGH treatment although it should be noted that four out of the six patients showed a slight decrease in percentage body fat, but the difference did not reach significance. Regarding the effects of rhGH treatment on the parameters of glucose metabolism, neither fasting plasma glucose, nor insulin or c-peptide concentrations, nor insulin-stimulated glucose disposal rate during the clamp were modified after one year of rhGH treatment. These results indicated, however, that rhGH therapy, at the low doses that were used, has no deleterious effect on glucose metabolism.

Little is known about the influence of GH deficiency and GH replacement therapy on gene expression in muscle and adipose tissue. It has recently been reported that insulin-resistant GHD patients show a reduced stimulation by insulin of skeletal muscle glycogen synthase activity (Hew *et al.* 1996, Christopher *et al.* 1998), a defect which is reminiscent of that observed in insulin-resistant type 2 diabetic patients (Damsbo *et al.* 1991). This defect was not corrected by rhGH treatment (Christopher *et al.* 1998). In the present study we report, for the first time, the expression profiles of eight genes coding important metabolic enzymes and proteins in skeletal muscle and in adipose tissue of GHD patients. The mRNA levels of p85 α PI3-K seemed to be reduced in GHD muscle (Andreelli *et al.* 2000) and the expression of the HSL seemed to be higher in GHD adipose tissue than in controls (Lefebvre *et al.* 1998). However, because the quantitation of the target mRNAs were not made at the same time and in parallel runs of RT-cPCR in GHD and in control subjects, and also because the number of GHD patients was low, we preferred not to draw any definite comparative conclusions. We may, however, conclude that there was no major abnormality in the relative levels of the different target mRNAs in the tissues of GHD when compared with those we have previously reported in healthy lean control subjects (Lefebvre *et al.* 1998, Andreelli *et al.* 2000). Interestingly, one year of rhGH

treatment increased significantly the expression of LPL mRNA both in muscle and in adipose tissue without affecting the expression of genes involved in insulin action in muscle or HSL and PPAR γ mRNA levels in adipose tissue.

Adipose tissue and skeletal muscle are the main sources of LPL, a key enzyme in the regulation of the flux of fatty acids. Numerous studies have demonstrated that GH can participate in the regulation of LPL expression and activity (Richelsen 1999). In rats, hypophysectomy reduced LPL activity in muscle, and rhGH treatment increased LPL activity in heart and in gastrocnemius muscle (Oscarsson *et al.* 1999). It has also been demonstrated that GH can stimulate LPL gene transcription *in vitro* in a time- and dose-dependent manner (Francis *et al.* 1993). However, in obese subjects with (Oscarsson *et al.* 1996) or without (Richelsen *et al.* 1994) GHD, treatment with GH resulted in a reduction in adipose tissue LPL activity without a change in mRNA levels. In obese GHD patients treated for 4 months with GH, Richelsen (1999) found no effect on skeletal muscle LPL activity or mRNA level. This discrepancy with our findings may result from differences in patient selection (obese vs nonobese GHD), in the doses of GH used (markedly higher in Oscarsson *et al.* 1996 and Richelsen *et al.* 1994 than in our work) as well as in the associated treatments that can affect LPL gene regulation (Fried *et al.* 1993). Despite the increased LPL mRNA expression, triglyceride concentrations did not change significantly during rhGH treatment in the investigated group of patients. This result suggests that the observed upregulation of LPL mRNA may not be associated with an increase in LPL activity in the blood vessels. Further investigations are thus required to verify whether the change in LPL expression could have a role in the previously documented beneficial effect of rhGH treatment on lipoprotein metabolism (Carroll *et al.* 1998, Christopher *et al.* 1998).

In insulin-resistant type 2 diabetes mellitus patients, defects in the acute regulation by insulin of the expression of some important genes have been documented in skeletal muscle (Andersen *et al.* 1993, Andreelli *et al.* 1999). We found in this work that insulin up-regulates p85 α PI3-K, Glut4 and HKII mRNA expression in the muscle of GHD patients. One year of treatment with rhGH did not modify the expression of these three genes in response to insulin infusion. Therefore, the regulation by insulin of important genes is not altered in the skeletal muscle of GHD patients.

In conclusion, our study suggests that adult GHD patients without upper-body obesity are not insulin resistant and shows that they have a normal response of gene expression to insulin in skeletal muscle. Growth hormone treatment (1.25 IU/day) for one year, which increases plasma IGF-I levels, induces LPL mRNA expression both in skeletal muscle and in subcutaneous adipose tissue without affecting whole body insulin sensitivity and the response of gene expression to insulin in muscle.

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