Individualized Low-Dose Growth Hormone (GH) Treatment in GH-Deficient Adults With Childhood-Onset Disease: Metabolic Effects During Fasting and Hypoglycemia

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Growth hormone (GH) has insulin-antagonistic effects, and GH secretion is augmented during fasting and hypoglycemia. In the present study, 10 patients aged 21 to 28 years with childhood-onset GH deficiency (GHD) were studied during a 24-hour fast and a hypoglycemic glucose clamp before and after 9 months of GH replacement. During the 24-hour fast, blood glucose, serum insulin, and serum free fatty acid (FFA) levels were measured. In the hypoglycemic clamp, the counterregulatory hormones (plasma catecholamines, serum glucagon, and serum cortisol), serum insulin-like growth factor (IGF) binding protein-1 (IGFBP-1), serum FFA, and glucose uptake were measured. The GH dose was adjusted to the response of serum IGF-I, and the median GH dose was 0.14 IU/kg/wk (range, 0.08 to 0.19). At the end of the study, serum IGF-I levels were normalized in all but one patient, in whom serum IGF-I was above the normal range. Nine months of GH treatment did not cause any significant changes in the blood glucose level, insulin to glucose ratio, or serum FFA level during the 24-hour fast, and none of the patients experienced hypoglycemia either before or after GH treatment. However, GH therapy resulted in increased insulin resistance during hypoglycemia, without changes in the counterregulatory hormonal responses, serum IGFBP-1, or serum FFA.

GROWTH HORMONE (GH) has insulin-antagonistic effects and is normally released in response to stress, fasting, and a rapid decline in blood glucose.[1,2] Children with GH deficiency (GHD) have an increased risk of fasting hypoglycemia,[3-5] but with increasing age and height, the risk of hypoglycemia appears to be reduced.[3,6] Recently, it was suggested that adults with childhood-onset versus adult-onset GHD were somatically underdeveloped,[7] being shorter and having a lower lean body mass since glucose turnover is correlated with lean body mass,[8] it would be of interest to investigate whether adults with childhood-onset GHD have low blood glucose levels during fasting. In adult-onset GHD patients without GH substitution compared with normal subjects, changes in glucose and fuel metabolism have been documented,[8] but in patients with childhood-onset GHD, corresponding studies have not been performed.

GH replacement to adult GHD patients resulted in an increase in insulin resistance[9] and overnight fasting blood glucose levels,[10-12] possibly through the strong lipolytic effect of GH to cause an increase in free fatty acids (FFAs), which inhibit insulin-stimulated peripheral glucose uptake.[13] In GHD patients, the duration of GH treatment seems to influence the increase in FFA levels,[9,14] and probably also the GH dose. Furthermore, fasting is shown to increase the lipolytic responsiveness to GH in healthy subjects.[15] Hitherto, the effect of GH substitution on FFA levels during more prolonged fasting has only been investigated in GHD dwarfs.[16]

Maintenance of a normal glucose concentration is crucial to survival, and a rapid decrease in blood glucose causes the release of counterregulatory hormones: glucagon, catecholamines, cortisol, and GH.[17] Following hypoglycemia, there is also a rapid increase in serum insulin-like growth factor binding protein-1 (IGFBP-1),[18] which modulates the free fraction of insulin-like growth factor-I (IGF-I),[19] suggesting that IGFBP-1 has a counterregulatory role in glucose homeostasis.[20] GH prevents hypoglycemia via its insulin-antagonistic effects mainly in the peripheral tissues,[21] and it has been shown that subjects with GHD recover more slowly from hypoglycemia.[22] However, it is not known whether the effect of GH during hypoglycemia is a direct effect or an indirect effect, eg, via changes in counterregulatory hormonal responses or FFA levels.

The aim of the present study was to assess whether young adults with childhood-onset GHD have a risk of fasting hypoglycemia, as well as to determine if individualized GH replacement increases blood glucose, serum insulin, and serum FFA levels during fasting or influences insulin resistance during hypoglycemic conditions.

SUBJECTS AND METHODS

Patients

Ten patients (eight men and two women) with childhood-onset GHD were studied. Three patients had isolated GHD, and seven had multiple pituitary deficiencies. Patient characteristics are shown in Table 1. All patients were treated with GH during childhood. The median time of GH therapy discontinuation before the study was 7 years (range, 5 to 10). When required, the patients received stable replacement therapy for at least 6 months before the study with glucocorticoids (cortisone acetate 10 to 30 mg/d), thyroid hormone (levothyroxine 0.1 to 0.2 mg/d), antidiuretic hormone analog (desmopressin 0.2 to 1.6 mg/d orally), and gonadal steroids (in men, testosterone enanthate 250 mg every 3 to 4 weeks intramuscularly). One of the female patients received treatment with conjugated estrogen (0.625 mg, days 1 to 21) plus medroxyprogesterone acetate (10 mg, days 11 to 21). The other female patient had no gonadal insufficiency but used oral contraceptives (levonorgestrel plus ethinylestradiol).

Study Design

The study used an open design. The patients were studied during a 24-hour fast and in a hypoglycemic clamp model before and after 9

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Table 1. Clinical and Endocrine Characteristics of Patients With Childhood-Onset GHD at Inclusion in the Study (N = 10)

Patient No.	Sex	Age (yr)	Diagnosis	Hormone Replacement	Height (cm)	Weight (kg)	BMI (kg/m²)	GH Peak During Hypoglycemia (mIU/L)
1	M	28	Craniopharyngioma	C/T/G/ADH	177	78	24.9	<0.1
2	M	27	Suprasellar cyst	C/T/G	167	101	36.2	<0.1
3	M	27	Prolactinoma	T/G	174	71	23.5	0.1
4	M	21	Optic glioma	—	162	70	26.7	1.6
5	M	26	Idiopathic hypopituitarism	C/T/G	172	70	23.7	<0.1
6	M	25	Craniopharyngioma	C/T/G/ADH	189	124	34.7	0.1
7	M	27	Craniopharyngioma	C/T/G/ADH	178	81	25.6	<0.1
8	M	21	Idiopathic GHD	—	161	87	33.6	<0.1
9	F	27	Idiopathic hypopituitarism	T/G	158	54	21.6	0.1
10	F	22	Idiopathic GHD	—	160	79	30.9	0.9
Median		27			170	79	26.2	0.1
Range		21-28			158-189	54-124	21.6-36.2	<0.1-1.6

Abbreviations: C, cortisone acetate; T, levothyroxine; G, gonadal steroids; ADH, antidiuretic hormone analog.

months of GH treatment. To adjust for the influence of gonadal steroids on insulin sensitivity,[23,24] the 24-hour fast and the hypoglycemic clamp were performed with the same time interval after a given injection of testosterone in men and in the same phase of the female gonadal hormone substitution. Serum levels of IGF-I and thyroid hormones and body composition were measured before and after 1.5, 3, 6, and 9 months of GH treatment. Before GH treatment, all patients but two had subnormal serum IGF-I (median, 51 μg/L; range, <20 to 172) (Table 2). After 9 months of GH treatment, serum IGF-I levels were normalized in nine patients and above the normal range in one (median, 275 μg/L; range, 148 to 506).

Replacement therapy with glucocorticoids, levothyroxine, and sex hormones was kept constant during the study in all patients except one (patient no. 5), in whom the levothyroxine dose was increased from 0.15 mg/d to 0.20 mg/d due to a low serum free thyroxine (T_4) concentration. Median serum thyrotropin (TSH) was 0.2 mU/L (range, <0.01 to 3.5) before GH treatment and 0.08 mU/L (range, <0.01 to 2.5) after 9 months of GH treatment. Median serum free T_4 and free triiodothyronine (T_3) before GH therapy were 14.6 pmol/L (range, 11.0 to 24.0) and 5.2 pmol/L (range, 4.2 to 6.3), respectively, and after 9 months of GH treatment, 15.5 pmol/L (range, 11.0 to 23.0) and 5.4 pmol/L (range, 3.5 to 8.4), respectively.

Table 2. Individual Final GH Dose and Changes in Serum IGF-I, Body Insulin Resistance, and Percentage Body Fat Mass in Patients With Childhood-Onset GHD After 9 Months of GH Treatment (N = 10)

Patient No.	Final GH Dose (IU/kg/wk)	Serum IGF-I Before/After GH (μg/L)	Change in Whole-Body Resistance Measured by BIA (%)	Change in Percentage Body Fat Mass (%)
1	0.18	35/231	−11.6	−1.0
2	0.10*	30/148	−10.7	−4.9
3	0.10†	140/372	−7.4	−3.2
4	0.14	172/506	−10.4	−4.2
5	0.14	82/231	−8.7	−2.3
6	0.09	38/331	+1.2	−2.5
7	0.17	<20/375	−9.7	−6.6
8	0.08	83/229	+0.2	−1.6
9	0.19	<20/313	−8.9	−2.6
10	0.18	63/236	−22.6	−5.5
Median	0.14	51/275	−9.3	−2.9
Range	0.08-0.19	<20-172/148-506	+1.2-−22.6	−1.0-−6.6

NOTE. The reference range for serum IGF-I for adults aged 21-28 years is 129-385 μg/L.

Abbreviation: BIA, bioelectric impedance analysis.

Dose reduction due to *edema and †arthralgia and edema.

The study protocol was approved by the Ethics Committee of Lund University.

Body Mass Index and Body Composition

The body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Body composition was measured in the supine position by bioelectric impedance analysis (BIA) using the BIA 101-S technique (RJL Systems, Detroit, MI). A 50-KHz, 800-μA current was applied.

24-Hour Fast

Before each fasting period (8 AM to 8 AM), the patients were served their ordinary breakfast and any medication was taken at the usual times. Blood glucose levels were measured at 10 AM, 2 PM, 6 PM, 10 PM, 12 midnight, and 2, 4, 5, 6, 7, and 8 AM. Serum levels of insulin and FFA were measured at 10 AM, 5 AM, and 8 AM. At the same time points, to adjust for differing glucose levels and to evaluate the degree of insulin resistance, the insulin to glucose ratio was calculated.

Hypoglycemic Clamp

The patients fasted since 8 PM the evening before the investigation. Before the start of the clamp at 8 AM, they took their ordinary morning medication. One vein was cannulated for blood sampling, and one vein was cannulated in the contralateral arm for infusion of insulin and glucose. Insulin was infused at a constant rate of 2.5 mIU/kg/min. By a graded infusion of 20% glucose, blood glucose was decreased for 46 to 80 minutes (mean, 64.8) to a nadir of 2.10 ± 0.10 mmol/L (phase A) (Fig 1). Blood glucose was then kept constant at a level of 2.28 ± 0.11 mmol/L for 45 minutes (phase B). After the 45-minute plateau phase, the insulin infusion was stopped. After 9 months of GH treatment, the hypoglycemic clamp was repeated. Glucose uptake (M) was calculated for each patient during phase A and phase B before and after GH treatment as the glucose infusion rate (I) + glucose space correction (S).[25] Plasma epinephrine and norepinephrine, serum cortisol (in patients without cortisol substitution), and serum glucagon levels were measured before the start of the clamp and after 45 minutes of hypoglycemia. Serum levels of IGFBP-1 and FFA were measured before the start of the clamp, at the start of phase B, and after 45 minutes of hypoglycemia.

GH Treatment

Biosynthetic human GH (Genotropin; Pharmacia & Upjohn, Stockholm, Sweden) was administered in the evening by subcutaneous

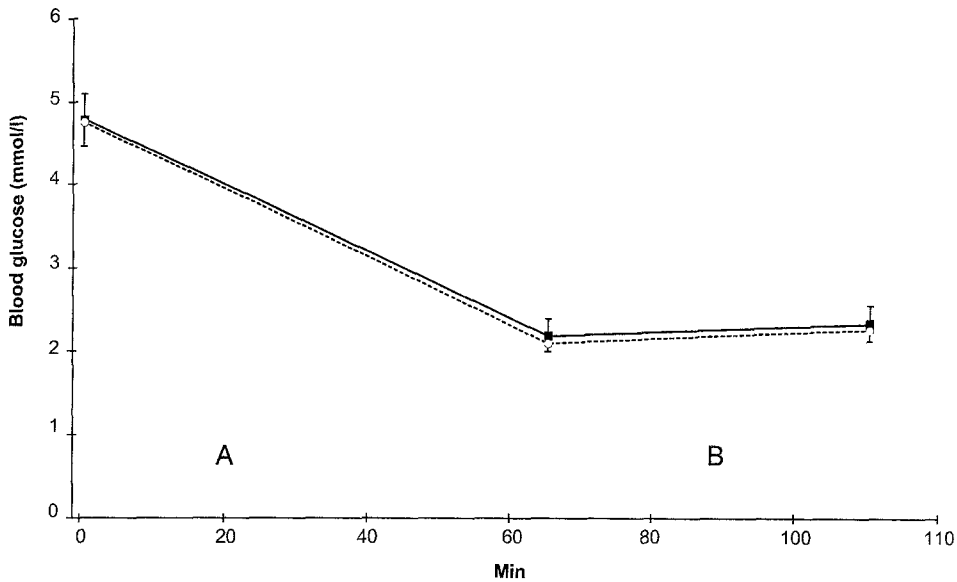

Fig 1. Blood glucose concentration (mmol/L) before and after the decrease of blood glucose (A) and during hypoglycemia (B) in 10 patients with childhood-onset GHD before (○) and after (■) GH treatment for 9 months. Values are the mean ± SD.

injection with a commencing dose of 0.5 IU/d. The dose was increased over 2 weeks to 1.5 IU/d and thereafter adjusted to achieve normal serum IGF-I levels. The median GH dose at the end of the study was 0.14 IU/kg/wk (range, 0.08 to 0.19). The individual final GH doses are listed in Table 2.

Analytical Techniques

Except for serum GH, serum IGF-I, and thyroid hormones, which were analyzed currently, samples were stored at −70°C until analysis. Samples from before and after GH treatment were mixed and analyzed in the same assay. Serum GH was analyzed by an immunofluorometric method (Wallac Oy, Turku, Finland). At 4 mU/L, the interassay and intraassay coefficient of variation (CV) was 4.3% and 5.0%, respectively. Serum IGF-I levels were measured by radioimmunoassay after formic acid–ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA). At an IGF-I level of 87 μg/L, the intraassay CV was 3.6% and interassay CV 9.1%, and at an IGF-I level of 200 μg/L, the intraassay CV was 1.9% and interassay CV 7.9%. The detection level for serum IGF-I was 20 μg/L, and the reference range for adults aged 21 to 28 years was 129 to 385 μg/L. Serum TSH and serum free T_4 were analyzed with an immunofluorometric technique (AutoDelfia; Wallac Oy) Serum free T_3 was analyzed by radioimmunoassay. The reference range for serum free T_4 and free T_3 was 9 to 22 and 3.4 to 7.2 pmol/L, respectively. The serum insulin level was measured by a competitive radioimmunoassay.[26] The intraassay CVs at different serum insulin levels (18 to 157 mIU/L) were 7.1% or less and the detection level was 3 mIU/L. Plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography with electrochemical detection.[27] The intraassay CV was 8% for epinephrine and 5.6% for norepinephrine. The serum cortisol level was measured by a radioimmunological method (Orion Diagnostica, Espoo, Finland). The intraassay CV was 5% or less at serum cortisol levels of 95 to 800 mmol/L. The reference range for serum cortisol at 8 AM was 200 to 800 nmol/L. The serum glucagon level was measured by a competitive radioimmunoassay.[28] The intraassay CV was 24% at a serum glucagon level of 25 pmol/L, 16% at 64 pmol/L, and 21% at 160 pmol/L The serum IGFBP-1 level was measured by radioimmunoassay (In-House Method; Pharmacia & Upjohn), which is linearly correlated ($r = .68$) to a previously published method[29] (Pharmacia & Upjohn, unpublished data, October 1994) The intraassay CV was 3.2%. Serum FFA levels were measured by an enzymatic colorimetric method using a commercially available kit (Wako Chemicals, Neuss, Germany). The CV for serum FFA was 2.7% or less. Venous blood glucose levels were measured every 3 to 5 minutes during the hypoglycemic clamp with a Yellow Springs Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH) and during the fast with a HemoCue Blood Glucose Analyzer (HemoCue, Ängelholm, Sweden)[30] The instrument was controlled daily using a standard microcuvette and weekly using a hemolysate (Eurotrol, Wageningen, The Netherlands) with known glucose concentration.

Statistical Analysis

Data are presented as the median and range, except for blood glucose levels during the 24-hour fast and the hypoglycemic clamp (mean ± SD). The area under the curve for glucose (AUC-glucose) during the 24-hour fasting period was calculated using the trapezoidal method. Intraindividual comparisons of data before and after GH treatment were made with the Wilcoxon matched-pair signed-rank test. The Mann-Whitney *U* test was used for comparison of patients with and without corticotropin (ACTH) deficiency. Univariate correlations were assessed using Spearman's rank order correlation test. Significance was set at a P level of .05 or less.

RESULTS

BMI and Body Composition

The BMI before GH treatment was 26.2 kg/m^2 (range, 21.6 to 36.2; Table 1), and there was no change in the BMI after GH treatment (26.8 kg/m^2; range, 21.6 to 38.0; $P > .5$). Nine months of GH therapy reduced the whole-body resistance measured by BIA (−9.3%, $P = .01$), and the percentage of body fat mass decreased in all patients (−2.9%, $P = .005$) (Table 2).

24-Hour Fast

Blood glucose. The lowest blood glucose level in any patient before GH treatment was 3.3 mmol/L. GH therapy did not cause any significant changes in blood glucose during the fasting period (Fig 2), and the AUC-glucose did not change significantly after GH treatment (Table 3). Even during the night (12 midnight to 8 AM), no significant change in AUC-glucose was observed after GH treatment ($P > .5$). There was a

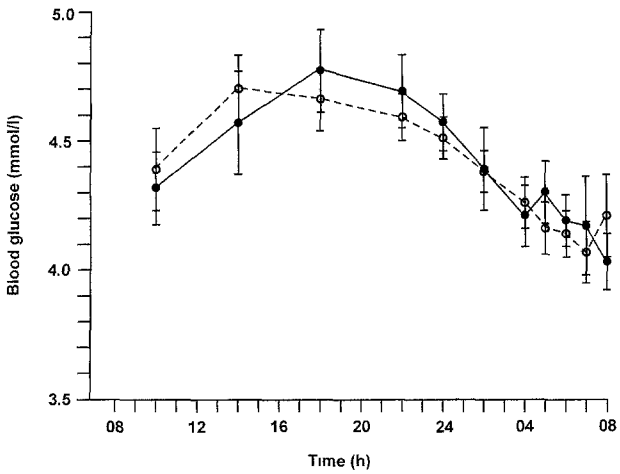

Fig 2. Blood glucose concentration (mmol/L) during 24 hours of fasting in 10 patients with childhood-onset GHD before (○) and after (●) GH treatment for 9 months. Values are the mean ± SD.

negative association, albeit not statistically significant, between the percentage of body fat mass and AUC-glucose before ($r = -.52$, $P = .13$) and after ($r = -.50$, $P = .14$) GH treatment. There was no significant difference in AUC-glucose between five patients with ACTH deficiency and five patients with normal ACTH function before ($P = .22$) or after ($P > .5$) GH treatment.

Insulin to glucose ratio. The insulin to glucose ratio decreased significantly during the fasting period both before and after GH treatment, but GH therapy did not significantly change the ratio (Table 3).

Serum FFA. Serum FFA levels increased significantly during the 24-hour fast both before and after GH treatment (Table 3). However, GH treatment did not cause any changes in FFA concentrations. The percentage of body fat mass was not associated with FFA levels after 24 hours of fasting either before ($r = .21$, $P > .5$) or after ($r = -.32$, $P = .37$) GH treatment.

Table 3. AUC-Glucose, Insulin to Glucose Ratio, and Serum FFA Levels in Patients With Childhood-Onset GHD During a 24-Hour Fast Before and After Nine Months of GH Treatment (N = 10)

	Before GH Treatment		After 9 Months of GH Treatment		
Parameter	Median	Range	Median	Range	*P*
AUC-glucose (mmol/L · h)	98.6	89.2-106.7	98.7	83.0-112.4	.39
Insulin/glucose ratio (mIU/mmol)					
10 AM (2 hours of fasting: I)	5.0	2 7-31.6	3.0	1.7-24.9	.39
5 AM (21 hours of fasting)	1.3	0.7-28.8	1.3	0 7-2.3	.10
8 AM (24 hours of fasting: II)	1.3	0.7-4.8	1.1	0.8-2 7	>.5
P (I *v* II)		.005		.005	
Serum FFA (mmol/L)					
10 AM (2 hours of fasting: I)	0.34	0.13-0.78	0.31	0.13-0.48	.11
5 AM (21 hours of fasting)	0.57	0.32-0.72	0.61	0.25-0.79	>.5
8 AM (24 hours of fasting: II)	0.72	0.38-0.85	0.67	0.32-0.80	>.5
P (I *v* II)		.008		.005	

Hypoglycemic Clamp

Glucose infusion rate and glucose uptake. GH therapy was associated with a significant decrease in the rates of glucose infusion and glucose uptake compared with baseline values in both phase A and phase B (Table 4). There were no associations between the percentage of body fat mass and the rates of glucose infusion and glucose uptake during hypoglycemia (phase B) before ($r = -.006$, $P > .5$ and $r = .16$, $P > .5$, respectively) or after GH treatment ($r = .04$, $P > .5$ and $r = .06$, $P > .5$, respectively). The five patients with ACTH deficiency were more insulin-resistant during phase B in comparison to patients with normal ACTH function both before ($P = .06$) and after ($P = .22$) GH treatment, but this was not statistically significant.

Counterregulatory hormones. Plasma levels of epinephrine and norepinephrine increased significantly during hypoglycemia, and 9 months of GH treatment did not change these responses. Serum cortisol increased in the five patients with normal ACTH function before GH treatment, but GH therapy had no significant effect on this response. Serum glucagon levels increased significantly during hypoglycemia both before and after GH treatment, with no significant difference in this response after GH treatment (Table 5).

Serum levels of IGFBP-1 and FFA. Both before and after GH treatment, serum IGFBP-1 and FFA levels decreased significantly during hypoglycemia. No differences in these responses were observed before versus after GH treatment (Table 5).

DISCUSSION

In the present study, the effects of GH substitution were investigated during conditions in which GH has pronounced metabolic effects, ie, fasting and hypoglycemia. Nine months of treatment with low-dose GH (median, 0.14 IU/kg/wk) in 10 young patients with childhood-onset GHD increased serum IGF-I levels in all patients, without significant changes in blood glucose levels, during a 24-hour fast. Furthermore, none of the patients experienced hypoglycemia either before or after GH

Table 4. Glucose Infusion Rate and Glucose Uptake During the Period of Reduction of Blood Glucose and During Hypoglycemia in Patients With Childhood-Onset GHD Before and After 9 Months of GH Treatment (N = 10)

	Before GH Treatment		After 9 Months of GH Treatment		
Parameter (mg/kg/min)	Median	Range	Median	Range	*P*
Phase A (blood glucose reduction)					
I	1.96	0.91-3.35	1.21	0.16-2.69	.02
M	6.36	4.37-8.86	5.79	3.51-8.41	.005
Phase B (hypoglycemia)					
I	1.36	0.46-5.20	0.31	0-2.81	.005
M	1.02	0.03-4.31	0.14	−0.54-2.46	.01

Abbreviations: I, glucose infusion rate; M, glucose uptake.

Table 5. Plasma Epinephrine and Norepinephrine and Serum Cortisol, Glucagon, IGFBP-1, and FFA Levels Before and During Hypoglycemia in Patients With Childhood-Onset GHD Before and After 9 Months of GH Therapy

Parameter	Before GH Treatment		After 9 Months of GH Treatment		
	Median	Range	Median	Range	*P*
Plasma epinephrine (nmol/L, n = 9)*					
Before hypoglycemia (I)	0.11	0.08-0.26	0.06	0.03-0.92	
After 45 minutes of hypoglycemia (III)	4.88	1.16-7.41	3.41	0.99-8.30	>.5
P (I *v* III)	.008		.008		
Plasma norepinephrine (nmol/L, n = 9)*					
Before hypoglycemia (I)	1.43	0.23-2.85	1.30	0.45-2.76	
After 45 minutes of hypoglycemia (III)	3.04	0.72-6.43	2.61	0.97-3 42	.14
P (I *v* III)	.008		008		
Serum cortisol (nmol/L, n = 5)					
Before hypoglycemia (I)	357	250-582	547	209-641	
After 45 minutes of hypoglycemia (III)	911	622-1,234	890	587-1,480	>.5
P (I *v* III)	.04		.08		
Serum glucagon (pmol/L, n = 10)					
Before hypoglycemia (I)	39.5	27-47	36.5	27-68	
After 45 minutes of hypoglycemia (III)	81.5	45-142	76.5	33-144	.17
P (I *v* III)	.005		01		
Serum IGFBP-1 (ng/mL, n = 10)					
Before hypoglycemia (I)	8.1	5.0-20 4	12.0	3.5-23.6	.26
At start of hypoglycemia (II)	8.3	2.7-16.9	9.5	<1.8-18.2	>.5
After 45 minutes of hypoglycemia (III)	5.3	2.9-9.8	6.2	<1.8-15.0	>.5
P					
I *v* II	.17		.005		
I *v* III	.005		.005		
Serum FFA (mmol/L, n = 10)					
Before hypoglycemia (I)	0.39	0.22-1.16	0.56	0.11-0.71	>.5
At start of hypoglycemia (II)	0.16	0.06-0.34	0.19	0.07-0.28	>.5
After 45 minutes of hypoglycemia (III)	0.15	0.12-0.42	0.20	0.14-0.20	.39
P					
I *v* II	.009		.03		
I *v* III	.01		.03		

*Values are missing in 1 patient due to analytical problems.

treatment. However, in a hypoglycemic clamp model, a significant increase in insulin resistance was observed after GH treatment.

In children with GHD, hepatic glucose production is decreased,[4] and especially in young children, fasting hypoglycemia is commonly observed.[3,5] In adult GHD dwarfs without GH substitution, 4 days of fasting caused hypoglycemia.[16] However, in adults with GHD, only sporadic cases of hypoglycemia have been reported.[12] Glucose turnover is correlated with lean body mass,[8] and compared with adult-onset GHD patients, childhood-onset GHD patients have been found to be significantly shorter and to show a significant decrease in lean body mass.[7] However, in the present study, a 24-hour fast did not result in hypoglycemia even at dawn, when the insulin-antagonistic effect of GH is more pronounced.[31] Children with GHD can become hypoglycemic within 24 hours,[3] but in adult GHD patients, this period might not be sufficient for the development of spontaneous hypoglycemia.

Al-Shoumer et al[32] showed that compared with normal subjects, adult-onset hypopituitary patients on cortisone replacement therapy had low serum levels of cortisol throughout the night,[32] which could be of importance for the alterations in glucose metabolism. In the present study, no difference was found between patients with ACTH deficiency and those without for AUC-glucose during 24 hours of fasting either before or after GH replacement, but the small study group could be of importance in this finding.

Except for one study, which investigated only overnight fasting levels of glucose and insulin in adults with childhood-onset GHD after GH treatment,[33] the study groups have been heterogenous with respect to current age[9,34] and age at onset of GHD (childhood or adulthood).[10-12,35] The patients in the present study were fairly homogenous, as all were treated with GH during childhood and the duration of therapy withdrawal before restarting GH treatment was between 5 and 10 years. Moreover, the current age range was narrow (21 to 28 years). Furthermore, the 9-month follow-up investigations were performed in exactly the same time in relation to gonadal hormone substitution as in the primary investigation. This has not been a consideration in previous studies, but nevertheless may be of importance. In normal women, there are differences in glucose homeostasis between the follicular and luteal phases of the menstrual cycle,[23] and in men, the testosterone concentration varies with the time of injection[36] and there is an association between the total testosterone level and insulin sensitivity.[24]

In contrast to previous studies in which GH therapy in adult GHD patients caused an increase in insulin resistance[9,34] and in overnight fasting blood glucose levels,[10-12,37] the present study found no significant changes in blood glucose or the insulin to glucose ratio during the entire 24-hour fast. In most previous

studies, a higher GH dose has been used,[9-12,34] and it is probable that the presently used relatively low-dose GH could be of importance in the results. Recently, the importance of individualization of GH treatment has been reported,[37] and in the present study, the GH dose was not calculated from body weight or body surface area, but was adjusted according to the response in serum IGF-I. After 9 months, serum IGF-I levels were normalized in all patients except one, in whom serum IGF-I was above the normal reference range. In accordance with other studies,[10,38,39] GH substitution resulted in a significant decrease in whole-body resistance measured by BIA and the percentage of body fat mass.

The exact mechanism for the insulin-antagonistic effect of GH is still unclear. GH stimulates lipolysis, and in healthy subjects, FFA levels increase within hours after GH administration.[40] Increased FFA levels inhibit insulin-stimulated peripheral glucose uptake in a dose-dependent way.[41] Short-term, in contrast to long-term, GH treatment in GHD patients increases overnight serum FFA levels.[9,14] This is in accordance with the present study, in which GH treatment for 9 months had no influence on FFA levels even after an extended period of fasting. There was no association between the percentage of body fat mass and serum FFA, in agreement with a previous investigation in which total fat oxidation did not correlate with fat mass.[8]

The counterregulatory hormones efficiently prevent hypoglycemia. Glucagon and the catecholamines can rapidly influence hepatic glucose production,[17] while cortisol and GH are of importance for the insulin resistance observed later.[42,43] Previously, it was shown that discontinuation of GH treatment has effects on day-to-day fuel metabolism, including changes in insulin sensitivity during hypoglycemia.[44] In the present study, 9 months of GH treatment increased the resistance to insulin during hypoglycemia. Due to lack of a control group in the present study, it is not known whether the increase in insulin resistance is in fact a normalization or a further deterioration. However, in euglycemic conditions, GH treatment in the same group of patients with the presently used GH dose produced no impairment of glucose tolerance according to oral and intravenous glucose tolerance tests.[45] The five ACTH-deficient patients with glucocorticoid replacement had a nonsignificant tendency to be more insulin-resistant during hypoglycemia than the five patients with normal ACTH function. However, due to the small number of patients in each group, a difference cannot be ruled out.

Children with isolated GHD have a loss of the norepinephrine response and a smaller increase in epinephrine levels in response to insulin-induced hypoglycemia compared with healthy controls.[46] However, in the present study, GH replacement therapy for 9 months caused no significant alterations in the catecholamine, glucagon, or cortisol response to hypoglycemia, which could explain the augmented insulin resistance during hypoglycemia. However, since counterregulatory hormones act synergistically,[47] it is possible that the addition of GH may partially explain the pronounced increase in insulin resistance during hypoglycemia.

GH treatment also did not cause any significant alteration in serum IGFBP-1 levels during the hypoglycemic clamp, which could explain the increase in insulin resistance. Serum IGFBP-1 is inversely regulated by insulin,[48,49] and following hypoglycemia, there is normally a rapid increase in serum IGFBP-1.[18] But during a continuous insulin infusion as in the present study design, IGFBP-1 production is suppressed.[50]

Fowelin et al[9] showed that 6 weeks of GH treatment at a dosage of 0.5 IU/kg/wk in adult-onset GHD patients resulted in an increase in serum FFA levels during a hyperinsulinemic-euglycemic clamp.[9] The increase in FFA levels was suggested to explain the increased insulin resistance. However, in their study, GH treatment for 26 weeks resulted in normalization of serum FFA levels and of insulin resistance. In the present study, serum FFA levels were suppressed by insulin infusion, and the possibility of an increase in peripheral uptake of FFAs was not investigated.

In conclusion, in 10 young patients with childhood-onset GHD, treatment with low-dose GH for 9 months did not cause any significant changes in the blood glucose level, insulin to glucose ratio, or serum FFA level during a 24-hour fast. In contrast, in a hypoglycemic clamp model, GH treatment caused a significant increase in insulin resistance, which could not be explained by changes in the counterregulatory hormonal response or in serum IGFBP-1 levels. Determining whether the increased insulin resistance during hypoglycemia indicates a normalization or a deterioration requires additional investigation.

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