Insulin Secretion in Growth Hormone–Deficient Adults: Effects of 24 Months' Therapy and Five Days' Acute Withdrawal of Recombinant Human Growth Hormone

Norshinah Kamarudin, Fen Lee Hew, Michael Christopher, Janet Alford, Christian Rantzau, Glenn Ward, and Frank Alford

β-Cell function in growth hormone (GH)-deficient (GHD) adults is poorly documented. β-Cell function was therefore studied in 10 GHD adults (age, 40 ± 3 years; weight, 79.3 ± 4.8 kg; body mass index [BMI], 27.5 ± 1.3 kg · m^{-2}) before and after 6- and 24-month recombinant human GH (rhGH) therapy (0.24 IU · kg^{-1} · wk^{-1}) compared with 10 age-, sex-, weight-, and BMI-matched control subjects. With rhGH therapy, fat-free mass (FFM) increased (48.2 ± 4.9, 52.5 ± 4.8, and 59 ± 6.8 kg, respectively) and fat mass (FM) decreased (33.8% ± 2.8%, 28.0% ± 3.0%, and 29.4% ± 2.5%, respectively), as did serum cholesterol. Oral glucose tolerance initially deteriorated at 6 months, but improved toward the control value by 24 months. Fasting insulin (FI) increased significantly, as did the acute insulin response to oral glucose ($\Delta AIR_{OGTT}/\Delta G$) at 30 minutes (FI: pretreatment 9.8 ± 0.8, 6 months, 14.0 ± 1.8, 24 months 12.5 ± 1.6 *v* control 11.4 ± 1.9 mU · L^{-1}; $\Delta AIR_{OGTT}/\Delta G$: pretreatment 201 ± 24, 6 months 356 ± 41, 24 months 382 ± 86 *v* control 280 ± 47 mU · $mmol^{-1}$). However, the acute insulin response to intravenous (IV) glucose (AIR_G) and IV glucagon at euglycemia and hyperglycemia did not change with rhGH therapy and were similar to the control group values. Importantly, the expected reciprocal relationships (as observed for the control group) between the various insulin secretory parameters and insulin sensitivity (SI) either were not present or were statistically weak in GHD subjects, despite the 35% decrease in SI by 24 months of rhGH therapy. In particular, over time, there was an attenuation of insulin secretion with respect to the ongoing insulin resistance with rhGH therapy, particularly for AIR_G at 24 months. After 5 days of rhGH withdrawal, insulin secretion decreased and SI improved in GHD subjects. It is concluded that the current long-term rhGH treatment regimens appear to impact on insulin secretion such that the normal relationships between insulin secretion and SI are altered despite the favorable impact on body composition and serum lipid profiles.

THE GLUCOSE TOLERANCE of an individual depends on the endogenous insulin action and the amount of circulating insulin, ie, β-cell function, between which there is a close reciprocal relationship.[1] Growth hormone (GH)-deficient (GHD) subjects are known to be insulin-resistant[2-6] due to a major defect in the glucose storage–glycogen synthase activation pathway in muscle.[6] Glucose tolerance in GHD subjects is frequently impaired, and more so in female GHD subjects, although frank diabetes is uncommon.[7-9] However, only limited information is available on β-cell function in GH deficiency, and it is mainly restricted to fasting insulin (FI) and to insulin levels after an oral glucose tolerance test (OGTT).[7-12] In these studies, FI has been reported as normal[5-7] or elevated[10] compared with values in matched control subjects, as have post-OGTT insulin levels.[7] Importantly, insulin levels have not been examined with respect to insulin sensitivity.[1]

Similarly, few data are available on the interrelated responses of glucose tolerance, insulin sensitivity, and β-cell function in GHD adults treated with replacement doses of recombinant human GH (rhGH). Thus, in separate studies following rhGH therapy, glucose tolerance decreases somewhat,[8,13] although the emergence of frankly impaired glucose tolerance by World Health Organization (WHO) criteria is uncommon,[8] insulin sensitivity is unaltered[2,14] or, in longer-term studies, deteriorates significantly,[4,15-16] and fasting and oral glucose–stimulated insulin levels are usually elevated.[8] The increase in insulin levels after rhGH therapy may represent the body's response to the change in insulin sensitivity aimed at preserving glucose tolerance in such individuals.[8] It may also indicate a failure to significantly improve the hyperinsulinemia-insulin resistance–induced cardiac risk factors in rhGH-treated individuals,[4,15,16] despite the clear improvement in body composition parameters.[17]

Two further unresolved issues are whether GH itself has a direct effect on the β-cell and directly contributes to the ongoing insulin resistance in GH-treated subjects. First, an insulinotropic effect of GH on the β cell has been reported for both in vitro[18] and in vivo[19] studies. However, a chronic "toxic" effect of GH on β-cell function occurs in dogs following prolonged GH administration[20] and in chronically acromegalic patients.[21] Second, in our recent detailed study on insulin action in rhGH-treated GHD subjects, a significant correlation between the rhGH-induced increase in insulin-like growth factor-1 (IGF-1) and free fatty acid (FFA) levels versus the insulin resistance was noted, and we questioned whether the current doses of rhGH used in GHD subjects are excessive and contribute to the ongoing insulin resistance and fasting hyperinsulinemia.[15]

Therefore, the present study examined β-cell function in GHD adults before and after 6 and 24 months of rhGH therapy, and in age-, sex, and body mass index (BMI)-matched healthy control subjects using multiple tests for β-cell function (oral and intravenous [IV] glucose loads and IV glucagon at euglycemia and hyperglycemia). Insulin sensitivity was measured in all

From the Department of Endocrinology and Diabetes, St. Vincent's Hospital, Fitzroy; and the Department of Medicine, University of Melbourne, Parkville, Melbourne, Victoria, Australia.

Submitted November 10, 1998; accepted May 21, 1999.

Supported by the National Health and Medical Research Council of Australia and the Research Foundation of Diabetes Australia.

Presented in part at the Second International Growth Hormone Society Meeting, London, UK, November 15-17, 1996.

Address reprint requests to Frank Alford, FRACP, MD, Department of Endocrinology and Diabetes, St. Vincent's Hospital, Victoria Parade, Fitzroy, Victoria 3065, Australia.

0026-0495/99/4811-0010$10.00/0

individuals during a euglycemic-hyperinsulinemic clamp to establish whether the β-cell response was appropriate for each subject's insulin sensitivity or whether an altered β-cell "functional state" arose relative to the ongoing insulin resistance with rhGH therapy in GHD subjects. Finally, the tests were repeated 5 days after rhGH withdrawal, at a time when rhGH would disappear but body compositional changes remain.

SUBJECTS AND METHODS

Subjects

Ten subjects (five post–pituitary surgery for pituitary tumor, two idiopathic GH deficiency, one pinealoma, one Sheehan's syndrome, and one head injury) were recruited for the study. GH deficiency was confirmed in all subjects by insulin-induced hypoglycemia using the insulin tolerance test (ITT), with peak serum GH less than 7 $mU \cdot L^{-1}$. All except one (isolated GH deficiency) were on stable standard hormone replacement for at least 6 months (glucocorticoids, thyroxine [T_4], sex steroids, and/or desmopressin). All subjects had normal renal, liver, and cardiac status and no family history of diabetes. Ten healthy controls individually matched for age, sex, and BMI were also studied. Informed written consent was obtained, and the study was approved by the Human Research Ethics Committee of St. Vincent's Hospital in Melbourne. Ten subjects completed the 6-month study, and seven subjects completed the 24-month study. One subject unexpectedly committed suicide at 10 months, and two subjects withdrew for social reasons.

Study Design

The study was an open non–placebo-controlled trial with all GHD subjects receiving rhGH (Norditropin; Novo Nordisk, Copenhagen, Denmark) at a dose of 0.12 $IU \cdot kg^{-1} \cdot wk^{-1}$ during the first 2 weeks, increasing to 0.24 $IU \cdot kg^{-1} \cdot wk^{-1}$. The weekly dose was equally divided into seven daily subcutaneous injections administered at bedtime, and no dose-response titrations were performed. The dosage was subsequently reduced in most patients due to minor side effects and/or elevated IGF-1 above the normal range. By 6 and 24 months, the mean doses were 0.22 ± 0.03 and 0.17 ± 0.03 $IU \cdot kg^{-1} \cdot wk^{-1}$, respectively (mean ± SD). At 24 months, GH was discontinued, and subjects were studied on the morning of day 6 after withdrawal of rhGH.

Methods

Anthropometric variables (weight, height, BMI, and waist to hip ratio) and fat-free mass (FFM) and fat mass (FM) assessed by dual-energy x-ray absorptiometry (DEXA) (DPX; Lunar Radiation, Madison, WI) and bioelectrical impedance methods (model BIM3; SEAC, Baulkham Hills, Australia) with Lukaski's equation[22] were measured in the control subjects and GHD subjects at baseline, 6 months, and 24 months. Abdominal fat (by DEXA) was determined using an abdominal region of interest consisting of all abdominal tissues between the intervertebral disc at the first and second lumbar vertebrae and the anterior superior iliac spine as modified from Svendsen et al[23] and Carey et al.[24] The intraoperator (N.K.) coefficient of variation (CV) for the percent fat of the abdominal region was 1.9%.

The experimental protocol was performed on 2 separate days at 0, 6, and 24 months post–rhGH therapy and the day 2 protocol only for the 5-day post-study (Fig 1). All GHD subjects on stable hormone replacement therapy received their usual morning dose of medication 1 to 2 hours before the study. On study day 1 at 8:00 AM, after a 10-hour fast and a period of rest and arm-warming, an IV cannula was inserted into an arm vein (Intima, 18-gauge; Becton Dickinson, Sandy, UT). After collection of baseline samples (−10 and 0 minutes) for glucose, insulin, and C-peptide, a 0.5-mg IV bolus of glucagon (GlucaGen; Novo Nordisk) diluted in normal saline (5 mL) was administered, followed by rapid venous sampling at 1, 2, 3, 4, 5, 7, 10, and 15 minutes for glucose, insulin, and C-peptide. This test was used to calculate the acute insulin secretion response above baseline (AIR) to glucagon at euglycemia ($AIR_{G.GON}$). After a 60-minute rest period to allow the peripheral insulin concentration to return to the basal value (basal insulin *v* pre-OGTT insulin: 13 ± 1.5 *v* 10.7 ± 1.2 for GHD, 21.1 ± 5.1 *v* 17.5 ± 2.4 for rhGH-treated, and 12.6 ± 1.6 *v* 10.6 ± 1.1 $mU \cdot L^{-1}$ for control), a 75-g OGTT was performed. Blood samples for glucose, insulin, and C-peptide were taken at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 minutes after the oral glucose load. This test established each subject's glucose tolerance by WHO criteria and enabled calculation of the early-phase insulin response to oral glucose (ΔAIR_{OGTT}).[25] In addition, the insulin secretion rate (ISR) was calculated from the C-peptide data.[26] On study day 2, 5 to 10 days later, a hyperinsulinemic-euglycemic clamp[6] was performed. Two IV lines were inserted in opposite arms (Intima, 18-gauge); one arm was used for blood sampling and the other for infusion of glucose (10% dextrose) and insulin (60 $mU \cdot kg^{-1} \cdot h^{-1}$, Actrapid HM; Novo Nordisk).[6] Bedside glucose levels were determined at 5-minute intervals during the clamp using an electronic strip glucometer (Medisense 2; Medisense, Balwyn, Australia). Only subsequent laboratory biochemical values for blood glucose were used in the final calculations of insulin sensitivity and the glucose infusion rate (GINF). Steady state was defined when there was less than 10% variation in the GINF over the last 30 minutes of the clamp, usually at about 120 to 150 minutes of the clamp. Blood samples for glucose, insulin, and C-peptide were collected at 15, 30, 45, 60, 80, 90, 100, 110, 120, 130, and 140 minutes. The CV for the GINF at steady state was 3.1% ± 0.8% (mean ± SD) at baseline, 2.3% ± 0.4% at 6 months, 5.1% ± 0.9% at 24 months, 5.9% ± 1.4% at day 5, and 4.4% ± 0.9% in controls. The CV for serum insulin at steady state was 9.3% ± 1.5% at baseline, 6.9% ± 1.6% at 6 months, 8.9% ± 1.5% at 24 months, 10.5% ± 2.8% at day 5, and 8.8% ± 1.2% in controls. The CV for plasma glucose at steady state was 4.3% ± 0.8% at baseline, 6.4% ± 1.5% at 6 months, 4.9% ± 1.3% at 24 months, 5.5% ± 1.2% at day 5, and 7.1% ± 1.6% in controls.

At cessation of the clamp, the insulin infusion was discontinued and the glucose infusion was tapered over 60 minutes while maintaining euglycemia. At the end of this rest period, the GINF was adjusted to the rate achieved at steady state and a 25-minute hyperglycemic clamp was commenced by injecting a rapid IV glucose bolus (9 g/m^2) over 60 to 90 seconds, and then after 5 minutes, the GINF was increased 1.8-fold. From the bolus glucose data, the acute insulin response to IV glucose (AIR_G) was calculated.[27] At the 10-minute point of the hyperglycemic clamp, an IV glucagon bolus (0.5 mg) was injected (Fig 1). This enabled calculation of the acute insulin response to glucagon at hyperglycemia ($AIR_{HyperG.GON}$).

Assays

All samples collected were immediately placed on ice and centrifuged for 10 minutes at 4°C, and the plasma was stored at −20°C until assay. Bedside glucose, initially measured using Medisense 2 strips, was subsequently determined by the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). The interassay CV at 4.1 and 14.7 $mmol \cdot L^{-1}$ was 1.36% and 1.69%, respectively. Plasma insulin was determined by radioimmunoassay (RIA) as described previously.[28] The sensitivity of the insulin assay was less than 1.0 $mU \cdot L^{-1}$, with an interassay CV at 8.0 and 35 $mU \cdot L^{-1}$ of 7.7% and 6.8%, respectively, and an intraassay CV of 6% over the range of 5 to 35 $mU \cdot L^{-1}$. Plasma C-peptide was determined by an in-house RIA[15] using a secondary antibody separation procedure. The assay sensitivity was 0.01 $pmol \cdot mL^{-1}$, with an interassay CV at 0.32 and 1.0 $pmol \cdot mL^{-1}$ of 4.5% and 11.5%, respectively. Serum IGF-1 was determined by RIA using acid/ethanol extraction followed by a double-antibody RIA (Bioclone Australia, Marrackville, Australia). The interassay CV at 0.6

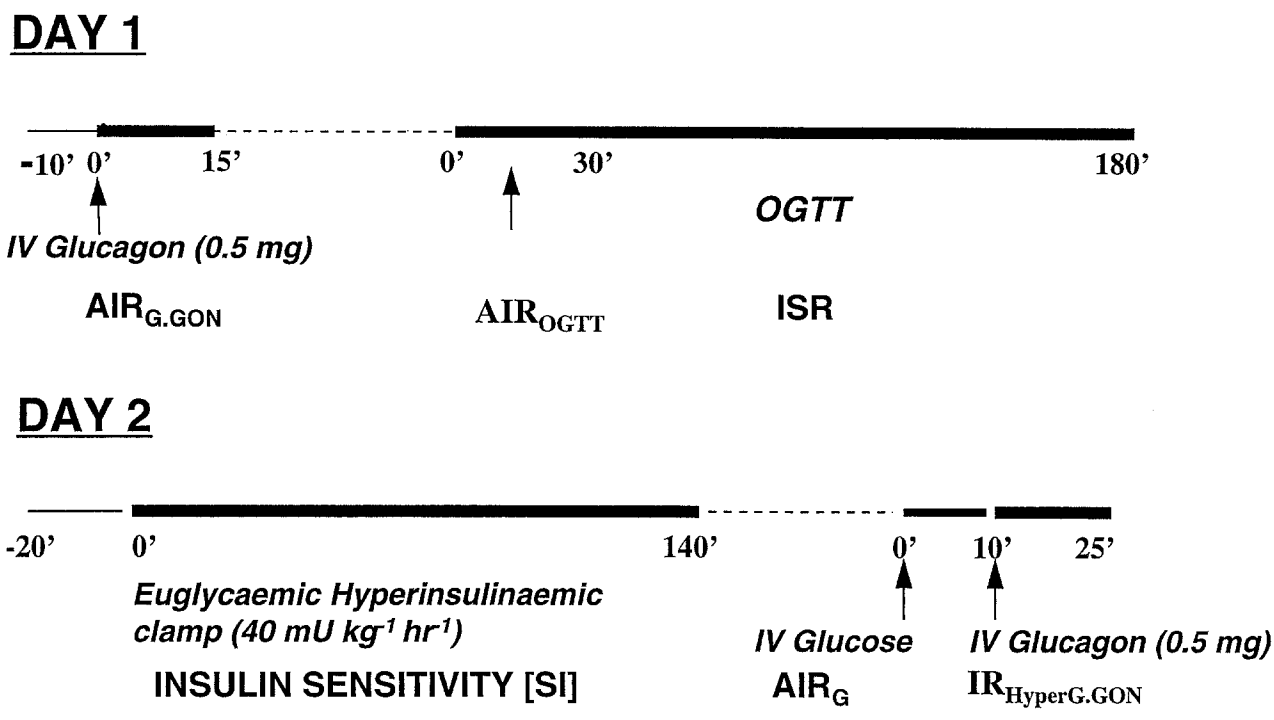

Fig 1. Study design for measurements of insulin secretion and insulin sensitivity. Day 1: IV glucagon bolus was followed by frequent venous sampling and a 1-hour rest period. OGTT was performed over the next 180 minutes for measurement of early-phase insulin response and ISR. Day 2: Hyperinsulinemic-euglycemic clamp was performed to measure insulin sensitivity, followed by a 1-hour rest period. Hyperglycemic clamp initiated by an IV glucose bolus with frequent venous sampling over 10 minutes was finally followed by IV glucagon bolus during the ongoing hyperglycemia.

and 2.2 $U \cdot mL^{-1}$ was 8.6% and 6.8%, respectively. Plasma total cholesterol (TC) and triglyceride (TG) levels were measured automatically using an Olympus AU800 (Integrated Sciences, Sydney, Australia) assay. High-density lipoprotein cholesterol (HDL-C) was determined by precipitation of β lipoproteins with polyethylene glycol 6000 followed by the analysis already described. Hemoglobin A_{1c} (HBA_{1c}) was determined using an in-house chromatographic method (normal range, 3.5% to 6.9%). Free T_4 was determined by competitive immunoassay (Ciba Corning Diagnostics, Medfield, MA).

Calculations and Statistical Analysis

The mean fasting serum glucose, insulin, and C-peptide concentrations shown in Table 1 were taken as the mean of values obtained on day 1 (−10 and −1 minutes) and day 2 (−20, −10, and −1 minutes).

$\Delta AIR_{OGTT}/\Delta G$ is the incremental elevation of insulin above basal (area under the curve) standardized for the change in the glucose concentration from baseline to 30 minutes of the OGTT. AIR_G is calculated as the time-averaged incremental elevation of insulin above basal from 0 to 10 minutes after IV glucose. $AIR_{G.GON}$ is the time-averaged incremental elevation of insulin above basal over 10 minutes after the IV glucagon bolus at euglycemia. $IR_{HyperG.GON}$ is the time-averaged incremental elevation of insulin after IV glucagon at hyperglycemia, above the prevailing insulin level from 0 to 10 minutes. The ISR basally and during the OGTT was calculated using the deconvolution method of Polonsky et al[29] based on peripheral C-peptide values. This method uses the standard kinetic parameters for C-peptide clearance adjusted for age, sex, height, and weight as implemented by the ISEC (Insulin Secretion) computer program of Hovorka and Jones.[26] $\Delta ISR_{0\text{-}30}$ is the incremental sum of the ISR above basal (area under the curve) over the first 30 minutes of the OGTT, given as picomoles per kilogram per minute. Insulin sensitivity (SI) was calculated from the average GINF at steady state per unit change of insulin, normalized for the glucose concentration at steady state.[1]

The data are presented as the mean ± SEM. Differences within matched groups were determined using the Wilcoxon matched-pairs signed-rank test and between groups using the Mann-Whitney *U* test. Correlations were performed using Spearman rank correlation analysis. A *P* level of .05 or less was considered significant. Statistical analyses were performed with the Minitab program (Minitab, State College, PA).

RESULTS

GHD Group Versus Controls

Baseline body composition and biochemistry. Despite matching the control and GHD groups for weight and BMI, the total-body FFM was lower in GHD subjects versus control subjects and total FM and abdominal FM were higher in the GHD group, although this did not reach statistical significance. The mean IGF-1 level was similar in both groups despite the proven GH deficiency by the ITT, with eight of 10 subjects having a GH level less than 5 $mU \cdot L^{-1}$ and one subject less than 7 $mU \cdot L^{-1}$ in the GHD group. Interestingly, although fasting glucose ($P < .003$) and HbA_{1c} ($P < .002$) were within the normal range, they were significantly lower than the control group values. The 2-hour glucose levels were similar. GHD subjects had higher TC than control subjects ($P < .02$), but TG and HDL-C were similar in the two groups. The TC/HDL-C ratio was higher in GHD subjects but did not reach statistical significance (Table 1).

Insulin secretion—OGTT. FI and fasting C-peptide levels were similar in the GHD and control groups. Following the oral

Table 1. Body Composition and Biochemical Profile of GHD Subjects and Matched Control Subjects (mean ± SEM)

Parameter	GHD Subjects			Control Subjects
	Baseline (n = 10)	6 Month rhGH (n = 10)	24 Month rhGH (n = 7)	(n = 10)
Age (yr)	40.1 ± 2.9		43.0 ± 3.4	40.7 ± 3.1
Sex (male/female)	7/3		5/2	7/3
Weight (kg)	79.3 ± 4.8	80.6 ± 4.6	74 ± 5.5	78.5 ± 5.1
WHR	0.89 ± 0.03	0.88 ± 0.02	0.87 ± 0.03	0.87 ± 0.02
BMI (kg/m^{-2})	27.5 ± 1.3	28.1 ± 1.3	26.0 ± 1.2	26.7 ± 1.2
FFM (kg)	50.5 ± 3.9	54.3 ± 3.8‡	59.0 ± 6.8¶	53.8 ± 3.2
FM (kg)	26.9 ± 2.6	24.5 ± 3.1†	21.1 ± 1.5¶	25.0 ± 2.8
FM %	35.0 ± 2.6	31.0 ± 3.0‡	29.4 ± 2.5¶	31.4 ± 2.1
Abdominal FM %	42.6 ± 2.3	35.9 ± 3.0‡	33.5 ± 2.2¶	36.3 ± 2.4
IGF-1 ($U \cdot mL^{-1}$)	0.5 ± 0.1	1.4 ± 0.2‡	1.20 ± 0.19¶	0.6 ± 0.1#
Free T_4 ($pmol \cdot L^{-1}$)	16 ± 3	13 ± 2	16.1 ± 1.6	13 ± 0.4#
Cholesterol ($mmol \cdot L^{-1}$)	5.9 ± 0.3	5.2 ± 0.2‡	5.3 ± 0.3	5.0 ± 0.2§
TG ($mmol \cdot L^{-1}$)	1.8 ± 0.3	1.4 ± 0.2*	1.5 ± 0.2	1.6 ± 0.3
HDL ($mmol \cdot L^{-1}$)	1.15 ± 0.07	1.19 ± 0.05	1.17 ± 0.10	1.11 ± 0.10
TC/HDL ratio	5.34 ± 0.48	4.45 ± 0.25†	4.65 ± 0.33	4.85 ± 0.57
HbA_{1c} (%)	4.5 ± 0.1	4.9 ± 0.2	5.55 ± 0.19¶	5.2 ± 0.1‖
Fasting glucose ($mmol \cdot L^{-1}$)	4.5 ± 0.1	5.1 ± 0.2‡	5.2 ± 0.3	5.1 ± 0.1‖
2-h glucose ($mmol \cdot L^{-1}$)	6.6 ± 0.5	7.7 ± 0.6†	7.4 ± 0.4	6.8 ± 0.4

*$P < .1 > .05$, †$P \leq .05$, ‡$P \leq .01$, 0 *v* 6 month rhGH.
§$P \leq .02$, ‖$P \leq .003$, 0 month *v* control.
¶$P < .05$, 0 *v* 24 month rhGH.
#$P < .05$, control *v* 24 month rhGH.

glucose load, GHD subjects had a lower glucose response than the control group over the initial 60 minutes, but after 90 minutes the glucose profiles were similar. The insulin and C-peptide profiles were flatter in GHD subjects, although over the final 90 minutes the responses were similar in the GHD and control groups. The mean peak insulin concentration during the OGTT in the control and GHD subjects was observed by 30 to 40 minutes and appeared lower in GHD subjects (not significant), but was prolonged in GHD subjects. In the control group, the mean peak C-peptide was observed at 60 minutes and appeared higher (not significant), but was delayed to 90 minutes in GHD subjects. When the ISR was calculated, the fasting ISR tended to be lower in GHD subjects, as did the post–oral glucose ISR profile over the initial 90 minutes of the OGTT (Fig 2 and Table 2).

Acute insulin responses. The acute insulin response to oral glucose expressed either as $\Delta AIR_{OGTT}/\Delta G$ or as $\Delta ISR_{0\text{-}30}$ was similar in both groups. The acute insulin responses to IV glucose (AIR_G) and to glucagon at euglycemia ($AIR_{G.GON}$) and hyperglycemia ($IR_{HyperG.GON}$) were also similar for the GHD subjects at baseline and the control group (Table 2).

Effects of rhGH Therapy

Body composition and biochemistry. A nonsignificant increase in weight was observed at 6 and 24 months of rhGH therapy, with no significant change in the BMI. Total FM, FM%, and abdominal FM were significantly decreased and FFM was significantly increased by rhGH therapy. These variables now more closely resembled those present in the control group. Serum IGF-1 increased almost threefold at 6 months ($P < .01$), and decreased slightly by 24 months with rhGH dose adjustment. Fasting glucose increased significantly ($P < .01$) at 6 months and thereafter remained stable and similar to the control group values. HbA_{1c} also increased slightly at 6 months and somewhat further at 24 months, but remained similar to the control values. TC decreased at 6 months ($P < .05$) and 24 months, whereas TG and HDL-C were unchanged over 24 months. There was a transient decrease in the TC/HDL-C ratio at 6 months, which then stabilized, similar to the value in control subjects (Table 1).

Insulin secretion—OGTT. Fasting insulin and C-peptide and the ISR increased significantly at 6 months in rhGH-treated GHD subjects but not at 24 months, although the 24-month levels were still elevated by approximately 35% compared with the baseline. Following the oral glucose load, there was a small significant delay in the glucose profile in GH-treated subjects at 6 months (Fig 2A), which was less evident by 24 months. The early insulin profiles also increased post–rhGH therapy, and these profiles (ie, 0 to 30 minutes) now more closely resembled the control values. Thereafter, the profiles were somewhat greater versus pre-rhGH but flatter than those of the control group. The C-peptide post–glucose load response profile was greatest at 6 months, particularly after 90 minutes, but decreased by 24 months, being closer to the C-peptide profile pre-rhGH. The ISR profile after rhGH therapy mirrored that found for C-peptide, with the greatest increases observed at 6 months. At no time did insulin, C-peptide, or ISR profiles exceed those obtained in the control subjects, apart from a trend for somewhat higher levels at 180 minutes (Table 2 and Fig 2).

Acute insulin responses. Early-phase insulin release following the oral glucose load ($\Delta AIR_{OGTT}/\Delta G$) and $\Delta ISR_{0\text{-}30}$ increased in the 6-month and 24-month treated groups. The acute insulin responses after IV glucose (AIR_G) and IV glucagon ($AIR_{G.GON}$ and $IR_{HyperG.GON}$) were also somewhat higher following rhGH therapy at 6 months, but decreased at 24 months, apart from $AIR_{G.GON}$, which remained elevated at 24 months. All

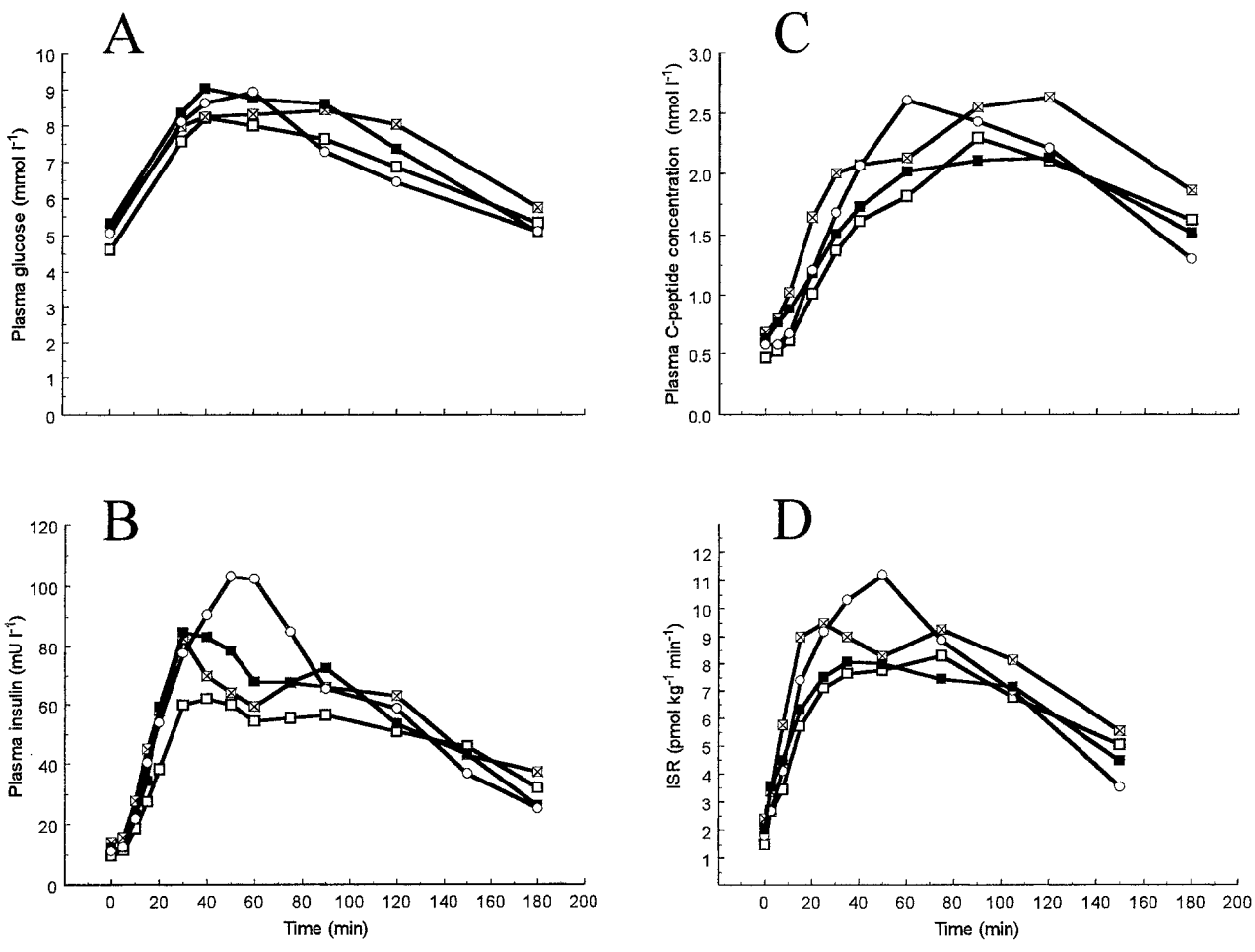

Fig 2. OGTT plasma glucose (A), insulin (B), and C-peptide (C) concentrations and ISR (D) measured over 180 minutes post-oral glucose load. Data are the mean values for all groups: (□) GHD at baseline, (⊠) GHD at 6 months, (■) GHD at 24 months, and (○) control group. For the sake of clarity, SEMs are not shown.

secretory parameters in the rhGH-treated groups did not differ significantly from the values in the control group (Table 2).

Relationships between insulin sensitivity and insulin secretion. In the control group, SI was strongly related to adiposity expressed as weight, FM, and abdominal FM ($r = -.78$, $r = -.71$, $r = -.90$, $P < .01$, respectively). In contrast, SI was not related to the absolute degree of obesity in GHD subjects before and after rhGH replacement. Age was a factor for SI only in GHD subjects before therapy ($r = -.75$, $P \leq .05$). Post–rhGH therapy IGF-1 levels at 6 and 24 months negatively

Table 2. Basal and Acute Insulin Secretory Responses and SI at 0, 6, and 24 Months of rhGH in GHD and Matched Control Subjects (mean ± SEM)

Parameter	GHD Subjects			Control Subjects (n = 10)
	Baseline (n = 10)	6 Month rhGH (n = 10)	24 Month rhGH (n = 7)	
FI (mU · L⁻¹)	12.7 ± 1.9	20.4 ± 4.1‡	12.5 ± 1.6	14.0 ± 2.2
Fasting C-peptide (pmol · L⁻¹)	540 ± 60	840 ± 110†	625 ± 115	620 ± 080
Basal ISR	1.49 ± 0.12	2.42 ± 0.24†	1.85 ± 0.40	1.80 ± 0.16
$\Delta AIR_{OGTT}/\Delta G$	318.4 ± 67.7	407.3 ± 58.5*	382 ± 86	353.9 ± 71.2
$\Delta ISR_{0\text{-}30}$	12.1 ± 1.4	13.4 ± 1.7	11.2 ± 2.3	13.8 ± 1.8
AIR_G	58.1 ± 22.8	62.5 ± 15.7	27.6 ± 3.9	59.0 ± 12.3
$AIR_{G.GON}$	48.4 ± 9.3	98.3 ± 28.9†	65.5 ± 11.6	56.7 ± 8.0
$IR_{Hyper.G.GON}$	197.3 ± 59.6	227.9 ± 43.3	170.1 ± 25.9	194.4 ± 31.3
SI	12.5 ± 1.8	7.8 ± 1.1†	10.2 ± 2.4	12.0 ± 2.7

NOTE. $\Delta AIR_{OGTT}/\Delta G$ in mU · mmol⁻¹, $\Delta ISR_{0\text{-}30}$ in pmol · kg⁻¹ · min⁻¹, AIR_G, $AIR_{G.GON}$, and $IR_{Hyper.G.GON}$ in mU · L⁻¹, SI in [L · min⁻¹ per mU · L⁻¹ · kg FFM⁻¹] × 10⁻⁵.

*$P < .01 > .05$, †$P \leq .05$, ‡$P \leq .01$, 0 *v* 6 month rhGH.

influenced SI ($r = -.79$ and $-.83$, $P < .05$, respectively), as did the incremental increase in IGF-1 following therapy ($r = -.76$ and $-.85$, $P < .05$, respectively) (Fig 3).

Early-phase insulin release ($\Delta AIR_{OGTT}/\Delta G$ and AIR_G) was related to adiposity (weight, total FM, and/or abdominal FM, $r = .67$, $P < .05$) and SI ($r = -.65$, $P < .05$) in control subjects. However, in GHD subjects pretreatment, $\Delta AIR_{OGTT}/\Delta G$ was related to weight and total FM ($r = .60$, $P < .05$ for both), and for AIR_G, weight ($r = .61$, $P < .05$) and SI ($r = -.64$, $P < .05$). Only IGF-1 levels, whether expressed as absolute or

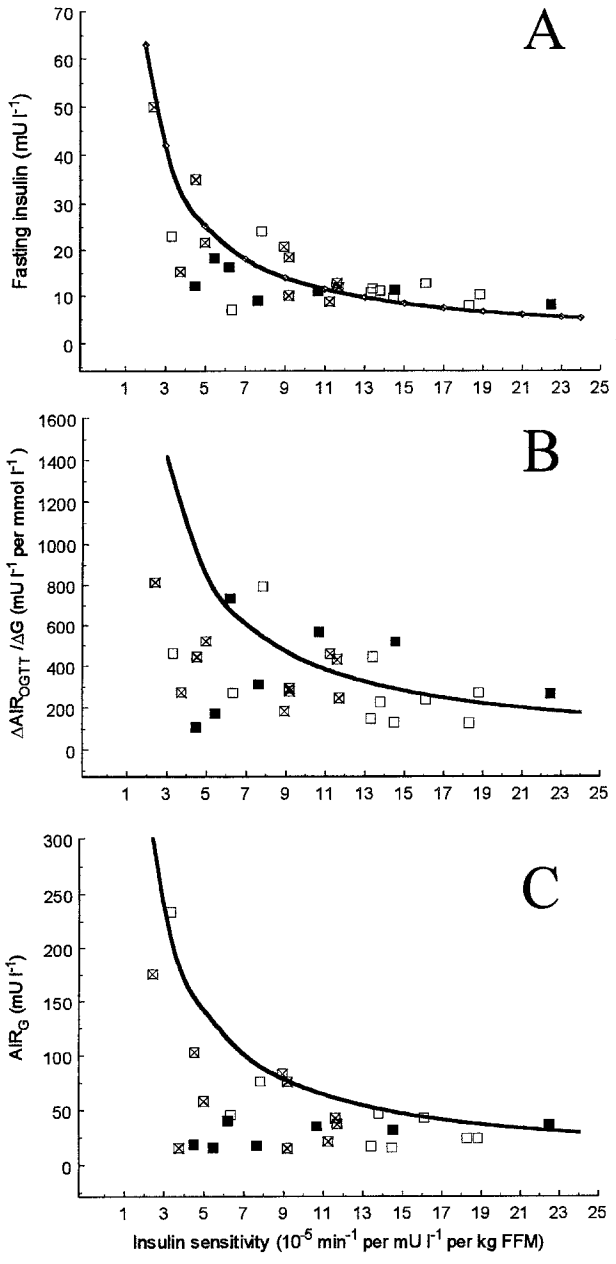

Fig 3. Relationships between SI versus FI (A), $\Delta AIR_{OGTT}/\Delta G$ (B), and AIR_G (C) before (□) and after 6 months (⊠) and 24 months (■) of rhGH treatment in GHD adults. Curved lines represent the mean for the relationship between insulin secretory parameters and insulin sensitivity for the control group.

incremental changes following rhGH therapy, did not influence early-phase insulin secretion, but age influenced AIR_G in 6-month rhGH-treated subjects ($r = -.61$, $P < .05$). There were no relationships between early-phase insulin release and any of these variables by 24 months.

SI in GHD subjects was similar to that in the matched control subjects. Following the 6-month rhGH therapy, SI was significantly reduced, but it increased slightly by 24 months, although remaining about 35% below the level in the matched control subjects (Table 2). There were the expected curvilinear relationships between FI ($r = -.90$, $P < .01$), $\Delta AIR_{OGTT}/\Delta G$ ($r = -.75$, $P < .01$), and AIR_G ($r = -.55$, $P < .05$) and SI for the control group. In contrast, at month 0 in GHD subjects, the relationship between insulin secretion and SI was statistically poor, there being no relationship between FI or $\Delta AIR_{OGTT}/\Delta G$ versus SI, although it was present for AIR_G versus SI ($r = -.64$, $P < .05$). For FI before and after 6 months of treatment, the majority of points were around the mean curvilinear control line for FI, but by 24 months of therapy, there was a downward and flatter trend for the FI-SI relationship compared with the control group, although FI was still related to SI ($r = -.75$, $P < .05$). During the OGTT, $\Delta AIR_{OGTT}/\Delta G$ did not correlate with SI at 6 or 24 months, and the majority of points were below the mean control line at 0 and 6 months but increased somewhat by 24 months (Fig 3). For AIR_G, there was a clear shift of individual points away from and below the mean of the control group at 0, 6, and 24 months of rhGH treatment, indicating a relatively inadequate response to IV glucose with respect to each individual's insulin sensitivity. This was most evident by 24 months.

Effect of 5-day rhGH withdrawal. As expected, there were no changes in body weight or body composition, particularly FM, or in serum lipids with 5-day withdrawal. IGF-1 decreased significantly but was still in the normal range for adults. FI, C-peptide, and the basal ISR decreased significantly in six of seven subjects following rhGH withdrawal, with a smaller decrease (~20%) in acute insulin secretion (AIR_G and $IR_{HyperG\text{-}GON}$), again in six of seven subjects. SI increased significantly with acute rhGH withdrawal, whether expressed as the GINF or the SI index (Table 3).

DISCUSSION

The present data demonstrate that in GHD subjects prior to treatment with rhGH, insulin secretion, whether expressed in absolute terms as FI or the acute response to oral and IV glucose or glucagon at euglycemia and hyperglycemia, was similar to that in matched control subjects. However, there was a subtle delay and prolongation of insulin and C-peptide secretion during the OGTT in the normal glucose-tolerant GHD group before rhGH therapy. Following exposure to rhGH for 6 months, glucose tolerance deteriorated and an apparent enhancement of basal insulin and insulin secretion during the OGTT occurred in association with the further significant worsening of insulin resistance.[15] Early-phase insulin release following IV glucose and glucagon also increased, although these latter increases were not significant. At 24 months, glucose tolerance improved slightly with no change in insulin secretion. However, in contrast to the control subjects, the expected relationships between the acute insulin secretion parameters and insulin sensitivity were poor or statistically nonsignificant both before

Table 3. Effects of Stopping rhGH for 5 Days in rhGH-Treated GHD Adults on Body Composition, Insulin Secretion, and SI (mean ± SEM)

Parameter	24 Month rhGH (n = 7)	5 Day Cessation (n = 7)
Weight (kg)	74.0 ± 5.5	73.9 ± 5.5
BMI (kg · m^{-2})	25.96 ± 1.22	25.68 ± 1
FM (kg)	20.05 ± 2.26	20.73 ± 2.12
FM %	26.98 ± 2.16	27.00 ± 2.28
IGF-1 (U · L^{-1})	1.20 ± 0.19	0.72 ± 0.10§
Cholesterol (mmol · L^{-1})	5.30 ± 0.30	5.35 ± 0.22
TG (mmol · L^{-1})	1.5 ± 0.2	1.2 ± 0.3
HDL-C (mmol · L^{-1})	1.17 ± 0.10	1.20 ± 0.11
Fasting glucose (mmol · L^{-1})	5.2 ± 0.3	5.0 ± 0.2
FI (mU · L^{-1})	12.54 ± 1.53	8.09 ± 1.15§
Fasting C-peptide (pmol · L^{-1})	625 ± 115	442 ± 88‡
AIR_G (mU · L^{-1})	27.56 ± 3.94	21.23 ± 5.12
$IR_{HyperG.GON}$ (mU · L^{-1})	170.1 ± 25.9	141.4 ± 21.5
GINF*	34.23 ± 3.85	47.44 ± 4.23§
SI†	10.21 ± 2.43	12.45 ± 2.39

*μmol · min^{-1} · kg FFM^{-1}.
†SI units as in Table 2.
‡$P < .1 > .05$, §$P \leq .05$, 24 month rhGH *v* 5 day cessation.

and after rhGH treatment in the GHD groups, with a downward shift in the insulin secretory parameters versus SI curves, particularly for AIR_G by 24 months (Fig 3). However, despite the favorable changes in body composition (FFM and FM) and in serum lipids induced by 6 and 24 months of rhGH therapy,[15] SI was the main determinant of AIR_G before treatment with rhGH, whereas after rhGH therapy, there were no relationships between early-phase insulin release and SI and/or obesity. These data support the notion that the altered in vivo metabolic environment induced by rhGH therapy (ie, changes in body composition and SI) impacts β-cell function. Finally, following acute withdrawal of rhGH therapy, insulin sensitivity improved and insulin secretion decreased, while body composition was maintained.

To date, there have been only a few studies detailing β-cell function in GHD subjects before and after rhGH therapy. These studies used oral[7,8] or IV[2,4] glucose but either did not determine concurrent insulin sensitivity for each individual[7,8] or failed to include matched control subjects.[2,4] Given the presence of severe insulin resistance and significant dyslipidemia[2,6,30] in GHD subjects and their relationship to the metabolic insulin resistance syndrome,[2,6,7] an accurate assessment of β-cell function and hyperinsulinemia in such subjects is important, particularly in view of the higher prevalence of atheromatous disease and premature atherosclerosis in young GHD subjects.[31] Thus, Beshyah et al,[7] in a large series of GHD subjects, reported normal insulin levels post–oral glucose but significant glucose intolerance in 40% of the subjects, particularly women, compared with an age-, sex-, and BMI-matched control group. In a subsequent report, these investigators demonstrated that following 6 to 18 months of rhGH therapy, their GHD subjects developed significant hyperinsulinemia during an OGTT but with a minor deterioration in overall glucose tolerance.[8] Similar post-glucose hyperinsulinemia was noted in 3- to 6-month rhGH-treated GHD subjects when IV glucose was used,[2,4] with the 25% to 40% glucose-induced increase in insulin being just significant. Only minor or no changes in glucose tolerance occurred.[2,4] In contrast, in the present study in which GHD subjects were a decade younger than Beshyah's patients (~40 years),[7,8] glucose tolerance was normal in all GHD subjects both before and after GH therapy, as were the absolute acute insulin responses to oral and IV glucose and IV glucagon. In fact, pre–rhGH treatment, fasting glucose and HbA_{1c} were significantly lower than in the matched controls. Taken together, the present data and previous data from OGTT, IV glucose, and IV glucagon studies indicate that β-cell function is probably adequate in pre– and post–rhGH-treated GHD subjects to ensure normal glucose tolerance in younger (≤ fourth decade) patients, despite the presence of insulin resistance and the disturbed relationship to insulin sensitivity but perhaps reflecting the improvement in body composition following rhGH treatment.

The importance of the fasting insulin data as a test of β-cell function is more controversial[32,33] since it may better reflect the subjects' insulin sensitivity status.[32] Fasting insulin in the pretreated GHD subjects was similar to that in the matched controls in the present study and previous studies,[2,3,6,7] and in fact was reported as reduced in GHD subjects when using a highly specific insulin immunoassay that distinguishes between true insulin, proinsulin, and proinsulin split products.[34] However, C-peptide levels were not measured in that study, and when C-peptide was measured, the levels have been similar to matched controls.[2,6] Following 3- to 6-month rhGH treatment, fasting basal insulin, C-peptide, and the ISR were initially increased, as noted here and in other studies,[2,4,8,15,34] which could be interpreted as indicating "normal" β-cell function, ie, an appropriate response to the documented diminished insulin sensitivity.[4,15] However, by 24 months, there was a downward and leftward shift of FI versus SI, consistent with an abnormality of basal β-cell function (Fig 3). Beshyah et al[34] showed that fasting pre– and post–rhGH therapy proinsulin and proinsulin split products comprised a greater proportion of net plasma insulin immunoreactivity (~11% and ~18.5%, respectively) than observed in the matched controls (~7.2%) using their specific insulin assay, which confirmed the findings of Weaver et al.[4] They argued that this signaled disordered β-cell function.[34] However, the relative proportion of proinsulin and proinsulin split products probably does not reflect a defect in β-cell function, given the considerable differences in the half-life of the circulating peptides.[35] What is important is that there is an apparent attenuation of basal and stimulated β-cell function in long-term rhGH-treated subjects.

Although in the present study SI in GHD subjects was not statistically different from our matched controls, SI was in the lower tertile compared with a normal population,[1,36,37] and this decreased significantly with rhGH therapy.[4,15] However, SI in the GHD subjects was identical to that reported in our prior GHD study (when expressed in the same units) in which similarly aged but leaner (BMI, 24.6 ± 1.1 kg/m^2) subjects were investigated.[6] Thus, it seems that the effect of obesity (27.5 ± 1.3 kg/m^2, present study) on SI is evident only in the healthy control subjects, not in GHD subjects. This confirms our original observation that the degree of obesity is only a minor factor in the pathogenesis of the insulin resistance of GHD.[6] Given the effect of obesity on SI in the control subjects, the

predicted inverse relationships between the various parameters of insulin secretion (FI, AIR_G, and $\Delta AIR_{OGTT}/\Delta G$) and SI[36,37] were present for the control group. These relationships either were not present or were poor in GHD subjects both before and particularly after rhGH therapy. The reason is not evident from the current study, but may be due to differences in insulin clearance (although insulin clearance is normal in rhGH-treated subjects[2,15]) or direct effects of rhGH on β-cell function.[18,20]

GH has been reported to have acute positive trophic effects on in vivo and in vitro β-cell function, including specific receptor binding of GH to the β cell, increased insulin biosynthesis and secretion, and enhanced β-cell DNA synthesis and β-cell growth, particularly for neonatal β cells.[18] This has led to the postulate that GH is essential for normal growth and maturation of neonatal β cells.[18] This trophic effect includes increased insulin and proinsulin production following short-term (~1 week) exposure to GH in normal dogs,[20] with processing of proinsulin to insulin probably being normal.[18] In contrast, chronic exposure (~1 month) of dogs to GH reduces insulin secretion despite the coexisting reduction of insulin sensitivity.[20] A similar situation occurs in acromegalic patients, who, early in the natural history of the disease, display enhanced basal and glucose-stimulated insulin secretion but later develop progressive β-cell failure.[21] These data are consistent with the present observation of attenuation of β-cell function over time in rhGH-treated subjects. An alternate explanation for the attenuation of insulin secretion could be that the chronic GH-induced increase in IGF-1 permanently influences β-cell function in an adverse manner,[18] although acute increases in IGF-1 are known to decrease insulin secretion in a reversible manner.[38] Interestingly, the β-cell responses observed here to chronic GH exposure are similar to the responses noted for the β cell following chronic exposure to excessive FFA and/or increased intracellular long-chain FFA,[39] ie, enhanced basal insulin levels accompanied by a blunted acute response of the β cell to various secretagogues.[39] Thus, it may be postulated that before treatment, the β cell is exposed to enhanced FFA flux from the excessive central fat deposits in the presence of low GH and low IGF-1 levels. In contrast, after treatment with GH, the enhanced availability of FFA on the β cells occurs with the increase in GH-induced lipolysis. These differences in the in vivo metabolic environment of the β cell may account for the apparent detrimental effect of long-term excessive GH exposure on β-cell function[20,21] and the loss of the expected relationship between β-cell function and insulin sensitivity.[1,36] Moreover, the importance of FFA metabolism to the ongoing insulin resistance observed in rhGH-treated GHD adults has been recently stressed,[15] and may suggest a common FFA mechanism for changes in insulin secretion and insulin action in rhGH-treated subjects.

As previously discussed for untreated and rhGH-treated subjects, other replacement hormones such as T_4, cortisol, and sex steroids may have influenced the degree of insulin resistance in these subjects, although the influence of these hormones is probably minor.[2,6,15] Whether these hormones directly influence β-cell function separate from the indirect effect of the rhGH-induced change in insulin sensitivity is uncertain. The cortisone replacement doses used are unlikely to directly affect β-cell function.[40,41] In established thyrotoxic patients, first- and second-phase insulin release are increased, but not to greater degree than expected for the accompanying insulin resistance.[42] It is also unlikely that replacement doses of sex steroids directly impact β-cell function or insulin action in these GHD adults.[43,44]

Following 5 days of rhGH withdrawal, there was a clear improvement in insulin sensitivity (expressed as FI, SI, or GINF) and a decrease in insulin secretion (expressed as fasting C-peptide, AIR_G, or $AIR_{HyperG.GON}$). Since body composition, particularly FM, and serum lipids did not change, these responses most likely represent a release from the tonic effects of rhGH and/or "elevated" IGF-1, the latter now being in the mid- to high-normal range for adults. Three other studies have found similar results but used pharmacologic short-term doses of rhGH.[45-47] The present study is the first to demonstrate that even after 2 years of "replacement" GH therapy, the acute metabolic impact of daily rhGH administration adversely affects insulin action and promotes tonic basal hyperinsulinemia in GHD-treated adults. These data also imply that the currently used doses of rhGH may be too high.[15,48]

In conclusion, the present data demonstrate that chronic rhGH therapy enhances fasting and glucose-induced insulin secretion in GHD subjects over the short-term but with probable attenuation of β-cell function in time. These changes occur despite the favorable effects on body composition and serum lipid status. Whether these changes induced by GH therapy in glucose and insulin secretion will lead to some "exhaustion" of the β cell following long-term exposure to rhGH is unclear. Also, determining whether these results arise from the dose or manner in which rhGH is currently administered deserves further study, as does determining whether the changes in the insulin sensitivity–β-cell relationship are directly attributable to the rhGH therapy and/or the ongoing insulin-resistant state.

ACKNOWLEDGMENT

This study forms part of the MD thesis requirement of N.K., who, with F.L.H., was a Novo Nordisk Research Fellow. The technical support of Marion Oakley and the untiring secretarial services of Elke Quennell are greatly appreciated.

REFERENCES

1. Bergman RN: Towards physiological understanding of glucose tolerance: Minimal model approach (Lily Lecture). Diabetes 38:1512-1527, 1989

2. O'Neal DN, Kalfas A, Dunning PL, et al: The effect of three months of recombinant human growth hormone (rhGH) therapy on insulin and glucose–mediated glucose disposal and insulin secretion in GH-deficient adults. J Clin Endocrinol Metab 79:975-983, 1994

3. Hew FL, Koschmann M, Kreiger T, et al: Insulin tolerance test in patients with anterior pituitary hormone deficiency: Reduced insulin sensitivity. Endocrinol Metab 2:93-98, 1995

4. Weaver JU, Monson JP, Noonan K, et al: The effect of low dose recombinant human growth hormone replacement on regional fat distribution, insulin sensitivity, and cardiovascular risk factors in hypopituitary adults. J Clin Endocrinol Metab 80:153-159, 1995

5. Johansson JO, Fowelin J, Landin K, et al: Growth hormone–deficient adults are insulin resistant. Metabolism 44:1126-1129, 1995

6. Hew FL, Koschmann M, Christopher M, et al: Insulin resistance in growth hormone deficient adults: Defects in glucose utilisation and glycogen synthase activity. J Clin Endocrinol Metab 81:555-564, 1996

7. Beshyah SA, Henderson A, Niththyananthan R, et al: Metabolic abnormalities in growth hormone–deficient adults. II. Carbohydrate tolerance and lipid metabolism. J Endocrinol Metab 1:173-180, 1994

8. Beshyah SA, Henderson A, Niththyananthan R, et al: The effects of short and long term growth hormone replacement therapy in hypopituitary adults on lipid metabolism and carbohydrate tolerance. J Clin Endocrinol Metab 80:356-363, 1995

9. Rosén T, Bosaeus I, Tölli J, et al: Increased body fat mass and decreased extracellular fluid volume in adults with growth hormone deficiency. Clin Endocrinol (Oxf) 38:63-71, 1993

10. Merrimee TJ, Rabinowitz D, Rimoin DL, et al: Isolated human growth hormone deficiency. III. Insulin secretion in sexual ateliotic dwarfism. Metabolism 17:1005-1111, 1968

11. Rosén T, Johansson G, Hallgren P, et al: Beneficial effects of 12-month replacement therapy with recombinant human growth hormone to growth hormone deficient adults. Endocrinol Metab 1:55-66, 1994

12. Russell-Jones DL, Watts GF, Weissberger A, et al: The effect of growth hormone replacement on serum lipids, lipoproteins, apolipoproteins and cholesterol precursors in adult growth hormone deficient patients. Clin Endocrinol (Oxf) 41:345-350, 1994

13. Whitehead HM, Boreham C, McIrath EM, et al: Growth hormone treatment of adults with growth hormone deficiency: Results of a 13-month placebo controlled cross-over study. Clin Endocrinol (Oxf) 36:45-52, 1992

14. Fowelin J, Attvall S, Bengtsson BA: Effects of treatment with recombinant human growth hormone on insulin sensitivity and glucose metabolism in adults with growth hormone deficiency. Metabolism 42:1443-1447, 1993

15. Christopher MJ, Hew FL, Oakley M, et al: Defects of insulin action and skeletal muscle glucose metabolism in growth hormone–deficient (GHD) adults persist following 24 month recombinant growth hormone (rhGH) therapy. J Clin Endocrinol Metab 83:1668-1681, 1998

16. Hew FL, O'Neal D, Kamarudin N, et al: Growth hormone deficiency and cardiovascular risk. Baillieres Clin Endocrinol Metab 12:199-216, 1998

17. Bengtsson BA, Eden S, Lonn L, et al: Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. J Clin Endocrinol Metab 76:309-317, 1993

18. Nielsen JH, Lernmark A, Ole D, et al: Effects of growth hormone on the endocrine pancreas, in Isaksson O (ed): Growth Hormone—Basic and Clinical Aspects. New York, NY, Elsevier Science, 1987, pp 227-237

19. Davidson M: Effect of growth hormone on carbohydrate and lipid metabolism. Endocr Rev 8:115-131, 1987

20. Pierluissi J, Campbell J: Metasomatotrophic diabetes and its induction: Basal insulin secretion and insulin release responses to glucose, glucagon, arginine and meals. Diabetologia 18:223-228, 1980

21. Sönksen PH, Greenwood FC, Ellis JP, et al: Changes of carbohydrate tolerance in acromegaly with progress of disease and in response to treatment. J Clin Endocrinol Metab 27:1418-1430, 1967

22. Lukaski HC, Johnson PE, Bolonchuk WW, et al: Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 41:810-817, 1985

23. Svendsen OL, Hassayer C, Bermann I, et al: Measurement of abdominal and intra-abdominal fat in postmenopausal women by dual energy x-ray absorptiometry and anthropometry: Comparison with computerised tomography. Int J Obes 17:45-51, 1993

24. Carey DG, Jenkins AB, Campbell LV, et al: Abdominal fat and insulin resistance in normal and overweight women; direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. Diabetes 45:633-638, 1996

25. Phillips DIW, Clark PM, Hales CN, et al: Understanding oral glucose tolerance: Comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. Diabet Med 11:286-292, 1994

26. Hovorka R, Jones RH: How to measure insulin secretion. Diabetes Metab Rev 10:91-117, 1994

27. Lerner RL, Porte D: Relationships between intravenous glucose loads, insulin responses and glucose disappearance rate. J Clin Endocrinol Metab 33:409-417, 1971

28. Christopher MJ, Rantzau C, Ward GM, et al: Impact of variable insulinaemia and glycaemia on in-vivo glycolysis and glucose storage in dogs. Am J Physiol 29:E62-E71, 1994

29. Polonsky KS, Licinio-Paixao J, Given BD, et al: Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. J Clin Invest 77:98-105, 1986

30. O'Neal DN, Hew FL, Sikaris K, et al: Low-density lipoprotein particle size in hypopituitary adults on conventional hormone replacement therapy. J Clin Endocrinol Metab 81:2448-2454, 1996

31. Markussis V, Beshyah SA, Fisher C, et al: Detection of premature atherosclerosis by high-resolution ultrasonography in symptom-free hypopituitary adults. Lancet 340:1188-1192, 1992

32. Matthews DR, Hosker JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419, 1985

33. McCulloch DK, Kahn SE, Schwartz MW, et al: Effect of nicotinic acid–induced insulin resistance on pancreatic β-cell function in normal and streptozocin-treated baboons. J Clin Invest 87:1395-1401, 1991

34. Beshyah SA, Gelding SV, Andres C, et al: B-cell function in hypopituitary adults before and during growth hormone treatment. Clin Sci 89:321-328, 1995

35. Ahmed S, Clark A, Matthews DR: Progressive decline of β-cell function in non–insulin dependent diabetes. Curr Opin Endocrinol Diabetes 4:300-307, 1997

36. Kahn SE, Prigeon RL, McCulloch DK, et al: Quantification of the relationship between insulin sensitivity and β-cell function in human subjects: Evidence for a hyperbolic function. Diabetes 42:1663-1672, 1993

37. Henriksen JE, Alford F, Handberg A, et al: Increased glucose effectiveness in normoglycaemic but insulin resistant relatives of patients with non–insulin dependent diabetes mellitus: A novel compensatory mechanism. J Clin Invest 94:1196-1204, 1994

38. Takano K, Hizuka N, Asakawa K, et al: Effects of sc administration of recombinant human insulin-like growth factor-I (IGF-I) on normal human subjects. Endocrinol Jpn 37:309-317, 1990

39. Prenki M, Corkey BE: Are the β-cell signalling molecules malonyl CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects in obesity and NIDDM? Diabetes 45:273-283, 1996

40. Martin IK, Christopher MJ, Alford FP, et al: Distinctive but nonadditive effects of epinephrine and cortisol on determinants of glucose tolerance in dog. Am J Physiol 260:E148-E153, 1991

41. Henriksen JE, Alford FP, Ward GM, et al: The risk and mechanism of dexamethasone induced deterioration of glucose tolerance in non-diabetic first degree relatives of NIDDM patients. Diabetologia 40:1439-1448, 1997

42. Pestell R, Alford FP, Ramos R, et al: Insulin secretion, insulin sensitivity and glucose-mediated glucose disposal in thyrotoxicosis: A minimal model analysis. Clin Endocrinol (Oxf) 33:481-493, 1990

43. Bhasin S, Storer TW, Berman N, et al: Testosterone replacement

increases fat-free mass and muscle size in hypogonadal men. J Clin Endocrinol Metab 82:407-413, 1997

44. O'Sullivan AJ, Ho KK: A comparison of the effects of oral and transdermal estrogen replacement on insulin sensitivity in post-menopausal women. J Clin Endocrinol Metab 80:1783-1788, 1995

45. Jorgensen JOL, Møller J, Alberti KGMM, et al: Marked effects of sustained low growth hormone (GH) levels on day to day fuel metabolism: Studies in GH-deficient patients and healthy untreated subjects. J Clin Endocrinol Metab 77:1589-1596, 1993

46. Rosenbaum M, Gertner JM, Leibel RL: Effects of systemic growth hormone (GH) administration on regional adipose tissue distribution and metabolism in GH-deficient children. J Clin Endocrinol Metab 69:1274-1281, 1989

47. Rosenbaum M, Gertner JM, Gidfar N, et al: Effects of systemic growth hormone (GH) administration on regional adipose tissue in children with non–GH deficient short stature. J Clin Endocrinol Metab 75:151-156, 1992

48. Burman P, Johansson AG, Siegbahn A, et al: Growth hormone (GH) deficient men are more responsive to GH replacement therapy than women. J Clin Endocrinol Metab 82:550-555, 1997