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Impact of 5 years of growth hormone replacement therapy on cardiovascular risk factors in growth hormone–deficient adults

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Abstract

The benefits of long-term effects of growth hormone (GH) substitution on carbohydrate and lipid metabolism in GH-deficient (GHD) adults are still controversial. The purpose of this study was to evaluate the effects of 5 years of GH substitution on body composition, glucose and lipid metabolism, and carotid artery intima-media thickness (IMT) in GHD adults. Fourteen patients were clinically assessed every 3 months for 5 years. Serum insulin-like growth factor 1 levels, lipid profile, oral glucose tolerance test, and ultrasonography of the carotid arteries were performed at baseline, 6 months, and every year during replacement. Visceral fat was measured by computed tomographic scan at baseline and at 6, 12, 24, and 60 months. The waist circumference was reduced after 6 months but increased during the next months toward baseline values. Visceral fat decreased during the study. Fasting glucose and insulin levels did not change, as well as the homeostasis model assessment of insulin resistance index. Despite an initial increase in frequency of abnormal glucose tolerance, mean 2-hour oral glucose tolerance test glucose levels decreased during the last 2 years. There was an increase in apolipoprotein A-1 levels during the treatment. Apolipoprotein B levels were reduced after 6 months and remained stable thereafter. A reduction in carotid artery IMT was observed during replacement. We concluded that 5 years of GH replacement therapy promoted positive effects on visceral fat, lipid profile, and carotid artery IMT in GHD adults. Long-term therapy improves insulin sensitivity through a reduction in visceral fat, and continuing monitoring is mandatory in terms of glucose metabolism.

1. Introduction

Hypopituitary adults have a reduced life expectancy, with a 2-fold higher risk of death related to cardiovascular disease compared with healthy controls [1]; and growth hormone deficiency (GHD) has been considered the underlying factor influencing this increased mortality. Growth hormone deficiency produces negative effects on cardiovascular function, directly on the heart and the endothelium and indirectly via its impact on hypercoagulability, abdominal obesity, insulin resistance (IR), unfavorable lipid profile with an increase in low-density lipoprotein cholesterol (LDL-C), apolipoprotein (apo) B, and triglycerides (TG) in association with decreased levels of high-density lipoprotein cholesterol (HDL-C), atherosclerosis, decreased exercise performance, and reduced pulmonary capacity [2-9]. These alterations may contribute to an increase in premature cardiovascular morbidity in patients with hypopituitarism receiving conventional full pituitary hormone substitution other than GH [3,10,11].

Several studies have indicated favorable effects of GH replacement on all of these changes [12-18], with the exception of glucose homeostasis. Few studies have documented positive effects of GH therapy on glucose metabolism and IR [12,14,19], whereas others have shown a deterioration [15-17,20-22] or no change in insulin sensitivity during treatment [23-26], even in cases of favorable changes in body composition [16,23]. Therefore, the effects of GH substitution on carbohydrate metabolism in GHD adults are still controversial and require further research.

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The purpose of this long-term prospective study was to assess the effects of 5 years of GH substitution on body composition, glucose metabolism, lipid profile, and carotid artery intima-media thickness (IMT) in Brazilian adults with GHD.

2. Materials and methods

2.1. Subjects

Fourteen GHD adults (4 men and 10 women; age range, 33-62 years; body mass index [BMI], 24.6 ± 4.3 kg/m^2) (Table 1) were studied between 1998 and 2006. All patients had multiple pituitary deficiencies and were undergoing stable conventional replacement therapy for at least 6 months before and during the study period. Prednisone (mean dosage, 2.5-5 mg/d), levothyroxine (132.3 ± 26.1 μg/d), desmopressin (15-40 μg/d), and gonadal steroids were used as necessary. All had severe GHD for at least 12 months before replacement (maximum peak serum GH response to insulin-induced hypoglycemia and glucagon test <3 ng/mL).

Exclusion criteria included the following: GH therapy in the last 12 months, any acute severe illness during the previous 6 months, pregnancy or lactation, chronic liver or renal disease, diabetes mellitus, prior acromegaly, severe hypertension, psychiatric disease, drug or alcohol abuse, history of malignancy, and use of chronic medication (except pituitary replacement therapy, contraceptives, and treatment of mild hypertension). Individuals who developed clinical asymptomatic diabetes during the trial remained in the study and received dietary instructions.

The causes of hypopituitarism were Sheehan syndrome (8 patients), nonfunctioning pituitary adenoma (2), idiopathic (2), histiocytosis X (1), and other pituitary pathologies (1) (Table 1). Three patients had family history of type 2 diabetes mellitus in first-degree relatives, and 4 had family history of dyslipidemia.

Table 1
Age, sex, BMI, and cause of hypopituitarism in the 14 GHD patients at baseline

Number	Age (y)	Sex	Cause of hypopituitarism	BMI (kg/m^2)
1	39	Male	Idiopathic	18.98
2	35	Female	Sheehan syndrome	25.68
3	50	Male	Histiocytosis X	27.16
4	58	Female	Sheehan syndrome	22.68
5	46	Female	Sheehan syndrome	25.61
6	37	Male	Nonfunctioning pituitary adenoma	29.54
7	38	Male	Idiopathic	25.71
8	62	Female	Sheehan syndrome	18.16
9	54	Female	Nonfunctioning pituitary adenoma	25.92
20	45	Female	Sheehan syndrome	26.10
11	33	Female	Sheehan syndrome	30.62
12	36	Female	Sheehan syndrome	17.21
13	61	Female	Sheehan syndrome	21.97
14	48	Female	Other pituitary pathology, vascular lesion	29.46

2.2. Ethical considerations

Informed written consent was obtained from each patient; and the study protocol was approved by the Human Research Ethics Committee of Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro, Brazil.

2.3. Study protocol

This was an open prospective study. Patients were evaluated each month during the period of dose adjustment and then every 3 months for 5 years. Growth hormone (Norditropin; Novo-Nordisk, Denmark, 3 IU/mg) was administered subcutaneously to the patient at bedtime. The initial dose was 0.015 mg/(kg wk). The injection site was either the abdomen or the anterior thigh, according to the patient's preference, but remained constant throughout the study. Although the body weight was used to define the initial dose of GH, the ideal dose was attained with a dose titration regimen, which was based on the analysis of adverse effects and serum insulin-like growth factor (IGF) 1 levels. The maintenance dose of GH was the one that kept IGF-I levels in the upper limit of the age-related reference range (defined by the IGF-1 assay manufacturer's instructions). The mean dose at the end of the period of dose titration was 0.83 ± 0.2 mg/d. Blood samples were drawn between 8:00 and 9:00 AM after 12 hours of overnight fast. Serum IGF-1 was assessed at baseline—along with total cholesterol (TC), TG, HDL-C, LDL-C, apo A and apo B, and lipoprotein (a) (Lp[a])—and every 4 weeks until maintenance dose was reached. Thereafter, lipid profile and serum IGF-1 levels were performed at 6, 12, 24, 36, 48, and 60 months during therapy. At baseline and after 6, 12, 24, 36, 48, and 60 months, the patients underwent an oral glucose tolerance test (OGTT) and ultrasonography of the carotid arteries. The visceral fat was measured by computed tomographic (CT) scan at baseline and at 6, 12, 24, and 60 months after GH replacement. Compliance was checked by vial count; and initially, all patients were asked not to change their diet and level of physical activity.

2.4. Body composition

Body weight was measured to the nearest 0.1 kg via mechanical scales with subjects wearing light clothes. Body height was assessed barefoot to the nearest 0.5 cm by stadiometer. Body mass index was calculated as body weight (in kilograms) divided by squared height (in square meters). Waist to hip ratio (WHR) was calculated as the ratio of the narrowest waist to the widest gluteal region circumference by soft tape in the standing position. Abdominal adipose tissue was measured by CT scan using a helicoidal scanner. The technique has already been described in detail in our previous study [20].

2.5. Glucose metabolism

An OGTT was performed at 8:00 AM after a 12-hour overnight fast with 75 g of glucose monohydrate in 300 mL of water. Blood samples were collected at 0 minute for measurement of plasma glucose and insulin levels and at 120 minutes for glucose levels.

The American Diabetes Association criteria [27] were used for oral glucose tolerance classification. The IR in the fasting state was estimated by the homeostasis model assessment (HOMA) according to the formula described by Matthews et al [28]: HOMA-IR = fasting insulin (in microunits per milliliter) × fasting glucose (in millimoles per liter)/22.5.

2.6. Carotid artery ultrasonography

The evaluation was performed using a high-resolution echo-color Doppler system. This technique has previously been described in our preceding study [29].

2.7. Biochemical assays

Serum IGF-I was measured by immunoradiometric assay (DSL-5600 Active; Diagnostic Systems Laboratories, Webster, TX) with an intraassay coefficient of variation (CV) of 1.5% and interassay CV of 3.7% and reference range of 80 to 500 ng/mL. The upper limit of the age-related reference range was as follows: 30 to 40 years = 494 ng/mL, 40 to 50 years = 303 ng/mL, and 50 to 60 years = 258 ng/mL. Growth hormone was determined using an immunometric chemiluminescent assay (IMMULITE; DPC, Los Angeles, CA). The intraassay and interassay CVs were 5.8% and 5.7%, respectively, at a mean GH concentration of 3.1 ng/mL; the lowest detection limit was 0.01 ng/mL. Plasma glucose was immediately measured by the glucose oxidase method. Insulin was measured by a 2-site immunometric assay (Auto-DELFIA; Wallac, Turku, Finland) with an intraassay CV of 2.4%, interassay CV of 2.5%, cross-reactivity with proinsulin <1%, reference range of 2.34 to 26.40 μU/mL, and detection limit of 0.5 μU/mL. The TC and TG were measured by an enzymatic method (Colestat enzimático, Wiener Laboratory, Rosario, Argentina, and Ecoline 25, Merck, Whitehouse Station, NJ, respectively). The interassay CVs for TC and TG were 2.32% and 2.81%, respectively. The cholesterol content of HDL was determined using an inhibition selective method (HDL LE; Labtest Diagnostica, Minas Gerais, Brazil); the interassay CV was 2.06%. Apolipoproteins A-1 and B were measured by immunonephelometry (N antisera to human apo A-1 and B; Dade Behring, Deerfield, IL). The apo A intraassay and interassay CVs were 2.2% and 5.7% at mean levels of 158 and 145 mg/dL, respectively. The apo B intraassay and interassay CVs were 1.9% and 2.4% at mean levels of 104 and 108 mg/dL, respectively. Expected values for apo A-1 and apo B were 110 to 215 mg/dL and 55 to 140 mg/dL, respectively. Lipoprotein (a) was measured by immunonephelometry (LPA; Beckman, Galway, Ireland). The intraassay and interassay CVs were ≤5% and ≤8%, respectively. The LDL-C concentrations were calculated using the Friedewald equation [30].

Blood samples were immediately centrifuged and stored at −20°C for analysis after a 6-month maximum period. Overall, the same assay was used at baseline and during follow-up.

2.8. Statistical analysis

Statistical analysis was performed with Stata software (College Station, TX; version 7.0, 2001). Data were expressed as mean ± SD. We used analysis of variance (ANOVA) for repeated measures to analyze changes over time after log transformation, with Friedman as a complementary test when there was a high degree of non-normality in the distribution. Student paired *t* test was used to compare each parameter in time. All statistical tests were conducted based on 2-tailed alternatives. A *P* value less than .05 was accepted as significant for all analysis in the study.

3. Results

Serum IGF-I concentration was significantly increased by GH replacement throughout the study period (from 79.1 ± 67 to 187.8 ± 137 ng/mL after 60 months, $P = .0001$). At the sixth month, mean GH dosage was 0.87 mg/d (range, 0.56-1.2). The dose of GH was gradually lowered during the study to keep IGF-I within normal levels adjusted for age. At the fifth year, mean GH dosage was 0.64 mg/d (range, 0.35-1.0) (Fig. 1). No major adverse effects were observed besides edema and arthralgia at the beginning of the study.

3.1. Body composition

Despite an initial reduction in body weight, there was an overall tendency of its increase throughout time ($p = .059$). The waist circumference reduced after the first 6 months of treatment but had progressively increased during

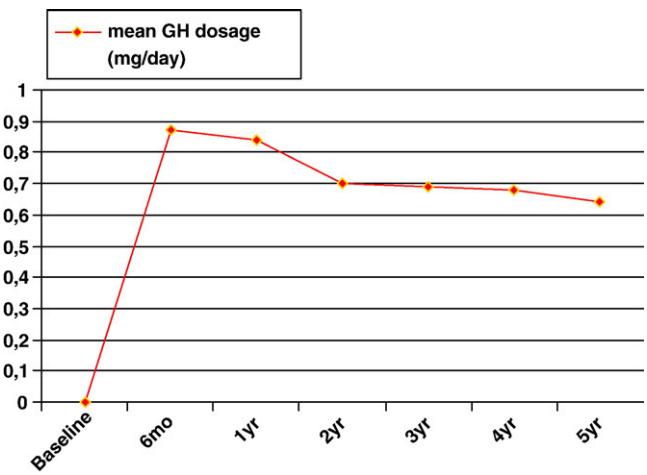

Fig. 1. The dose of GH during 5 years of GH replacement in 14 GHD adults.

Table 2
Body composition in GHD adults at baseline and after 6, 12, 24, 36, 48, and 60 months of GH replacement (N = 14)

	Baseline	6 mo	12 mo	24 mo	36 mo	48 mo	60 mo	*P*
Visceral fat (cm^2)	135 ± 55	91 ± 46	93 ± 45	95 ± 49	–	–	88 ± 42	**.001**
Waist (cm)	79 ± 9.9	76 ± 9.7	77 ± 8.2	78 ± 8.2	80 ± 9.1	78 ± 9.0	80 ± 10	**.039**
WHR	0.85 ± 0.08	0.80 ± 0.09	0.83 ± 0.07	0.82 ± 0.06	0.83 ± 0.08	0.81 ± 0.08	0.83 ± 0.08	.878
Body weight	61 ± 13	60 ± 12	60 ± 13	61 ± 13	62 ± 13	61 ± 13	63 ± 13	**.059**
BMI (kg/m^2)	24.6 ± 4.3	24.0 ± 4.2	24.2 ± 4.0	24.4 ± 3.8	25.0 ± 4.1	24.5 ± 4.1	25.4 ± 4.1	**.059**

Values are expressed as mean ± SEM. *P* values are based on ANOVA for repeated measurements (baseline vs 60 months). Boldfaced entries indicate levels of statistical significance or a tendency.

the next months toward baseline values ($P = .039$). There was no significant change in WHR. Visceral fat was reduced progressively during the 5 years of GH substitution ($P = .001$) (Table 2).

3.2. Glucose metabolism

Fasting glucose levels and the HOMA-IR did not change during the treatment period ($P = .39$ and 0.74, respectively) (Table 2). A considerable but non–statistically significant reduction in fasting insulin levels was observed in the 60th month ($P = .64$) (Table 3).

The prevalence of abnormal glucose tolerance increased from 2 patients (14.3%) at baseline evaluation to 5 (35.7%) after 6 months, 8 (57.1%) after 12 months, and 9 (64.3%) at the 24th month. Afterward, this prevalence declined to 6 patients (42.9%) after 36 months, 7 (50%) at the 48th month, and 4 (28.6%) after 60 months (Fig. 2). Therefore, regardless of the initial increase in the frequency of abnormal glucose tolerance ($p = .04$), mean 2-hour OGTT glucose levels decreased during the last 2 years of GH replacement ($p = .031$) (Table 3). Only one patient still had diabetes mellitus at the end of the study: a 59-year-old woman (BMI 25.9 kg/m^2) with impaired glucose tolerance at baseline and positive family history of diabetes.

3.3. Lipid profile and carotid artery IMT

There was a considerable increase in apo A-1 levels during the study ($p = .03$). Apolipoprotein B levels were reduced significantly after the first 6 months of treatment ($p < .001$) and remained stable thereafter. An increase in LDL-C levels occurred from 36 to 60 months ($p = .044$). No significant changes were observed in TC, HDL-C, TG, or Lp (a) levels after GH substitution (Table 4).

A progressive reduction in common carotid artery IMT and in carotid artery bifurcation IMT (Table 5) was observed during the 60 months of replacement ($p = .015$ and .002, respectively).

4. Discussion

In this open prospective study, 5 years of GH replacement therapy induced a sustained reduction in visceral fat measured by CT scan, which is in agreement with many studies [11,13,25,31]. Moreover, the waist circumference was reduced after the first months of treatment but showed a progressive increase throughout time toward baseline values. In addition, there was a tendency of an increase in body weight during the study. These findings are supported by another long-term study, in which continuous GH therapy had no effect on BMI but prevented the age-related increase in waist circumference [26].

Among numerous cardiovascular risk factors, abdominal obesity is a well-known predictor of subsequent coronary artery disease because centrally obese patients show atherothrombotic and proinflammatory abnormalities and a high risk of IR, diabetes mellitus, hypertension, and dyslipidemia [32]. It is now well established that GH replacement increases lean body mass by 2 to 5 kg while reducing body fat mass by 30%, or approximately 3 to 6 kg [31,33]. Improvement in body composition may be the single most important factor in reducing vascular risk, estimated by the Framingham model to correspond to a 3% to 4% decrease in the incidence of coronary heart disease over 10 years [25].

In the current study, fasting glucose levels and the HOMA-IR index did not change during the treatment period, whereas a considerable but non–statistically significant reduction in fasting insulin levels was observed in the 60th month. Furthermore, regardless of the initial increase in frequency of abnormal glucose tolerance, mean 2-hour OGTT glucose

Table 3
Glucose metabolism in GHD adults at baseline and after 6, 12, 24, 36, 48, and 60 months of GH replacement (N = 14)

	Baseline	6 mo	12 mo	24 mo	36 mo	48 mo	60 mo	*P*
Fasting glucose (mg/dL)	80 ± 9	91 ± 26	81 ± 10	87 ± 14	85 ± 11	82 ± 8	83 ± 9	.39
2-h OGTT glucose (mg/dL)	123 ± 62[a]	128 ± 39[a]	147 ± 49[a]	163 ± 69[a]	149 ± 56[b]	173 ± 61[b]	124 ± 53[b]	**.04[a] .03[b]**
Fasting insulin (μU/mL)	14 ± 26	11 ± 5	9 ± 6	13 ± 9	9 ± 6	14 ± 21	8 ± 7	.64
HOMA-IR	2.7 ± 5.0	3.6 ± 4.6	2.0 ± 1.5	2.8 ± 2.1	1.8 ± 1.1	2.9 ± 4.6	1.7 ± 1.6	.74

Values are expressed as mean ± SEM. *P* values are based on ANOVA for repeated measurements (baseline vs 60 months), except [a]Student paired *t* test: baseline vs 24 months and [b]Student paired *t* test: 24 months vs 60 months. Boldfaced entries indicate levels of statistical significance or a tendency.

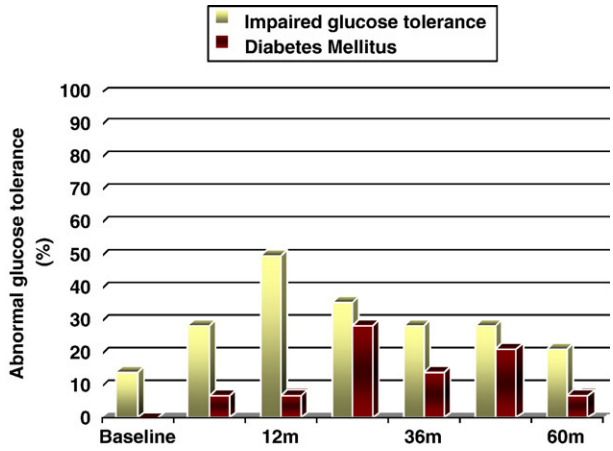

Fig. 2. Prevalence of impaired glucose tolerance and diabetes mellitus at baseline and after 6, 12, 24, 36, 48, and 60 months of GH replacement (N = 14) according to the American Diabetes Association criteria (1997).

levels decreased during the last 2 years of GH replacement. In our previous study, 24 months of GH substitution induced an increase in the prevalence of abnormal glucose tolerance, with a significant progressive increment in 2-hour OGTT insulin levels at 3, 12, and 24 months. Fasting plasma glucose levels, HOMA-IR index, and insulin sensitivity index composite did not alter during the study. Visceral fat was reduced at month 12 and remained decreased until the end of the study [34].

Insulin resistance, as determined by a decreased insulin-stimulated glucose uptake by fat and skeletal muscle, is a central feature of the metabolic syndrome associated with increased cardiovascular mortality. High levels of endogenous GH or high doses of recombinant human GH may antagonize the actions of insulin via an increase in hepatic gluconeogenesis and glycogenolysis in association with increased lipid oxidation as well a decrease in peripheral glucose utilization, which appears to be related to a postreceptor defect [35]. However, although this is a consistent effect of GH therapy, it does not mean per se that it leads to abnormal glucose tolerance and diabetes mellitus [36].

Several methods for measuring insulin sensitivity can be used in clinical research and routine clinical practice. We chose HOMA-IR index because it is a simple, low-cost method and correlates well with the glucose disposal rate derived from the hyperinsulinemic euglycemic clamp, being mostly useful for the evaluation of insulin sensitivity in euglycemic individuals and in persons with mild diabetes. Furthermore, the OGTT has still been considered a practical method for epidemiological studies, for population screening, and for large-scale intervention trials [37].

During the initial months of GH treatment, there is an initial deterioration of IR that could be restored to baseline values after several months of treatment. A generally accepted hypothesis for the return of insulin sensitivity in the direction of baseline values after 3 to 12 months of GH replacement is the favorable effects of GH on body composition, such as an increase in muscle mass and a sustained reduction in visceral fat [14], as well as improvements in well-being and physical activity level. Christopher et al [24] showed that insulin sensitivity appears to be unchanged, compared with baseline, for up to 2 years of GH substitution. In another 5-year prospective study [14], the blood glucose concentration was increased throughout the study period, whereas the serum insulin levels were unaffected by GH treatment. At 5 years, the serum glycated hemoglobin concentration was reduced compared with the baseline value. A meta-analysis [31] of blinded, randomized, placebo-controlled trials of GH in adults with GHD published up to August 2003 showed that GH therapy significantly increased plasma glucose and insulin levels and does not support the proposal that IR falls during low-dose and long-term GH treatment.

The doses of GH used in early studies were higher than the dose recommended now [38], and this fact may have impacted upon insulin sensitivity. Standard doses of GH replacement (GH doses titrated to normalize serum IGF-1 levels according to sex and age) lead to consistent reductions in truncal fat but have variable effects on insulin sensitivity because these doses can probably induce lipolysis. A recent open, prospective study has demonstrated that, in contrast to the standard GH dose (mean dose, 0.48 mg/d), administration of a fixed low dose of GH (0.10 mg/d) enhances insulin sensitivity and decreases fasting glucose levels with no apparent favorable effects on

Table 4
Lipid profile in GHD adults at baseline and after 6, 12, 24, 36, 48, and 60 months of GH replacement (N = 14)

	Baseline	6 mo	12 mo	24 mo	36 mo	48 mo	60 mo	*P*
TC (mg/dL)	197 ± 38	184 ± 31	194 ± 34	201 ± 31	205 ± 31	215 ± 46	217 ± 36	.19
HDL-C (mg/dL)	49 ± 14	50 ± 16	54 ± 18	57 ± 17	55 ± 19	55 ± 18	54 ± 21	.84
LDL-C (mg/dL)	120 ± 33	109 ± 35	112 ± 33	118 ± 28	122 ± 31[a]	135 ± 36[a]	141 ± 26[a]	**.06** **.04**[a]
TG (mg/dL)	141 ± 70	124 ± 67	125 ± 46	132 ± 60	139 ± 81	123 ± 68	107 ± 66	.85
Apo A (mg/dL)	152 ± 46	161 ± 35	160 ± 38	166 ± 53	162 ± 59	–	164 ± 81	**.03**
Apo B (mg/dL)	111 ± 23[b]	93 ± 16[b]	89 ± 22	94 ± 24	95 ± 30	–	89 ± 22	**.001**[b] .11
Lp(a) (mg/dL)	34 ± 27	43 ± 32	33 ± 27	35 ± 31	39 ± 32	–	24 ± 21	.80

Values are expressed as mean ± SEM. *P* values are based on ANOVA for repeated measurements (baseline vs 60 months), except [a]Student paired *t* test: 36 months vs 60 months and [b]Student paired *t* test: baseline vs 6 months. Boldfaced entries indicate levels of statistical significance or a tendency.

Table 5
Carotid artery IMT in GHD adults at baseline and after 6, 12, 24, 36, 48, and 60 months of GH replacement (N = 14)

	Baseline	6 mo	12 mo	24 mo	36 mo	48 mo	60 mo	*P*
Cca IMT (cm)	0.076 ± 0.02	0.077 ± 0.01	0.074 ± 0.01	0.07 ± 0.01	0.076 ± 0.03	0.067 ± 0.01	0.065 ± 0.02	**.015**
CaB IMT (cm)	0.084 ± 0.02	0.079 ± 0.02	0.079 0.02	0.077 ± 0.01	0.080 ± 0.02	0.068 ± 0.01	0.069 ± 0.01	**.002**

Values are expressed as mean ± SEM. *P* values are based on ANOVA for repeated measurements (baseline vs 60 months). Cca indicates common carotid artery; CaB, carotid artery bifurcation.

body composition, nonesterified fatty acid levels, and other surrogate cardiovascular risk markers in GHD patients [39]. Another study comparing 2 different GH doses (mean daily GH dose of 0.55 and 0.45 mg, respectively) has shown similar response to GH treatment in terms of body composition, glucose homeostasis, Lp(a) levels, and blood pressure [40]. Boguszewski et al [41] have demonstrated a decrease in waist circumference and total body fat in association with an increase in muscle mass with a fixed low dose of 0.2 mg GH per day administered for 1 year, independent of achieving normal serum IGF-1 levels. However, there were no changes in truncal fat; and HOMA-IR worsened with therapy because of elevations in insulin levels. A retrospective examination of GH dosing practices over a 5-year period included 102 GHD patients and recommended that mean GH doses seldom exceed 0.6 and 1.0 mg/d in adult men and women, respectively, or 2.0 mg/d in transition patients [42]. In the present study, the weight-based dose regimen was abandoned after 1 to 2 years of treatment. This gradual lowering and individualization of the GH dose could contribute to an amelioration of glucose homeostasis in the last years of therapy.

Patients with GHD have increased blood vessel IMT, which represents one of the earliest morphological changes in the arterial wall in the developmental process of atherogenesis. Growth hormone substitution for 6 to 120 months has been associated with reduced IMT at the common carotid artery [12,43-45]. Ultrasound Doppler techniques can be used to determine the severity of atherosclerotic changes present in a vessel, and carotid IMT provides a direct measure of risk of myocardial infarction and stroke [46]. In our study, a progressive reduction in carotid artery IMT was demonstrated.

In the current study, the patients showed a considerable increase in apo A-1 levels; apo B levels were reduced significantly after the first 6 months and remained stable subsequently. Moreover, an increase in LDL-C levels occurred from 36 to 60 months. No significant changes were observed in TC, HDL-C, TG, or Lp(a) levels after treatment. In our preceding 2-year study with 29 GHD patients [29], the apo B levels decreased significantly after the first 3 months of GH treatment and remained stable until the end of the study. In addition, the female subjects presented an increase in HDL-C levels; and no differences were observed in the other lipid measurements.

Growth hormone enhances the available intrahepatic lipid substrate through its lipolytic action on fat tissue, stimulating very low-density lipoprotein (VLDL) apo B secretion. Nevertheless, GH also up-regulates the hepatic LDL receptor [47], which increases the clearance of LDL-C as well as the hepatic uptake of partially de-lipidated VLDL particles, thereby reducing the conversion rate from VLDL cholesterol to LDL-C [48].

Many studies showed that GH substitution in GHD patients can induce a sustained reduction in TC and LDL-C [14,16,40,49,50], whereas others revealed no change in these parameters throughout the therapy [26,51]. In a different way, our study has demonstrated a significant increase in LDL-C in the last 2 years of treatment regardless of a progressive reduction in abdominal fat and carotid IMT. Besides, there were no changes in diet habits, physical activity, or medications taken by all patients during treatment. It is important to emphasize that LDL-C is usually calculated indirectly from measurements of TC, TG, and HDL-C using the Friedewald formula, which is valid only in fasting samples and if TG values are <4.5 mmol/L. Besides, the errors of that method are >5% to 20% [52,53].

Results are also variable regarding other lipid measurements: HDL-C levels have increased after GH therapy in some studies [12,14,23,47] but not in others [14,16,26,48,49], whereas mean serum TG concentration was reduced [14,50] or unchanged [12,16,23,26,48,49,51] after GH treatment. Lastly, Lp(a) levels increased in most studies [49-51] but not in all [54-56].

The highly atherogenic apo B, the principal protein component of LDL-C, has so far only been under-investigated in GHD patients receiving GH substitution, as well as apo A-1 levels, the major constituent of HDL-C. A recent placebo-controlled study [55] has shown improvement in apo B and no effect on apo A-1 during treatment. Other authors [16,20,49] have found no changes in apo A-1 and apo B levels after long-term GH replacement. Some studies [15,56-58] of 6 to 12 months' duration had shown a reduction in apo B and no change in apo A-1 levels after GH treatment. In contrast, we have found a favorable apolipoprotein profile characterized by a significant increase in protective apo A-1 levels and a substantial reduction in apo B levels. Studies in cultured liver cell lines support the proposal that apo B secretion is improved by increased fatty acid uptake [59,60]. Thus, the decrease in visceral fat observed in this study may result in less fatty acids to stimulate apo B formation. The ratio of apo B to apo A-1 has repeatedly been suggested to be a simple and more accurate predictor of the severity of

coronary artery disease than the ratio of corresponding HDL to LDL levels [61-63]. Direct methods for determining apo B and apo A-1 are internationally standardized, and the errors of the techniques are <5% [52,53]. A large prospective study [64] has linked the apo B/apo A-I ratio to the risk of fatal stroke in a similar fashion as for myocardial infarction and other ischemic events. Furthermore, a recent study has shown that the apo B/apo A-I ratio is strongly associated with the presence of individual metabolic syndrome components, with the metabolic syndrome itself, and with IR, providing an additional mechanism to explain the increased cardiovascular risk in subjects with this syndrome [65].

In summary, 5 years of GH replacement promoted positive effects on visceral fat and carotid artery IMT in GHD adults. Although a significant increase in LDL-C in the last 2 years of treatment was observed, there was a sustained increase in apo A-1 levels and a decrease in apo B levels throughout the study. In relation to glucose homeostasis, there was an initial deterioration followed by a decrease in mean 2-hour OGTT glucose levels during the last 2 years of replacement in association with a noteworthy but not statistically significant reduction in fasting insulin levels in the last year of treatment. We conclude that long-term GH replacement improves insulin sensitivity through a reduction in visceral fat, and our recommendation is that continuing monitoring is mandatory in terms of glucose metabolism. Furthermore, the GH dose should be individualized and adjusted to serum IGF-1 levels and the clinical response to avoid over-replacement and an increase in IR. Long-term individual patient follow-up will be required to determine whether GH prolongs life in hypopituitary patients.

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