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ORIGINAL ARTICLE

Reduction in insulin sensitivity and inadequate β-cell capacity to counteract the increase in insulin resistance in children with idiopathic growth hormone deficiency during 12 months of growth hormone treatment

A. Ciresi · M. C. Amato · C. Giordano

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Abstract
Purpose To evaluate the performance of various indexes of insulin sensitivity and secretion and to identify the most useful indicator of deterioration of glucose metabolism in a cohort of children with growth hormone (GH) deficiency (GHD) during GH treatment.
Methods In 73 GHD children (55 M, 18 F; mean age 10.5 years) at baseline and after 12 months of treatment, we evaluated a number of surrogate indexes of insulin secretion and sensitivity. In a subgroup of 11 children we also performed an euglycemic hyperinsulinemic clamp.
Results After 12 months, a significant increase in fasting glucose ($p < 0.001$) and HbA1c levels ($p < 0.001$) was documented, despite all children remained with a normal glucose tolerance. With regard the insulin secretion, Homa-β did not show any significant change ($p = 0.073$), while oral disposition index (DIo) showed a significant decrease ($p = 0.031$). With regard the insulin sensitivity, Homa-IR significantly increased ($p < 0.001$) with a concomitant decrease in QUICKI ($p < 0.001$). ISI Matsuda showed a decrease, although not statistically significant ($p = 0.069$). In the subgroup of 11 children, the *M* value derived from clamp showed a significant decrease ($p = 0.011$) and a significant positive correlation was found between *M* value and ISI Matsuda both at baseline (ρ 0.950; $p = 0.001$) and after 12 months (ρ 0.980; $p = 0.001$) but not with Homa-IR and QUICKI.
Conclusions 12 months of GH treatment lead to a decrease in insulin sensitivity and impairment in insulin secretion relative to insulin sensitivity even without evident changes in glucose tolerance. DIo has proven to be the most useful indicator of deterioration of glucose metabolism even in cases in which the overt glucose abnormalities have not yet appeared.

Keywords Growth hormone treatment · Children · Glucose metabolism · Insulin sensitivity · Insulin secretion

A. Ciresi · M. C. Amato · C. Giordano (✉)
Section of Cardio-Respiratory and Endocrine-Metabolic Diseases, Biomedical Department of Internal and Specialist Medicine (DIBIMIS), University of Palermo, Piazza delle Cliniche 2, 90127 Palermo, Italy
e-mail: sa.75@libero.it; carla.giordano@unipa.it

Introduction

Growth hormone (GH), in addition to promote linear growth during childhood, plays a key metabolic role [1]. Is well known that untreated GH deficiency (GHD) in children, as well as in adults, is associated with cardiovascular risk factors such as abnormalities in body composition with increased visceral fat, increased peripheral inflammatory markers and dyslipidemia and GH treatment seems to exert beneficial effects on most of these alterations [2–4]. On the other hand, the anti-insulin effect of GH can induce important changes in glucose metabolism. The acute administration of GH has an early insulin-like and a later insulin-antagonist effect on carbohydrate metabolism [5–7], while continuous GH infusion induces acute insulin resistance characterized by impaired suppression of hepatic glucose production and decreased insulin-dependent glucose disposal [8–10]. GH treatment has therefore been suggested to impair glucose metabolism [9, 11, 12] and monitoring of glucose levels during GH treatment has been recommended in GHD subjects with diabetes mellitus risk factors because of the evidence of higher incidence of diabetes than the general population [13, 14].

Exhaustive studies about the mechanism by which the glucose metabolism may worsen during GH replacement treatment are still scarce. Various and conflicting surrogate indices have been used to assess the best way to evaluate it, but with discordant results. Thus, the objective of this study was to assess any difference in insulin sensitivity and secretion indexes between untreated GHD and healthy children and to evaluate the performance of these indexes, identifying the most useful indicator of deterioration of glucose metabolism, in the GHD group during a follow-up of 12 months of GH treatment.

Materials and methods

We prospectively studied 73 children (55 M, 18 F; mean age 10.5 ± 2.8 years; range 4.3–15) with isolated idiopathic GHD consecutively admitted to the Section of Endocrinology of the University of Palermo during the years 2010–2012 and treated with GH for at least 12 months. Fifty healthy subjects, matched for sex (37 M, 13 F), age (mean age 10.3 ± 2.8 years; range 5.1–13.2) and pubertal status, were recruited among children referred for the assessment of short stature as a control group of GHD children at baseline. We excluded children affected by multiple pituitary hormone deficiency or receiving any other kind of hormonal replacement treatment or drug and with a follow-up of less than 12 months. All children, even the older ones, were in the first or second stage of sexual development according to the criteria of Marshall and Tanner [15] to avoid any interference of puberty on insulin sensitivity degree. In particular, among GHD children, the pubertal status was stage I in 63 (54 M, 9 F) and stage II in 10 (8 M, 2 F) subjects at baseline, and stage I in 54 (48 M, 6 F) and stage II in 19 (14 M, 5 F) subjects after 12 months, while in the control group 44 children (39 M, 5 F) were in the stage I and 6 (4 M, 2 F) in the stage II.

The diagnosis of GHD was established by the clinical, auxological and biochemical criteria of the GH Research Society [16]. GHD was demonstrated by failure of GH to respond to the two stimuli (arginine and glucagon test) with GH peaks below 10 μg/l. The patients received GH once daily at bedtime with a pen injection system. During the follow-up, IGF levels and the growth velocity have allowed us to utilize in all children the GH dose in line with our internal fixed protocol, with an initial daily dose of 0.025 mg/kg and a gradual increase of 0.004 mg/kg/day every 3–6 months. From months 1 to 3 all children were maintained at a mean dose of 0.025 mg/kg/day, from months 3 to 9 at 0.029 mg/kg/day and from months 9 to 12 at 0.033 mg/kg/day. IGF-I levels were maintained during the entire follow-up within the normal range for age.

Study protocol

In all patients, after the diagnosis of GHD was made, at baseline and after 12 months of GH treatment, according to our fixed internal protocol, we measured body height (standard deviation SD), body mass index (BMI and BMI z-score) and waist circumference (WC).

On day 1, blood sample was drawn after an overnight fast for the measurement of fasting glucose, fasting insulin, Hemoglobin A1c (HbA1c) and IGF-I. This sample also served as the baseline sample for an oral glucose tolerance test (OGTT). Blood samples were collected every 30 min for 2 h for glucose and insulin measurements. The area under the curve (AUC) of glucose (AUC_{GLU}) and insulin (AUC_{INS}) during OGTT was calculated using the trapezoidal rule.

Estimates of basal insulin secretion included fasting insulin and the homeostasis model assessment for β-cell function index (Homa-β) [17]. The stimulated total insulin secretion was evaluated by AUC_{INS}, while the oral disposition index (DIo) was used as index of the ability of the β-cell to regulate its insulin response to stimuli based on differences in insulin sensitivity. DIo was calculated at the time 0′ and 30′ during OGTT as described [18], using the following formula, where insulin levels are expressed in IU/ml and glucose levels in mmol/L: DIo = [Δ insulin 0′–30′/Δ glucose 0′–30′] × 1/fasting insulin.

As surrogate estimates of insulin sensitivity, we considered the homeostasis model assessment estimate of insulin resistance (Homa-IR) [17], the quantitative insulin sensitivity check index (QUICKI) [19] and the insulin sensitivity index (ISI), a composite index derived from the OGTT and validated by Matsuda and DeFronzo [20]. In the control subjects, this evaluation was performed only at baseline.

In a subgroup of 11 children, the following day (day 2) an euglycemic hyperinsulinemic clamp was used to determine the insulin sensitivity. One catheter was placed in a vein on the forearm for administration of insulin and glucose and the second catheter was placed in a vein of the contralateral forearm for blood samples. The clamp was performed under standard conditions, i.e. the plasma insulin concentration was acutely raised with an insulin priming (0–3 min: 113.6 mU/m^2, 3–6 min: 80.2 mU/m^2, 7–10 min: 50.4 mU/m^2 of body surface area) for the first 10 min of the test and maintained by a continuous infusion of insulin infusion (40 mU/m^2 for the remaining 110 min). The rate of peripheral glucose utilization (*M* value) was calculated by dividing the glucose amount infused during the last 40 min by body weight measured in kilograms (milligrams per kilogram per minute). The plasma glucose concentration was held constant at basal levels by a variable glucose infusion, and under the steady state conditions of euglycemia the glucose infusion rate equalled glucose

Table 1 Clinical and biochemical features of GHD children at diagnosis (baseline) and control subjects

	Control group ($N = 50$) Mean ± SD	GHD at baseline ($N = 73$) Mean ± SD	p
Height (SD)	−1.97 ± 0.64	−2.1 ± 0.7	0.646
Growth velocity (cm/year)	4.4 ± 2.1	3.1 ± 1.4	0.112
BMI (kg/m^2)	17.52 ± 2.76	17.6 ± 3.2	0.673
BMI (z score)	0.56 ± 0.02	0.52 ± 0.10	0.125
WC (cm)	62.03 ± 9.23	60.9 ± 10.8	0.510
IGF-1 (SD)	0.5 ± 0.2	−1.8 ± 0.5	0.002
GH peak[a] (μg/l)	15 ± 6.2	3.2 ± 2.5	<0.001
Fasting glucose (mmol/L)	4.5 ± 0.7	4.5 ± 0.5	0.870
Glucose after 120′ OGTT	5.8 ± 0.8	5.4 ± 0.7	0.196
AUC_{GLU} (mmol/L)	12,685 ± 4,090	12,215 ± 1,557	0.625
HbA1c (%)	5.3 ± 0.4	4.8 ± 0.5	0.189
Fasting insulin (IU/ml)	4.9 ± 2.9	5.6 ± 6.4	0.319
AUC_{INS} (IU/ml)	5,758 ± 2,383	4,259 ± 3,868	0.697
Homa-β	117.2 ± 62.8	73.7 ± 22.6	0.268
Oral Disposition Index (DIo)	7.2 ± 8.78	9.2 ± 9.9	0.549
Homa-IR	0.99 ± 0.54	1.1 ± 1.2	0.206
QUICKI	0.4 ± 0.13	0.4 ± 0.06	0.675
ISI-Matsuda	12.7 ± 15.3	15.3 ± 11.9	0.539

AUC area under the curve, *OGTT* oral glucose tolerance test

[a] Mean GH peak after glucagon and arginine test

uptake by all the tissues in the body and it was therefore considered a measure of tissue sensitivity to exogenous insulin [21].

The institutional Ethics Committee of the University of Palermo approved this study. At the time of hospitalization, an informed consent for the scientific use of the data was obtained from both the participants and their parents.

Hormone and biochemical assays

All biochemical data were collected after overnight fasting. Glycemia and HbA1c were measured in the centralized accredited laboratories with standard methods. Serum insulin was measured by ELISA (DRG Instruments GmbH, Germany). The sensitivity of the method was 1 IU/ml. The normal insulin range (IU/ml) was 5–19. GH levels were assayed by immunoradiometric assay (Radim, Pomezia, Italy) and the sensitivity of the assay was 0.05 μg/l. The intra- and inter-assay coefficients of variation (CV) were 2.5–3.9 and 3.8–5.0 %, respectively. Serum total IGF1 was assayed in the same laboratory with the ELISA method (OCTEIA IGF-I kit, IDS Inc., Fountain Hills, AZ, USA). The sensitivity of the method was 1.9 μg/l. The inter- and intra-assay CV values were 7–7.1 and 2.3–3.5 %, respectively, at IGF-I levels of 90.7–186 and 66.7-120.9 μg/l, respectively. The normal ranges (males and females combined) of total IGF-I levels (μg/l) were: 12–108 (0–1 years); 13–100 (1–3 years); 26–280 (3–6 years); 85–230 (6–9 years); 98–404 (9–12 years); 142–525 (12–15 years); 146–415 (15–20 years).

Statistical analysis

The Statistical Packages for Social Sciences SPSS version 17 was used for data analysis. Baseline characteristics were presented as mean ± standard deviation (SD) for continuous variables. Normality of distribution for quantitative variables was assessed with the Kolmogorov–Smirnov test. Only the M value did not show a normal distribution. The differences between paired continuous variables (before and after 12 months of therapy) were analyzed by the paired t test. Pearson's correlation was performed among continuous variables with normal distribution; correlations among continuous variables without normal distribution were determined using the Spearman's test (non-parametric equivalent for Pearson test). A p value <0.05 was considered statistically significant.

Results

The clinical and biochemical features of control subjects, GHD children at diagnosis and after 12 months of GH treatment are shown in Tables 1 and 2.

Clinical and hormonal profile

No significant difference in height, growth velocity, BMI and WC between GHD children at baseline and control subjects has been found (Table 1). Conversely, GHD children at baseline showed significantly lower IGF-I levels

Table 2 Clinical and biochemical features of children at diagnosis and after 12 months of GH treatment

	Baseline ($N = 73$) Mean ± SD	12 months ($N = 73$) Mean ± SD	p
Height (SD)	−2.1 ± 0.7	−1.6 ± 0.7	<0.001
Growth velocity (cm/year)	3.1 ± 1.4	8.4 ± 2.5	<0.001
BMI (kg/m^2)	17.6 ± 3.2	18.2 ± 2.8	0.002
BMI (z score)	0.52 ± 0.10	0.59 ± 0.04	<0.001
WC (cm)	60.9 ± 10.8	64.9 ± 9.8	0.015
IGF-I (SD)	−1.8 ± 0.5	1.6 ± 1.5	<0.001
Glucose metabolism			
Fasting glucose (mmol/L)	4.5 ± 0.5	4.9 ± 0.5	<0.001
Glucose after 120′ OGTT	5.4 ± 0.7	5.8 ± 1.1	0.256
AUC_{GLU} (mmol/L)	12,215 ± 1,557	12,820 ± 3,123	0.491
HbA1c (%)	4.8 ± 0.5	5.1 ± 0.4	<0.001
Insulin secretion indexes			
Fasting insulin (IU/ml)	5.6 ± 6.4	9 ± 6.1	<0.001
AUC_{INS} (IU/ml)	4,259 ± 3,868	4,594 ± 2,439	0.536
Homa-β	73.7 ± 22.6	143.6 ± 144.5	0.073
Oral Disposition Index (DIo)	9.2 ± 9.9	4.6 ± 6.8	0.031
Insulin sensitivity indexes			
Homa-IR	1.1 ± 1.2	2 ± 1.4	<0.001
QUICKI	0.4 ± 0.06	0.3 ± 0.04	<0.001
ISI-Matsuda	15.3 ± 11.9	12.5 ± 7.3	0.069
M value[a]	7.2 ± 2.4	4.4 ± 1.5	0.011

AUC area under the curve, *OGTT* oral glucose tolerance test

[a] Euglycemic hyperinsulinemic clamp performed in a subgroup of 11 patients

(111.3 ± 56.5 vs. 207.8 ± 190.7 μg/l; $p = 0.026$) and mean GH peak after stimulus (3.2 ± 2.5 vs. 15 ± 6.2 μg/l; $p < 0.001$) than controls.

In the GHD children group the growth significantly increased after 12 months of treatment (height −1.6 ± 0.7 vs. −2.1 ± 0.7 SD; $p < 0.001$; growth velocity: 8.4 ± 2.5 vs. 3.1 ± 1.4 cm/year; $p < 0.001$), with a concomitant significant increase in BMI (18.2 ± 2.8 vs. 17.6 ± 3.2 kg/m^2; $p = 0.002$), WC (64.9 ± 9.8 vs. 60.9 ± 10.8; $p = 0.015$) and IGF-I levels (309.1 ± 173.9 vs. 111.3 ± 56.5 to μg/l; $p < 0.001$).

Glucose metabolism

No significant difference in glucose metabolism parameters between GHD children at baseline and control subjects has been found (Table 1).

All GHD children at baseline showed a normal glucose tolerance. The mean fasting and after 120 min during OGTT glucose levels were, respectively, 4.5 ± 0.5 and 5.4 ± 0.7 mmol/L, with a mean HbA1c of 4.8 ± 0.5 %. After 12 months of GH treatment, a significant increase in fasting glucose (4.9 ± 0.5 vs. 4.5 ± 0.5 mmol/L; $p < 0.001$) and HbA1c levels (5.1 ± 0.4 vs. 4.8 ± 0.5 %; $p < 0.001$) was documented, despite all children remained with a normal glucose tolerance (Fig. 1). No significant difference was found in AUC_{GLU} (12820 ± 3123 vs. 12215 ± 1557 mmol/L; $p = 0.491$).

IGF-I levels significantly correlated with HbA1c (r 0.375; $p < 0.001$) and glucose after OGTT (r 0.300; $p = 0.040$) at baseline and with HbA1c (r 0.121; $p = 0.047$) and fasting glucose (r 0.199; $p = 0.010$) at 12 months of treatment (Table 3).

Insulin secretion indexes

No significant difference in insulin secretion indexes between GHD children at baseline and control subjects has been found (Table 1).

At baseline, the mean fasting insulin levels were 5.6 ± 6.4 IU/ml, with a significant increase after 12 months of GH treatment (9 ± 6.1 IU/ml; $p < 0.001$), but without significant increase in AUC_{INS} (4,594 ± 2,439 vs. 4,259 ± 3,868 IU/ml; $p = 0.153$).

Homa-β did not show any significant change from baseline to 12 months (143.6 ± 144.5 vs. 73.7 ± 22.6; $p = 0.073$), while DIo showed a significant decrease (4.6 ± 6.8 vs. 9.2 ± 9.9; $p = 0.031$). No significant correlation between DIo and the other insulin secretion indexes evaluated was found (data not shown).

IGF-I levels significantly and positively correlated with fasting insulin both at baseline (r 0.280; $p = 0.041$) and after 12 months (r 0.395; $p < 0.001$) and negatively with DIo both at baseline (r −0.171; $p = 0.031$) and after 12 months (r −0.665; $p = 0.021$) (Fig. 1). No significant

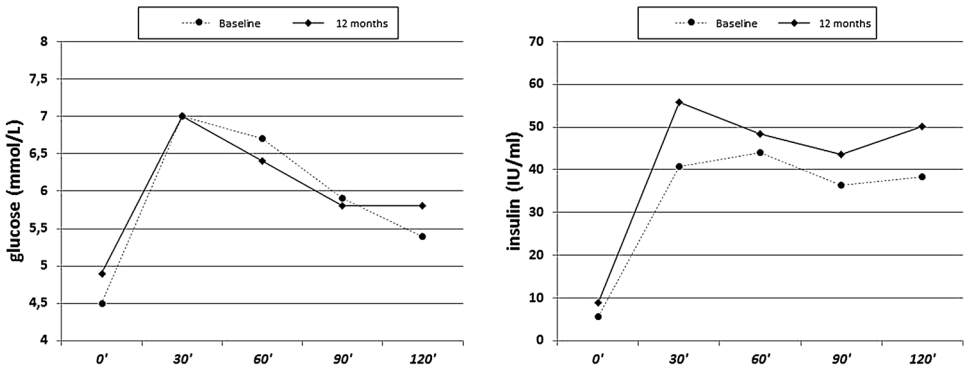

Fig. 1 Time response of glucose (mmol/L) and insulin (IU/ml) levels during oral glucose tolerance test at baseline and after 12 months of GH-treatment

correlation was found between IGF-I and the other insulin secretion indexes (Table 3).

Insulin sensitivity indexes

No significant difference in insulin sensitivity indexes between GHD children at baseline and control subjects has been found (Table 1).

Homa-IR significantly increased (2.04 ± 1.4 vs. 1.1 ± 1.2; $p < 0.001$) from baseline to 12 months of GH treatment, with a concomitant decrease in QUICKI (0.35 ± 0.04 vs. 0.41 ± 0.06; $p < 0.001$). ISI Matsuda showed a decrease, although not statistically significant (12.5 ± 7.3 vs. 15.3 ± 11.9; $p = 0.069$). A significant negative correlation was found between Homa-IR and ISI Matsuda at baseline (r −0.420; $p = 0.001$) but not after 12 months (r −0.787; $p = 0.114$) (data not shown).

IGF-I levels significantly and positively correlated with Homa-IR both at baseline (r 0.318; $p = 0.033$) and after 12 months (r 0.357; $p = 0.004$) and negatively with QUICKI (r −0.397; $p = 0.007$) and ISI Matsuda (r −0.429; $p = 0.007$) at baseline (Table 3).

Euglycemic hyperinsulinemic clamp

In the subgroup of 11 children, the *M* value derived from clamp showed a significant decrease from baseline to 12 months (4.4 ± 1.5 vs. 7.2 ± 2.4; $p = 0.011$). In addition, *M* value significantly and negatively correlated with IGF-I levels at baseline (ρ −0.845; $p = 0.008$) and after 12 months (ρ −0.788; $p = 0.012$) (Table 2). In these patients, a significant positive correlation was found between *M* value and ISI Matsuda both at baseline (ρ 0.950; $p = 0.001$) and after 12 months (ρ 0.980; $p = 0.001$) but not with Homa-IR and QUICKI (Table 4).

As additional analysis, we grouped all GHD children according to the family history of diabetes and we did not find any difference in all metabolic parameters between children with or without it. Similarly, when we performed the same analysis by grouping all patients according to gender, we did not find significant difference between males and females (data not shown).

Table 3 Correlation (univariate analysis) between IGF-I and glucose metabolism, insulin sensitivity and secretion indexes at baseline and after 12 months of GH treatment in GHD children

Independent variables	Dependent variable: IGF-I			
	Baseline		12 months	
	r	*p*	*r*	*p*
Fasting glucose	0.041	0.584	0.199	0.010
Glucose after 120′ OGTT	0.300	0.040	0.122	0.679
AUC_{GLU}	0.270	0.066	0.158	0.664
HbA1c	0.375	<0.001	0.121	0.047
Fasting insulin	0.280	0.041	0.395	<0.001
AUC_{INS}	0.312	0.057	0.103	0.934
Homa-β	0.011	0.944	0.322	0.061
Oral Disposition Index (DIo)	−0.171	0.031	−0.665	0.021
Homa-IR	0.318	0.033	0.357	0.004
QUICKI	−0.397	0.007	−0.262	0.293
ISI-Matsuda	−0.429	0.007	−0.586	0.601
M value	−0.845	0.008	−0.788	0.012

Table 4 Correlation (univariate analysis) between the gold standard euglycemic hyperinsulinemic clamp (*M* value) and the other insulin-sensitivity indexes at baseline and after 12 months of GH treatment in GHD children

Independent variables	Dependent variable: *M* value (clamp)			
	Baseline		12 months	
	ρ	*p*	ρ	*p*
Homa-IR	−0.567	0.112	−0.280	0.208
QUICKI	0.566	0.112	0.500	0.667
ISI-Matsuda	0.929	0.003	0.980	0.006

Discussion

In this prospective study performed in a large cohort of prepubertal children affected by idiopathic GHD we showed that after 12 months of GH treatment a decrease in insulin sensitivity and impairment in insulin secretion relative to insulin sensitivity occur, even without evident changes in glucose tolerance.

Decreased insulin sensitivity and impaired pancreatic β-cell function are the two key components in the pathogenesis of type 2 diabetes mellitus. Therefore, assessment of insulin sensitivity and secretion in children potentially at risk of glucose metabolism impairment, as during GH treatment, is of crucial importance. As several studies have reported, GH plays an important role in glucose and insulin metabolism, but the data about the metabolic effects of GH treatment in GHD patients are controversial.

A degree of insulin resistance in untreated GHD adults with an increased insulin sensitivity after GH therapy has been documented [22, 23] while other studies did not find any metabolic difference between untreated GHD adults and control subjects, or any variation after GH therapy [24]. In GHD children, the effect of GH therapy on insulin levels has also been reported. Indeed, a trend towards reduced insulin sensitivity with a compensatory hyperinsulinemic response or increased insulin levels, but with normal glucose levels, after GH treatment has been demonstrated [12, 25]. We already demonstrated an increase in HOMA-IR, related to increased insulin levels and without any untoward effects on glucose metabolism, in a small group of GHD children after GH treatment [26]. Our current results are in line with these data. We found an increase in insulin and Homa-IR values, correlated with IGF-I levels, with a concomitant decrease in QUICKI, after 12 months of GH treatment. The concomitant increase in fasting glucose and HbA1c levels, correlated with IGF-I, does not reach clinically significant values, as they remained within the limits of normality. In addition, only fasting glucose showed a significant increase, while no significant change was found in glucose after OGTT and in AUC_{GLU}, and these data could explain the maintenance of normal glucose tolerance after 12 months of GH treatment. These data are not consistent with those of Cutfield et al. [14], which reported from a retrospective analysis of data from an international pharmacoepidemiological survey an increased incidence of diabetes mellitus or impaired glucose tolerance in population of children and adolescents at higher risk for glucose intolerance receiving GH treatment, as if GH treatment may be an acceleration of the disorder in predisposed individuals and the metabolic impairment condition may be reversible by stopping or reducing the dose. This difference is probably also due to the short follow-up in our cohort of patients. Indeed, given the limited period of observation, our data do not exclude the possibility that a clear glucose intolerance may develop with longer duration of GH treatment. Notably, the estimates of insulin sensitivity, as well as of pancreatic β-cell function, derived from fasting insulin and glucose have not always been demonstrated to be useful surrogate measures of insulin sensitivity and secretion. The gold standard for measuring insulin sensitivity and secretion is hyperinsulinemic-euglycemic and hyperglycemic clamps, respectively [21, 27], but very few studies used these methods in GHD children. Indeed, these invasive and expensive procedures are not applicable to routine clinical practice and in a large number of patients. In our study, we also used the euglycemic clamp in a subgroup of children to evaluate the insulin-stimulated glucose utilization ant to compare it with the other insulin sensitivity indexes assessed. Our data derived from the clamp support the evidence of a decrease in insulin sensitivity after GH treatment. In healthy subjects, the insulin sensitivity state assessed by euglycemic clamp seems to strongly correlate with Homa-IR and QUICKI [28, 29]. Conversely, Schwartz et al. [30] found that surrogate measures of insulin sensitivity are only modestly correlated with the clamp measures and they concluded that these indexes do not offer any advantage over fasting insulin. In our study, among the insulin sensitivity indexes, we found a strong correlation between M value and ISI Matsuda both at baseline and after 12 months of GH treatment, while no correlation was found between the gold standard clamp and the other indexes evaluated. These data lead to the conclusion that, in our cohort of GHD children and during a follow-up of 12 months, the surrogate indexes Homa-IR and QUICKI probably do not represent useful and reliable indexes of the real degree of insulin sensitivity.

Also with regard to the evaluation of insulin secretion, the existing data in the literature about the effect of GH treatment are controversial. Heptulla et al. [12] demonstrated with the hyperglycemic clamp procedure an increase in insulin secretion after GH therapy, without change in fasting glucose. The authors concluded that glucose-stimulated insulin responses are increased in children treated with GH and that hyperinsulinemic responses compensate for reductions in insulin sensitivity and could be useful to amplify insulin effects on protein metabolism. Conversely, in adult GHD subjects GH treatment has been demonstrated to have a negative effect on the β-cell function, in terms of an inadequate adaptation in insulin secretion as the insulin sensitivity deteriorates [31]. A correlation between the insulin secretion assessed by hyperglycemic clamp and fasting insulin or Homa-β has been documented by some studies [29, 30]. Our data are partially in line with these results. Indeed, if the significant increase in fasting insulin concentrations could indicate that insulin secretion was increased by GH treatment, in our patients Homa-β did

not show a significant change after GH treatment, despite its trend to increase, as well as the total insulin secretion (AUC_{INS}). Conversely, when we analyzed the insulin secretion with DIo, which has been tested few times in GHD children, a worsening of insulin secretion relative to insulin resistance, correlated with IGF-I levels, was observed. DIo, which expresses the ability of β-cells to adequately compensate for insulin resistance through increased insulin secretion, has been shown to be a predictor of glucose levels after OGTT in obese adolescents [32, 33] and of development of diabetes in adults [18] and it has been shown to decline well before glucose levels significantly rise into the diabetic range [34, 35]. Our results are in line with these data. Indeed, we observed that after 12 months of GH treatment the impairment in insulin secretion relative to insulin sensitivity is apparent even with normal glucose tolerance. These data are also in agreement with those of Burns et al. [36], which showed a decline in β-cell function relative to insulin sensitivity with increasing fasting glucose levels in the non-diabetic range in children, and those of Jensen et al. [37], which demonstrated reduced DIo in a large cohort of children born small for gestational age after the first year of GH treatment.

In our hypothesis, if a direct trophic effect of GH on the pancreatic β-cells can not be ruled out to explain the increase in fasting insulin secretion [38, 39], the decrease in DIo can be considered an early marker of inadequate β-cell compensation to decreased insulin sensitivity. More exhaustive information about the effect of GH on β-cell function can come from future studies that will also analyze the secretion of basal and stimulated C-peptide.

A limit of this study is represented by the lack of data of the control group after 12 months of follow-up. Indeed, we can not exclude with certainty that the results are due to some other factor, in addition to the treatment with GH. For example, the impact of the deletion of exon 3 in the GH receptor gene (GHRd3) on insulin secretion and sensitivity has been documented both in healthy subjects and in GHD children, adolescents and adults [40–42] and the presence of the GHRd3 allele was associated with higher DIo [40, 41].

In addition, even if the pubertal stage did not change in all children during the entire follow-up, a role of some slightest change in DHEAS, estrogen or testosterone levels can not be ruled out, although nobody changed pubertal stage at visual inspection and no difference was found between males and females. Additional case–control studies with a larger number of patients will better clarify this aspect.

Lastly, in line with the discordant data that exist about the different metabolic effects of GH treatment according to the different dose used [43, 44], a larger prospective study where patients are randomized to different GH doses can give more complete information.

In conclusion, 12 months of GH treatment in GHD children, regardless of family history of diabetes, lead to a trend of worsening of glucose metabolism, which still remains in the normal range, associated with a significant reduction in insulin sensitivity and with an inadequate β-cell capacity to counteract the increase in insulin resistance. The surrogate Homa-IR and QUICKI probably do not represent useful and reliable indexes of the real degree of insulin sensitivity. Conversely, DIo has proven to be the most useful indicator of deterioration of glucose metabolism. However, data about the cut-offs expressing the normal range of DIo in children and adolescents with different age and pubertal stage and strong suggestions about the behavior of this index during the pediatric age are missing. Therefore, the use of DIo, which in the current study seemed useful to show a degree of glucose metabolism impairment even in cases in which the overt glucose abnormalities have not yet appeared during the follow-up of GH-treated children, must be validated in additional prospective larger studies with longer follow-up.

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Conflict of interest All the authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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