

# Effects of GH replacement therapy on thyroid volume and nodule development in GH deficient adults: a retrospective cohort study

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## Abstract

**Objective:** Despite the well-known effects of GH/IGF1 signaling on the thyroid, few data are available on the risk of developing nodular goiter in hypopituitary subjects during GH replacement therapy (GHRT). We aimed to define the effects of GH therapy on thyroid volume (TV) and nodular growth.

**Design:** The records of 96 subjects (47 males and 49 females, median age 48 years) with GH deficit (GHD) were investigated. Seventy also had central hypothyroidism (CH). At the time of our retrospective evaluation, median treatment duration was 5 years.

**Results:** Pre-treatment TV was smaller in GHD patients than in healthy subjects ( $P=0.030$ ). During GH treatment, TV significantly increased ( $P=0.016$  for the entire group and  $P=0.014$  in euthyroid GHD patients). Before starting GH therapy, 17 patients harbored thyroid nodules. During GH therapy, nodule size increased slightly in seven patients, and new thyroid nodules occurred in nine patients. Among the 79 patients without pre-existing thyroid nodules, 17 developed one or more nodules. There was no difference in the prevalence of CH in GHD patients with or without thyroid nodules ( $P=0.915$ ;  $P=0.841$ , when patients with pre-therapy nodular goiter were excluded), the main predictor for nodule development being serum IGF1 ( $P=0.038$ ).

**Conclusions:** GHRT is associated with TV's increase in GHD patients. Thyroid nodules developed in 27% of patients, mainly in relation to pre-therapy IGF1 levels, independently of normal or impaired TSH stimulation.

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## Introduction

Growth hormone (GH)/insulin-like growth factor 1 (IGF1) signaling influences thyroid function and morphology. IGF1 receptors are abundantly expressed in thyroid cells (1, 2), and IGF1 is a proliferative factor for thyrocytes (3, 4, 5, 6, 7, 8). Nevertheless, growth of thyroid cells is thought to be promoted both *in vivo* and *in vitro* by growth factors only in the presence of thyrotropin (TSH), which represents the main factor (4, 5, 8, 9, 10).

The prevalence of diffuse or nodular goiter in acromegaly is dramatically high (up to 70–100% of patients, in different studies) and the risk of developing thyroid cancer is increased (up to 6%) in patients with active disease (11, 12, 13, 14, 15, 16, 17, 18). IGF1 excess is implicated in the development of goiter, as sera of active and untreated acromegalic patients potentiate the proliferative effect of TSH on thyrocytes *in vitro* (19). However,

the occurrence of goiter in acromegaly was reported even when serum TSH levels were low, as in central hypothyroidism (CH), thus suggesting that increased GH/IGF1 levels *per se* may lead to thyroid growth (11, 14).

On the other hand, the consequences of GH deficiency (GHD) are difficult to assess, given that it is often associated with a partial or complete failure of TSH secretion. Cheung *et al.* (20) reported that thyroid size in a group of hypopituitary adults, lacking both GH and TSH, was smaller than that in controls (20). In such patients, 6–12 months of GH replacement therapy (GHRT), with restoration of IGF1 levels to normal, did not result in an increase in thyroid size or thyroid nodule development, in the absence of TSH. The authors concluded that IGF1 cannot independently stimulate thyroid growth *in vivo*, but exerts a promoting effect on thyroid cell proliferation by increasing the mitogenic action of TSH. Nevertheless, the presence of smaller thyroid volume (TV) in patients with isolated GHD compared with normal subjects and its correlation with serum IGF1 levels were demonstrated in the adult members of a Brazilian kindred with isolated GHD (21), suggesting that GH/IGF1 may affect normal thyroid growth even in the presence of normal TSH. In a subsequent study carried out on a small cohort of 20 patients from the same Brazilian pedigree, the authors demonstrated that TV significantly increased after a 6-month-duration GHRT and remained elevated also after a 6-month wash-out, confirming the important role of the GH-IGF1 axis on thyroid cell proliferation, but further underlying how thyroid growth needs the presence of TSH, which enhances the effect of IGF1 (22).

No data are available from literature concerning the risk of developing thyroid nodules or tumors during long-term GHRT. Herein, we retrospectively investigated the effects of GHRT on thyroid morphology in a series of hypopituitary adults affected by GHD.

## Subjects and methods

### Patients

The records of 96 patients (47 males and 49 females, median age 48 years, range 16–78 years) with childhood-onset (CO,  $n=28$ ) and adulthood-onset (AO,  $n=68$ ) GHD were studied. Diagnosis had been made, according to the current guidelines (23). GHD was idiopathic in 13 CO patients (one patient being affected by congenital panhypopituitarism due to septo-optic dysplasia diagnosed at age 6), and in the other 83 cases acquired after surgery and/or radiotherapy in the pituitary region ( $n=15$ , of

whom 12 affected by pituitary adenomas, two by craniopharyngiomas, and one by meningioma), or due to pituitary macroadenomas ( $n=28$ ), empty sella ( $n=19$ ), craniopharyngiomas ( $n=15$ ), granulomatous or autoimmune hypophysitis ( $n=4$ ), or Rathke's cleft cysts ( $n=2$ ). GHD was isolated in 22 patients and associated with other deficiencies in the remaining 74. The 19 patients with empty sella were all affected by multiple pituitary hormones deficiencies; of them, seven had a post-traumatic hypopituitarism and three females were affected by Sheehan syndrome. Seventy patients had CH. The diagnosis of CH was made based on low free thyroxine ( $FT_4$ ) levels and normal or low TSH levels in most patients, or based on TSH response after a thyrotropin-releasing hormone test in a minority of patients with moderate CH ( $n=16$ ), according to the currently accepted criteria (24). Five out of 96 patients (5.2%) showed positive Tg-Ab and/or TPO-Ab, the female:male ratio being 4:1.

Recombinant GH was started at a dose of 4–6  $\mu\text{g}/\text{kg}$  per daily. When necessary, the dose was increased in order to achieve and maintain a serum IGF1 level between 0 and 2 SDS for gender and age, with no evident side effects being present. The median GH dose was 4.30  $\mu\text{g}/\text{kg}$  per daily in men and 5.65  $\mu\text{g}/\text{kg}$  per daily in women; the maximal dose was 6.0  $\mu\text{g}/\text{kg}$  per daily in men and 8.0  $\mu\text{g}/\text{kg}$  per daily in women. At the time of our retrospective evaluation, median duration of disease was 7 years (range 1–34) and median GHRT duration was 5 years (range 1–30). While patients were under GHRT, conventional hormone replacement therapy with levothyroxine ( $L-T_4$ ; mean dosage,  $1.5 \pm 0.5$   $\mu\text{g}/\text{kg}$  body weight), cortisol, sex steroids, and desmopressin was also administered when appropriate (25).

In these patients, data on thyroid morphology both before starting and during GHRT were evaluated in order to assess: i) changes in TV; ii) *de novo* appearance of nodules and/or volume increase in pre-existent nodules; and iii) occurrence of thyroid cancer, during a prolonged follow-up. All patients were from areas of mild-to-moderate iodine deficiency in Southern Italy (North-Eastern Sicily). In this area, the estimated frequency of thyroid nodules in the general population is about 13%, ranging from 4% up to 28% in an age-dependent manner (26). For this reason, we routinely perform a thyroid ultrasonography (US) in the setting of endocrine evaluation of patients referred to our Outpatient Clinic. Unfortunately, due to the retrospective nature of our study, data on urinary iodine content were unavailable for most patients.

A group of 100 age- and sex-matched healthy subjects from the same geographic area, in which TV had been previously estimated (27), were used as controls.

Anthropometric parameters of these subjects have been recorded in our database and were not statistically different from those of our study population (median BMI: 26.5 kg/m<sup>2</sup> vs 29 kg/m<sup>2</sup> respectively;  $P=NS$ ). The study was approved by the Local Ethics Committee.

### Hormone measurements

Serum GH and IGF1 levels were measured by chemoluminescent immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The mean of at least four determinations of serum IGF1, assayed during the last year before our retrospective evaluation, was calculated for each patient. To compare IGF1 levels in patients of different ages, these levels were normalized and expressed as s.d. from the mean of age-matched healthy subjects (IGF1-s.d.), as previously reported (28). Calculation of IGF-s.d. was based on the normally distributed serum IGF1 values in our population, as demonstrated by Kolmogorov–Smirnov test ( $P=0.239$ ).

Serum FT<sub>4</sub>, free tri-iodothyronine (FT<sub>3</sub>), and TSH concentrations were measured by immunoenzymatic method (commercial kits by Medical Systems (Genoa, Italy); normal values in our laboratory: 10.3–24.6 pmol/l for FT<sub>4</sub>, 2.18–4.2 pg/ml for FT<sub>3</sub>, and 0.4–4.0 mU/l for TSH). Tg-Ab and TPO-Ab were measured by IRMA (DiaSorin, Saluggia, Italy; normal values <100 and <10 U/ml respectively).

Finally, serum fasting insulin was measured by immunoassay method with commercial kits (normal values in our laboratory 3–35  $\mu$ IU/ml).

All samples were processed centrally in the laboratory of our University Hospital of Messina. For all assays, the intra- or the inter-assay CV were <5 and <10% respectively.

### Thyroid imaging

Thyroid US was routinely performed in such patients every 12 months for the entire duration of follow-up, using a real-time 2D apparatus (General Electric Healthcare, Fairfield, CT, USA) with a 7.5–10 MHz linear transducer at the Radiology Unit of our University Hospital of Messina. Owing to the long duration of follow-up, US was not always performed by the same person on every patient, but all exams were recorded in the database of the Radiology Unit and were, therefore, re-read and compared by the same operator, who was unaware of the therapy assumed by the patients, at the time of our retrospective evaluation. The US data obtained during the last year before our retrospective evaluation were used for comparison with the pre-therapy data.

TV was calculated as the sum of volumes of the two lobes, each estimated with the ellipsoid formula ( $\pi/6 \times \text{height} \times \text{width} \times \text{depth}$ , each diameter being expressed in centimetres). Any nodular lesions were recorded, and the nodule sizes were reported in terms of their largest diameter in millimetres. When US revealed nodule(s) of  $\geq 10$  mm in maximum diameter, a thyroid scintigraphy was performed in order to exclude autonomously functioning nodules, as commonly used in iodine deficient areas (27).

### Statistical analyses

Numerical data are expressed as median and interquartile difference (ID), and categorical variables as number and percentage. The examined variables did not present normal distribution, as verified by the Kolmogorov–Smirnov test; consequently, the non-parametric approach was used. When comparing data among patient groups, we used the Mann–Whitney  $U$  test and  $\chi^2$ -tests, as appropriate. The Wilcoxon's test was applied to evaluate the differences between pre- and post-GHRT data. Correlations were estimated by the Spearman's rank coefficient.

Logistic regression models (LRM) were estimated (29). In univariate and multivariate analyses by LRM, the dependent variables were the variation in TV and the appearance of thyroid nodules during GHRT. The independent variables of adjustment were age, sex, AO or CO onset of the disease, coexistence of CH, serum levels of TSH, insulin and IGF1 at diagnosis, duration of the disease, GH dose and treatment duration. Firstly, we estimated univariate models, subsequently a multivariate model was estimated inserting only the significant variables that resulted from the univariate approach.

Statistical analyses were performed using SPSS 11.0 for Window package.  $P < 0.05$  was considered to be statistically significant.

## Results

### Hormonal data

Hormonal data before starting GHRT and at last evaluation are reported in Table 1. Serum basal levels of IGF1 were under the normal range for age and sex in all patients (Table 1). As expected, IGF1 levels significantly increased during GHRT. At final analysis, 82 patients had reached normal IGF1 range (i.e., IGF1-s.d. between 0 and 2.0 s.d.), while eight patients had not (IGF1-s.d. <0 s.d.). Six patients showed IGF1-s.d. >2.0 s.d. No differences

**Table 1** Patients' hormonal data before and after long-term GH replacement therapy<sup>a</sup>.

	Before treatment	After recombinant GH treatment	<i>P</i> *
IGF1 <sup>b</sup>	-2.16 (0.55)	-0.05 (1.87)	<b>0.001</b>
Insulin (μU/l) <sup>c</sup>	8.20 (11.3)	8.65 (6.64)	<b>0.014</b>
TSH (mIU/l) <sup>c</sup>	0.10 (1.13)	0.05 (0.76)	0.423
FT <sub>3</sub> (pg/ml) <sup>c</sup>	3.0 (1.1)	2.88 (0.71)	0.439
FT <sub>4</sub> (pmol/l) <sup>c</sup>	14.2 (6.41)	15.8 (3.38)	<b>0.003</b>

\**P* values typed in bold are significant ( $P \leq 0.05$ ).

<sup>a</sup>Data are expressed as median and interquartile difference (ID). Comparison between medians was made by the Mann-Whitney *U* test.

<sup>b</sup>IGF1 is expressed as s.d., as specified under 'Subjects and methods'. Calculation of IGF-s.d. was based on normally distributed serum IGF1 values in our population. Serum IGF1 normal values were calculated for each decade of life, based on 485 healthy subjects, and were 89.3–457.3, 115.1–336.7, 115.0–287.8, 99.3–255.5, 85.2–228.4, 73.5–202.9, and 62.6–179.8 mg/ml for subjects in the second, third, fourth, fifth, sixth, seventh, and eighth decade of life respectively.

<sup>c</sup>Normal values in our laboratory: 3–25 μU/ml for insulin, 0.4–4.0 mIU/l for TSH, 2.18–4.2 pg/ml for FT<sub>3</sub>, and 10.3–24.6 pmol/l for FT<sub>4</sub>. Most patients were under L-T<sub>4</sub> therapy.

in IGF1 levels emerged between GHD patients with and without associated CH under replacement therapy (median -0.02 s.d. vs -0.16 s.d.;  $P=0.735$ ).

Compared with basal values, serum TSH levels did not show any significant modification during the whole period of observation (Table 1). As expected, fasting insulin levels significantly increased during GHRT (Table 1).

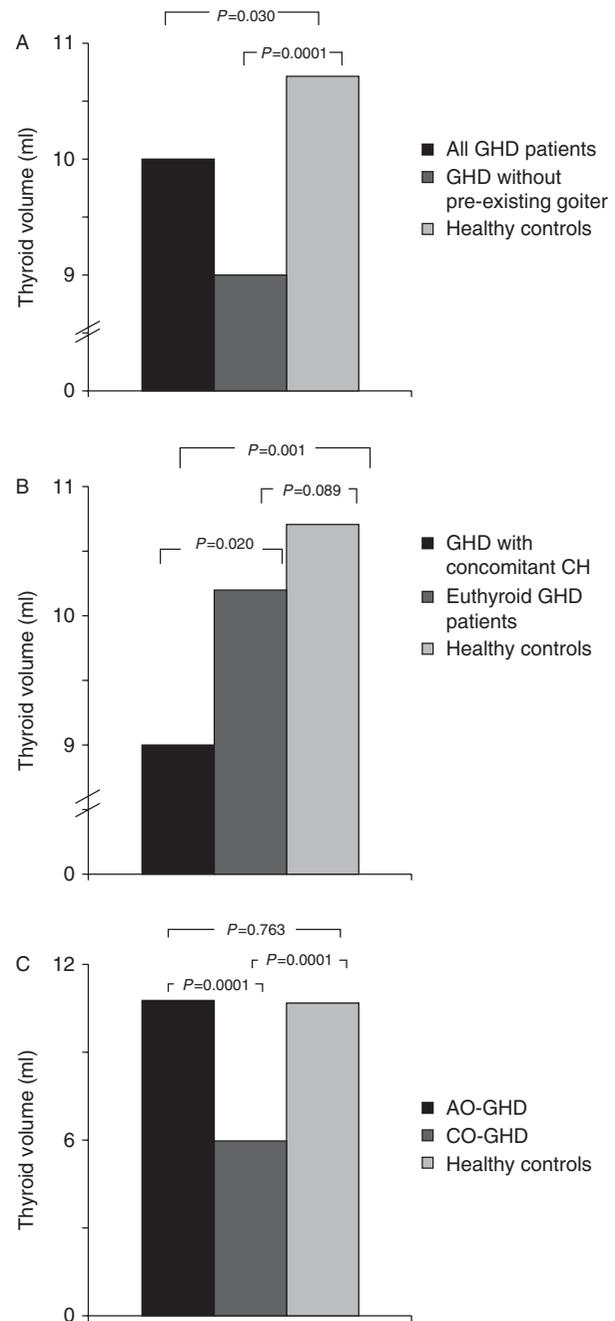
### Thyroid volume

At baseline, GHD patients had significantly smaller thyroid glands than the normal population from the same geographic area (mean ± s.d.  $9.9 \pm 5.0$  ml, median (ID) 10.0 (6.3) ml vs  $11.0 \pm 3.1$  ml, 10.7 (8.3) ml, respectively;  $P=0.030$ ). Moreover, excluding 17 patients in whom a nodular goiter was detected (see below), TV was even more significantly reduced than controls (mean ± s.d.  $8.8 \pm 4.3$  ml, median (ID) 9.0 (7.3) ml vs  $11.0 \pm 3.1$  ml, 10.7 (8.3) ml;  $P=0.0001$ ) (Fig. 1A).

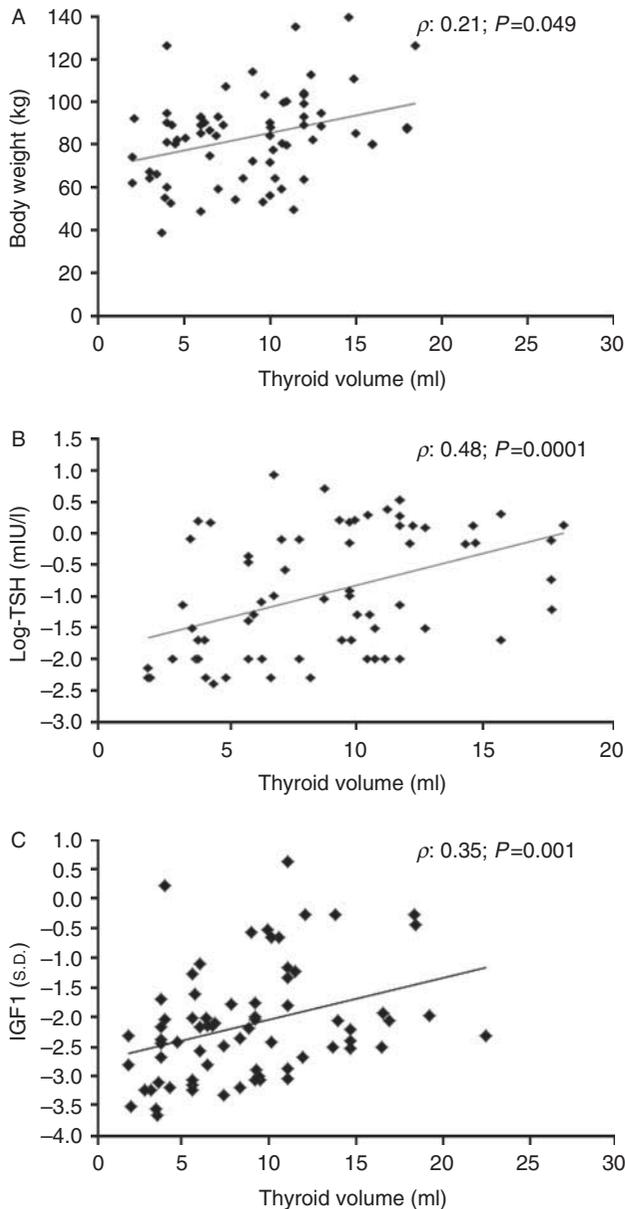
TV was significantly smaller in GHD patients with concomitant CH compared with euthyroid GHD patients (mean ± s.d.  $8.9 \pm 4.8$  ml, median (ID) 9.0 (7.4) ml vs  $10.9 \pm 4.7$  ml, median (ID) 10.2 (7.7) ml;  $P=0.020$ ), as well as to controls ( $P=0.001$ ). Also in euthyroid GHD patients, TV was smaller than that in controls, but did not reach statistical relevance ( $P=0.089$ ; Fig. 1B).

When patients were subdivided into CO- and AO-GHD, a significant difference in TV emerged. TV of CO-GHD patients (mean ± s.d.  $7.0 \pm 3.9$  ml, median (ID) 6.0 (6.1) ml) was significantly smaller than that in controls

( $P=0.0001$ ), as well as than that in AO-GHD patients (mean ± s.d.  $11.0 \pm 5.0$  ml, median (ID) 11.0 (6.1) ml;  $P=0.0001$ ), while there was no difference between AO-GHD patients and healthy subjects ( $P=0.763$ ) (Fig. 1C).

**Figure 1**

Pre-therapy thyroid volume in GHD patients, considered as a whole (A) or subdivided into patients with or without central hypothyroidism (B) and into childhood- and adulthood-onset GHD patients (C), compared with healthy subjects.

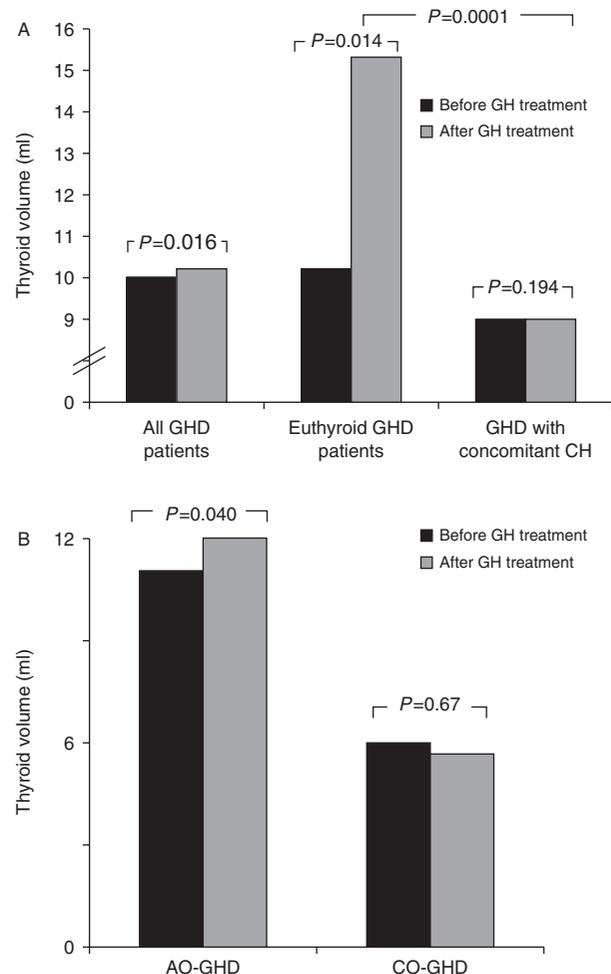
**Figure 2**

Positive correlation between thyroid volume and body weight (A), log-TSH (B), and serum levels of IGF1 (C) in GHD patients.

TV was positively correlated with body weight ( $\rho=0.21$ ;  $P=0.049$ ), serum IGF1 ( $\rho=0.35$ ;  $P=0.001$ ), and log-TSH ( $\rho=0.48$ ;  $P=0.0001$ ) levels (Fig. 2). Moreover, TV was directly correlated with the age of onset of GHD ( $\rho=0.44$ ;  $P=0.0001$ ) and inversely with disease duration ( $\rho=-0.34$ ;  $P=0.001$ ).

In our series, TV significantly increased during GHRT (mean  $\pm$  s.d.  $9.9 \pm 5.0$  ml, median (ID) 10.0 (6.3) ml vs  $10.6 \pm 5.7$  ml,  $10.2$  (8.3) ml;  $P=0.016$  for all study populations

and mean  $\pm$  s.d.  $10.9 \pm 4.7$ ,  $10.2$  (7.7) ml vs  $14.8 \pm 5.7$  ml,  $15.3$  (9.8) ml;  $P=0.014$  for GHD patients without concomitant CH) (Fig. 3A). However, the change in TV under GHRT was not significant in patients lacking both GH and TSH (mean  $\pm$  s.d.  $8.9 \pm 4.8$  ml, median (ID) 9.0 (7.4) ml vs  $9.3 \pm 5.1$  ml,  $9.0$  (7.4) ml;  $P=0.194$ ). As a consequence, after GHRT, TV was smaller in GHD patients with CH compared with those without CH (median 9.0 ml vs 15.5 ml;  $P=0.0001$ ; Fig. 3A). Moreover, subdividing the patients in CO- and AO-GHD, we found that TV significantly increased under GHRT only in AO-GHD patients (mean  $\pm$  s.d.  $11.1 \pm 5.0$  ml, median (ID) 11.0 (6.1) ml vs  $11.7 \pm 5.7$  ml,  $12.0$  (7.4) ml;  $P=0.040$ ), while it did not change in CO-GHD

**Figure 3**

Thyroid volume changes under GH therapy in GHD patients, considered either as a whole or subdivided into patients with or without central hypothyroidism (A) or into childhood- and adulthood-onset GHD patients (B).

patients (mean  $\pm$  s.d.  $7.0 \pm 3.9$  ml, median (ID) 6.0 (6.1) ml vs  $7.8 \pm 5.1$ , 5.7 (6.7) ml;  $P=0.67$ ) (Fig. 3B).

In the univariate analysis, the variables that were significantly associated with an increase in TV (the dependent variable being the post-therapy:pre-therapy TV ratio) were serum TSH values ( $\beta$ , 0.087; CI, 0.004 and 0.170;  $P=0.040$ ) and the presence of CH ( $\beta$ ,  $-1.648$ ; CI,  $-3.176$  and  $-0.120$ ;  $P=0.035$ ). In the multivariate analysis adjusted for serum TSH and presence/absence of CH, serum TSH levels were the main predictor for TV changing during GHRT ( $\beta$ , 1.009; CI, 0.034 and 1.984;  $P=0.043$ ).

### Thyroid nodules

Before starting GHRT, a nodular goiter was detected in 17 patients (12 females and five males, median age 64.0 years, range 40–78 years). These patients were older than the GHD patients without thyroid nodules ( $n=79$ , 37 females and 42 males, median age 43.0 years, range 16–76 years;  $P=0.001$ ). All these patients were affected by AO-GHD, and eight also by CH. In 11 patients, the thyroid nodules were diagnosed before onset of GHD (and of TSH deficit, when present). In this cohort of patients, TV was higher compared with GHD patients without any evidence of pre-existing thyroid disease (mean  $\pm$  s.d.  $14.9 \pm 5.2$ , median (ID) 14.2 (8.2) ml vs  $8.8 \pm 4.3$  ml, 9.0 (7.3) ml;  $P=0.0001$ ), as well as healthy controls ( $P=0.001$ ). Among these patients, ten had a single nodule and seven had a multinodular goiter. Most ( $n=14$ ) had nodules of  $\geq 10$  mm in maximum diameter (range 10–28 mm), scintigraphically 'cold', and underwent fine-needle aspiration cytology (FNAC) before starting GHRT, with the following results: colloid goiter (THYR2 according to the Bethesda system) (30) in 12 cases and lymphocytic thyroiditis in two. During GHRT, nodule size increased slightly in seven patients and appearance of new nodules was recorded in nine (two with nodule diameter  $\geq 10$  mm). The patients in whom the pre-existing nodule had increased in size, and the two patients in whom a new nodule  $\geq 10$  mm had appeared underwent FNAC, which was conclusive for colloid goiter (THYR2). None of the patients with CO-GHD was found to have goiter and/or nodules before starting GHRT.

Among the 79 patients in whom no thyroid lesions were detected before starting GHRT, 17 (ten females and seven males, median age 65.0 years, range 16–75 years) developed one ( $n=12$ ) or more ( $n=5$ ) nodules. Of these patients, 14 were affected by AO-GHD and three by CO-GHD, and 15 had also CH (13 AO and two CO).

In most, the nodule was  $< 10$  mm in maximum diameter. Seven patients who developed *de novo* thyroid nodules  $\geq 10$  mm (range 10–40 mm), scintigraphically 'cold', underwent FNAC. Cytology demonstrated colloid goiter (THYR2) in five cases. One patient had cytological features suggesting a follicular adenoma (THYR3 at FNAC), but pathological evaluation after thyroidectomy was consistent with a benign lesion. Another patient, a female affected by CO-GHD and CH, developed a papillary carcinoma (pT1<sub>b</sub>N1<sub>a</sub>Mx at histopathological evaluation).

Overall, at the last visit, thyroid nodules were detected in 34 patients (12 males and 22 females, median age 64.5 years, range 16–78 years), three CO-GHD and 31 AO-GHD. Twenty-three patients also had CH. Thus, there was no difference in the prevalence of TSH deficit in GHD patients with or without nodules (23/34 (68%) vs 44/62 (71%);  $\chi^2=0.01$ ;  $P=0.915$  or 11/17 vs 44/62 excluding patients with pre-therapy nodular goiter;  $\chi^2=0.04$ ;  $P=0.841$ ).

We divided our study population into subgroups for further evaluation. The 79 patients without pre-existing nodular goiter were subdivided in two groups based on the appearance (group A) or not (group B) of thyroid nodules during the period of observation. At the same manner, the 17 patients with pre-existing thyroid nodules were subdivided into patients in whom nodules size and/or number did not change (group C,  $n=5$ ), and patients in whom nodules increased in size or number (group D,  $n=12$ ) during GHRT. As reported in Table 2, we found that both the age at diagnosis and pre-treatment TV were significantly higher in group A compared with group B, as well as serum IGF1 levels at diagnosis. Also, despite the disease duration being similar, GHRT duration was significantly longer in group A than that in group B. Similarly, GHRT duration was significantly higher in group D compared with group C (Table 2), despite no other differences emerging, probably due to the small number of patients. Moreover, in GHD patients with pre-existing goiter, changes in the number and/or size of nodules occurred for a shorter duration of GHRT than observed in group A (3.0 years vs 8.5 years;  $P=0.001$ ).

In the univariate analysis, during GHRT the appearance of thyroid nodules was associated with the following variables: female sex (OR, 0.421,  $P=0.050$ ), current age (OR, 1.066;  $P=0.0001$ ), age at diagnosis (OR, 1.069;  $P=0.0001$ ), AO-GHD (OR, 0.211;  $P=0.009$ ), disease duration (OR, 0.916;  $P=0.030$ ), and serum IGF1 levels at diagnosis (OR, 1.786;  $P=0.017$ ) (Table 3). In the multivariate analysis adjusted for sex, age, serum IGF1, CO- or AO-GHD, and duration of disease, the main predictor for

**Table 2** Comparison between the study variables, subdividing the study cohort of patients in groups based on the occurrence or not of new thyroid nodules.

	Patients without pre-existing thyroid nodules (n=79)			Patients with pre-existing thyroid nodules (n=17)		
	Group A (n=17)	Group B (n=62)	P*	Group C (n=5)	Group D (n=12)	P†
Age of diagnosis (years)	57.5	34.0	<b>0.010</b>	56.0	56.5	0.597
Pre-treatment thyroid volume (ml)	11.4	7.3	<b>0.015</b>	15.2	13.1	0.865
Baseline IGF1 (s.d.)	-2.02	-2.37	<b>0.049</b>	-2.07	-1.55	0.246
Baseline insulin (μIU/l)	12.1	9.89	0.863	2.17	6.29	0.115
Baseline TSH (mIU/l)	0.67	0.07	0.363	0.83	0.44	0.752
Disease duration (years)	9.0	8.0	0.696	5.0	4.0	0.456
Treatment duration (years)	8.5	5.0	<b>0.043</b>	1.0	3.0	<b>0.024</b>
GH dose (μg/kg per daily)	5.3	5.0	0.682	3.5	3.7	0.161

P: \*group A vs group B and †group C vs group D; P value is typed in bold when statistically significant.

nodule development during GHRT was pre-treatment IGF1 levels (OR, 1.856;  $P=0.038$ ; Table 3).

## Discussion

To our knowledge, this is the first study focusing on a large number of adult GHD patients treated with GH for an

extended period of time, investigating both euthyroid and CH patients, the latter on adequate L-T<sub>4</sub> therapy. Previous studies had evaluated small numbers of patients, who were treated with GH for a short period (6–12 months), demonstrating no changes in TV in patients who were lacking both GH and TSH in one study (20), and a significant and stable TV increase in isolated GHD patients

**Table 3** Univariate and multivariate comparisons between the study variables, divided into patients with and without thyroid nodules<sup>a</sup>.

Variable	Development of nodules, n (%)	Absence of nodules, n (%)	Odds ratio (95% CI)	P*
<b>Univariate</b>				
Sex				
Male	12 (35.3%)	35 (56.5%)		
Female	22 (64.7%)	27 (43.5%)	0.421 (0.177–0.999)	<b>0.050</b>
Current age (years)	64.5	42.5	1.066 (1.032–1.102)	<b>0.0001</b>
Age of diagnosis (years)	57.0	34.0	1.069 (1.035–1.104)	<b>0.0001</b>
Onset of the disease				
CO	4 (11.8%)	24 (38.7%)		
AO	30 (88.2%)	38 (61.3%)	0.211 (0.066–0.675)	<b>0.009</b>
Baseline IGF1 (s.d.) <sup>b</sup>	-1.93	-2.37	1.786 (1.109–2.876)	<b>0.017</b>
Baseline insulin (μIU/l) <sup>b</sup>	6.29	9.89	0.955 (0.902–1.011)	0.115
Baseline TSH (mIU/l) <sup>b</sup>	0.66	0.07	1.008 (0.706–1.439)	0.965
Central hypothyroidism	23 (32.9%)	47 (67.1%)	0.667 (0.265–1.681)	0.391
Weight (kg) <sup>b</sup>	77.5	84	0.990 (0.969–1.013)	0.390
Disease duration (years) <sup>b</sup>	6.0	8.0	0.916 (0.846–0.992)	<b>0.030</b>
Treatment duration (years) <sup>b</sup>	5.0	5.0	0.950 (0.876–1.031)	0.218
GH dose (μg/kg per daily) <sup>b</sup>	5.3	5.0	1.002 (0.942–1.065)	0.957
<b>Multivariate</b>				
Sex			0.403 (0.140–1.159)	0.092
Current age (years)			1.097 (0.826–1.458)	0.521
Age of diagnosis (years)			0.980 (0.737–1.303)	0.887
Onset of the disease (CO/AO)			2.145 (0.333–13.826)	0.422
Baseline IGF1 (s.d.)			1.856 (1.036–3.327)	<b>0.038</b>
Disease duration (years) <sup>b</sup>			0.907 (0.682–1.207)	0.504

\*P values typed in bold are significant ( $P \leq 0.05$ ). P values typed in italics are borderline significant ( $P \leq 0.10$  but  $P \geq 0.05$ ).

<sup>a</sup>The 17 patients with pre-existing nodular goiter were excluded in order to avoid bias. Thus analysis was performed on 79 patients, of whom 70 had concomitant central hypothyroidism.

<sup>b</sup>Data are expressed as median and interquartile difference (ID).

in the other one (22). Despite the limitations due to its retrospective nature, our study provides novel data documenting the effects of GHRT on thyroid morphology during prolonged follow-up.

In our study, GHD patients had smaller thyroid glands than healthy euthyroid controls, and TV was positively correlated with serum IGF1 levels, further emphasizing the role of GH/IGF1 signaling in normal thyroid growth. The earlier the onset of GHD, the greater the effect on thyroid size. Indeed, patients affected by CO-GHD displayed a smaller TV than both healthy controls and AO-GHD patients. However, when the GHD patients were subdivided into CH and euthyroid subjects, we found that GHD patients with CH had a significantly smaller TV than GHD patients without CH, as well as healthy subjects, while in the euthyroid GHD group TV was smaller than that in healthy controls, but did not reach statistical significance. Our *in vivo* data are in accordance with experimental studies showing that IGF1 potentiates the mitogenic effects of TSH (4, 5, 6, 7, 8), and further underline that IGF1 and TSH act synergistically on normal thyroid growth (20).

In our study population, TV significantly increased during GHRT, but once again, subdividing GHD patients into CH and euthyroid subjects, the change in TV reached statistical significance only in GHD patients without CH, the main predictor for TV increase being serum TSH.

It is reasonable to suppose that GH/IGF1 deficit affects normal growth of the thyroid gland when it occurs early in life, also independently from the influence of TSH, but the effect is amplified if associated with TSH deficit. Moreover, restoration of IGF1 levels to normal range during GHRT did not result in an increase in thyroid size in CH subjects, suggesting a leading role of TSH in the stimulation of normal thyroid growth.

On the contrary, the situation is quite different when considering the risk of developing thyroid nodules. First, we found that in our cohort of adults affected by GHD, the prevalence of thyroid nodules before starting GH therapy was 17.7%, thus higher than the 4% reported in a previous study carried out in adult members of a large Brazilian kindred of comparable age ( $43.7 \pm 15.8$  years) affected by isolated GHD (21). This might be related to the different severity of iodine deficiency in the geographic areas of origin. In fact, such prevalence is not different from that reported in the healthy Italian population in other studies carried out during the same period of time (26). Moreover, 17 patients were affected by AO-GHD and in most of them the nodular goiter was diagnosed before onset of GHD (and of TSH deficit, when present), indicating that the thyroid

gland was presumably still exposed to normal levels of GH/IGF1 and TSH, when nodular growth occurred. On therapy, nodule size increased slightly in seven patients (41%), but the cytological features did not change.

During long-term GHRT, a non-negligible proportion of patients developed *de novo* thyroid nodules: 17 out of 79 patients without evidence of pre-existing thyroid disease, and nine out of the 17 patients with pre-existing nodular goiter, that is, overall, 27% of our GHD patients. The appearance of *de novo* nodules seems essentially to depend on serum pre-therapy IGF1 levels and duration of GHRT, but not on GH dosage or increase in IGF1 levels due to the therapy, nor on TSH levels. In particular, there was no difference in the prevalence of CH in GHD patients with or without thyroid nodules. Thus, although GH supplementation cannot restore normal TV in the absence of TSH, it can induce proliferation of thyrocytes and the appearance of thyroid nodules, due to its well-known proliferative and mitogenic effects, regardless of TSH levels. This novel finding fits with recent *in vitro* studies that proved that goiter development under experimental conditions was completely abrogated in IGF1 receptor knock-out mice, revealing an essential, autonomous role for IGF1 receptor signaling in the regulation of goitrogenesis (31).

Given the age-dependent development of nodules, we also evaluated whether age could be a confounding factor or a bias in the evaluation of our data. We found a significant difference regarding the age at diagnosis between GHD patients who developed *de novo* thyroid nodules and those who did not, the latter being significantly younger than the former. Moreover, in the univariate analysis, patients' age – as well as the adult onset of the disease – was significantly associated with the appearance of thyroid nodules. However, in multivariate models, both age and AO-GHD did not result as the independent risk factors for developing thyroid nodules, confirming the main role of IGF1 levels. Of course, we are aware that the assessment of the spontaneous course of TV and nodular disease in untreated GHD patients would be important to better understand the relevance of the present data, but unfortunately the retrospective nature of our study did not allow us to have an appropriate control group.

In our study population, only a single patient, a female who also suffered from CH, harbored a papillary thyroid cancer. This single piece of evidence is not sufficient to indicate a possible risk of malignant transformation during GHRT, even taking into consideration the relatively short period of observation (mean  $5.0 \pm 4.5$  years). This finding is in line with data from literature, showing a very low risk of developing malignancies

during long-term GHRT (32). However, no specific data are available on thyroid cancer in the above-mentioned meta-analysis (32).

In conclusion, we found that TV was smaller in GHD patients compared with healthy euthyroid subjects from the same geographic area of mild-to-moderate iodine deficiency, but significantly increased under GHRT, only in GHD patients without a concomitant CH. GHRT was associated with an increased risk of developing thyroid nodules regardless of the presence of a concomitant CH, mainly depending on IGF1 values and treatment duration. Also the age of such patients should be taken into account, because elderly GHD patients seem to be more at risk than younger ones. Thus, such risk should be considered when starting GHRT in GHD patients, and this caution might be more relevant in elderly patients with AO-GHD. Moreover, in GHD adults with a pre-existing goiter, increase in both the number and size of thyroid nodules could occur after a shorter duration of therapy. We propose that such patients could be more responsive to the stimulus exerted by GH/IGF1 signaling on thyroid cell proliferation. However, long-term GHRT does not seem to be associated with an increased risk of malignant transformation of thyroid nodules.

#### Declaration of interest

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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#### Author contribution statement

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