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Comparison of glucose and lipid metabolism and bone mineralization in patients with growth hormone deficiency with and without long-term growth hormone replacement

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Abstract

The effects of long-term growth hormone (GH) substitution in pituitary-insufficient patients with GH deficiency (GHD-pats) on glucose and lipid metabolism and bone mineral density (BMD) have yet to be ascertained. We performed this cross-sectional study comparing GHD-pats with and without long-term GH substitution. We measured lipid parameters at baseline and glucose and insulin concentrations for 3 hours during oral glucose tolerance test in 52 GHD-pats (21 female and 31 male; median age, 51.5 years [27-82]). Twenty-two GHD-pats were on constant GH substitution (GH-Subs) for a median of 10 years (2-42 years). Thirty GHD-pats had not been substituted for at least 2 years (non-Subs). For analyses of β-cell function, insulin resistance (IR), and sensitivity, homeostatic model assessment (HOMA)–β , HOMA-IR, and insulin sensitivity index were used, respectively. Body composition and BMD were measured by dual-energy x-ray absorptiometry. Age and body mass index did not differ significantly between groups. Fasting glucose was significantly lower for GH-Subs than non-Subs (87 mg/dL [71-103] vs non-Subs 89 mg/dL [71-113], $P < .05$), whereas basal insulin did not differ significantly (10 μU/mL (4-42) vs non-Subs 10μU/mL [4-63]). Glucose and insulin levels at 120 minutes as well as patients' area under the curve, C-peptide, hemoglobin A_{1c}, waist-hip ratio, HOMA-β, HOMA-IR, insulin sensitivity index, lipid parameters, and BMD did not differ significantly; but total fat mass was significantly higher in non-Subs (37% [20%-52%] vs GH-sub 31% [13%-54%], $P < .01$). More non-Subs had abnormal glucose tolerance (19 [63%] vs GH-Subs 9 [41%]). Long-term GH substitution trends to beneficially influence fasting glucose and glucose tolerance, although differences are sparse. Growth hormone substitution alone does not seem to significantly impact on insulin sensitivity, lipid metabolism, and BMD in patients with pituitary insufficiency.

1. Introduction

Growth hormone (GH) is a hormone produced in the pituitary with many metabolic effects such as affecting body composition, mineralizing bone mass, and influencing glucose and lipid metabolism. It antagonizes insulin action by reducing peripheral glucose uptake and promoting liver gluconeogenesis and glycogenolysis [1]. Furthermore, it stimulates lipolysis, which leads to an increase in lipid oxidation and a decrease in glucose oxidation [2,3]. Growth hormone application in obese, insulin-resistant adults has been shown to decrease insulin sensitivity during the first 6 weeks of therapy; but after 9 months of GH therapy, insulin sensitivity increased compared with baseline [4]. Patients with growth hormone deficiency (GHD-pats) have increased fat mass and often an impaired glucose metabolism [5,6]. β-Cell function is inadequately low in the presence of increased insulin resistance (IR) [7]. Growth hormone substitution in GHD-pats has been shown to worsen [3,8-10], to not influence [6,11,12], and to improve glucose metabolism and insulin sensitivity [13-15]. In a study of 23 333 children

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receiving GH treatment, a 6-fold higher occurrence of type 2 diabetes mellitus (DM) compared with children without GH treatment was observed [16]. One reason for the contradictory results on glucose metabolism seems to be the duration of GH substitution. Short-term substitution seems to deteriorate glucose metabolism [3,17-19], whereas long-term substitution does not lead to any differences in glucose and insulin metabolism [11,20] or has been reported to slightly improve it [14,20]. However, deteriorated glucose levels have also been observed after long-term GH substitution [21].

Lipid metabolism has been reported to be improved after GH substitution. Total cholesterol and low-density lipoprotein (LDL) decreased after GH substitution, whereas high-density lipoprotein (HDL) increased [14,21,22]. Triglycerides are described to decrease [22] or to not change after GH substitution [21]. Bone mineral density (BMD) increased during GH substitution [9,17,23], but patients with adult onset of GHD without GH substitution were also reported to have similar BMD compared with controls [24]. After 10 years of GH substitution, BMD did not change significantly from baseline [9] or was significantly higher in GH-substituted patients [25,26].

It is still a matter of debate whether GH substitution in patients with GHD does have beneficial effects on metabolism, in particular on glucose metabolism and insulin sensitivity, but also on lipid metabolism and bone mineralization, and should therefore be recommended to all GHD patients. To further clarify this question, we performed this cross-sectional study on a large group of GHD-pats with and without long-term GH substitution. The reason for using a cross-sectional design was to diminish the influence of age on glucose metabolism and insulin sensitivity. Our main question was whether significant differences in glucose and insulin metabolism could be evaluated in GHD-pats in relation to GH substitution. Furthermore, differences in lipid metabolism and BMD were studied.

2. Subjects and methods

2.1. Subjects

We informed all patients (N = 75, 30 female and 45 male) with GHD who recently presented in our outpatient clinic about this cross-sectional study. Patients with DM on insulin therapy were excluded from the study (n = 1). Fifty-three patients gave informed consent and were included into the study (median age, 51.5 years; range, 27-82 years). Twenty-one patients were female; 31, male. The study was reviewed and approved by the Ethics Committee of the Medical Faculty of the LMU Munich. Twenty-two GHD patients were on constant GH substitution (GH-Subs) over a median period of 10 years (2-42 years; 3 patients, <5 years; 1 patient, >15 years; mean, 11 years [SD ±5 years]). One female patient received GH substitution for 42 years, as she was first treated with human pituitary extracts until recombinant GH was available. The attending physicians made the decision to substitute a GHD patient at the time of diagnosis in agreement with the patient independent of this study. All evaluated substituted patients had an insulin-like growth factor (IGF)–I within the age- and sex-adjusted normal value, with reference to the published normative data for this method [27]. Growth hormone substitution was adjusted to reach an IGF-I level between mean and 1 SD. Median GH replacement dose was 0.3 mg (0.2-0.9 mg) per day. Growth hormone was injected every day in the evening. One GH-substituted female patient was excluded from data evaluation because her IGF-I level was beneath the age- and sex-adjusted normal value. The other 30 patients with GHD had not been substituted for at least 2 years before the study (non-Subs), and only 3 of these patients had received GH substitution after childhood. Six patients of the GH-Sub group and 9 patients of the non-Sub group had childhood onset of GH deficiency. In 1 male non-Sub patient, dual-energy x-ray absorptiometry (DXA) could not be performed because of adiposity (139 kg). One patient of the non-Sub group had diagnosed type 2 DM treated with the oral antidiabetic medication metformin; none of the GH-Sub group had diagnosed DM. Five patients of the GH-Sub group and 4 patients of the non-Sub group received medical treatment of lipid metabolism (GH-Subs: 4 on statins, 1 on fibrate; non-Subs: 3 on statins, 1 on fibrate). One of the non-Sub patients was on bisphosphonate therapy for treatment of osteoporosis. All patients were on constant and sufficient hormone replacement for pituitary insufficiency. In the GH-Sub group, 20 (91%) patients received thyroid hormones; in the non-Sub group, 24 did (80%). Seventeen (77%) patients of the GH-Sub group received hydrocortisone replacement, whereas 27 (90%) of the non-Subs did. The dose of hydrocortisone replacement per patient was between 15 and 25 mg/d. All patients with substituted insufficiency of the corticotropic axis received 10 mg. Twenty-nine (94%) of all male patients had an insufficiency of the gonadotropic axis; 28 (90%) of them received testosterone replacement. Eleven (50%) of the male GH-Subs and 15 (50%) of the male non-Subs were on testosterone substitution. Twenty-one (100%) of all female patients had insufficiency of the gonadotropic axis; 12 (57%) of them received sex hormone replacement. Five (23%) female GH-Subs and 7 (23%) non-Subs received sexual hormone replacement. Four (GH-Subs, 18%; non-Subs, 13%) patients in each GHD-pat group were on dehydroepiandrosterone substitution. Additional patient characteristics at study entry are in Table 1.

2.2. Methods

The study started between 7:00 and 9:00 AM in all 53 patients after an overnight fasting. First, patients' characteristics, clinical measurements, and baseline blood samples including IGF-I levels, hemoglobin A_{1c} (HbA_{1c}), C-peptide, and lipid profile were taken. Afterward, an oral glucose tolerance test (OGTT) was performed according to the World

Table 1
Patients' characteristics of GHD-pat groups

	GH-Sub	Non-Sub	*P* value
Patients (n)	22	30	
Age (years)	51.5 (33-75)	52 (27-82)	NS
Sex (female/male)	8/14 (36%/64%)	13/17 (43%/57%)	
Pituitary insufficiencies			
Corticotropic (n)	17 (77%)	27 (90%)	NS
Thyrotropic (n)	18 (82%)	23 (77%)	NS
Gonadotropic (n)	19 (86%)	29 (97%)	NS
Diabetes insipidus (n)	7 (32%)	7 (23%)	NS
Cause of insufficiency			
Tumor (n)	15 (68%)	23 (77%)	
Congenital (n)	3 (14%)	4 (13%)	
Brain injury (n)	2 (9%)	1 (3%)	
Others (n)	2 (9%)	2 (7%)	
Childhood onset of GH	6 (27%)	9 (30%)	
Pituitary radiation	4 (18%)	9 (30%)	
GH substitution (mg)	0.3 (0.2-0.9)	-	
BMI (kg/m^2)	26 (21-42)	29 (22-46)	NS
IGF-I (μg/L)	167 (74-319)	54 (25-104)	<.001
Fat mass (%)	31 (13-54)	37 (20-52)	<.01
Waist-hip ratio	0.96 (0.81-1.11)	0.97 (0.9-0.93)	NS
BMD (g/cm^2)	1.18 (0.97-1.39)	1.14 (0.92-1.32)	NS
T-score	-0.3 (-2.4-2)	-0.2 (-2.7-1.3)	NS
Osteopenia/osteoporosis (n)	8/0 (36%/0%)	6/2 (20%/7%)	NS

Data are given as median and range.

Health Organization criteria. Glucose and insulin levels were measured at time points −15, 0, 30, 60, 90, 120, 150, and 180 minutes after 75-g glucose administration (Dextro OGT; Roche, Mannheim, Germany).

2.3. Body composition

Body composition as fat mass, muscle mass, and BMD was measured by DXA (Lunar Prodigy; General Electric, Nuernberg, Germany; software version, Encore 9.30) in 52 patients. *Osteoporosis* was defined according to the World Health Organization. A T-score less than −1 SD and greater −2.5 SD was defined as *osteopenia*; a T-score less than or equal to −2.5 SD, as *osteoporosis*. Waist and hip circumferences were measured by one person using the same tape.

2.4. Laboratory values

The IGF-I concentrations were measured using an automated chemiluminescent immunoassay (Immulite; Diagnostic Products, Los Angeles, CA). Immulite IGF-I is a 2-site, solid-phase, chemiluminescent enzyme immunometric assay and is standardized according to the World Health Organization's second IS 87/518 [28].

Glucose levels were measured from whole venous blood by an automated glucose analyzer (Care Eco solo I, Care Diagnostic, Voerde, Germany); serum insulin and C-peptide levels were determined by the Adaltis Italia (Casalecchio di Reno, Italy) radioimmunoassay. Hemoglobin A_{1c} levels were analyzed from whole blood, standardized to the International Federation of Clinical Chemistry (IFCC), and calculated according to Diabetes Control and Complication Trial/National Glycohemoglobin Standardization Program (DCCT/NGSP) (Integra 700; Roche, Basel, Switzerland). Total cholesterol, HDL, and triglyceride were determined from heparin plasma by standardized enzymatic colorimetric test CHOD-PAP (cholesterol oxidase phenol-6-aminoantipyrine peroxidase), homogenous colorimetric test, and GPO-PAP (glycerol phosphate phenol-6-aminoantipyrine peroxidase), respectively (Integra 800, Roche). Low-density lipoprotein concentrations were calculated according to the Friedewald formula using the other 3 lipid parameters.

Leptin concentration in serum was measured using an immunofluorometric in-house assay as described previously [29]. The lower limit of quantification was 0.1 μg/L, and the linear working range was 0.2 to 40 μg/L. Intraassay variability was 7.4%, 4.3%, and 5.6% at leptin concentrations of 0.8, 2.5, and 15.3 μg/L, respectively. Interassay variability at the same concentrations was 8.3%, 5.2%, and 5.9%, respectively.

2.5. Assessment of glucose and insulin metabolism

Abnormal glucose tolerance (GT) as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and DM was estimated by OGTT according to the World Health Organization criteria for whole venous blood. We determined IR and β-cell function (β) by homeostatic model assessment (HOMA; therefore, HOMA-IR and HOMA-β, respectively). This is a computer model for predicting IR and β-cell deficiency (percentage β). The values for a patient can be assessed from fasting glucose and insulin concentrations as previously described [5]. Thereby, it is assumed that normal-weight control subjects older than 35 years have a β-cell function of 100% and a HOMA-IR of 1. High HOMA scores denote increased IR and improved β-cell function. The HOMA method has been shown to highly correlate with IR index assessed by euglycemic-hyperinsulinemic clamp technique [5]. Insulin sensitivity was determined by the insulin sensitivity index composite (ISI) derived from OGTT as described by Matsuda and DeFronzo [30]. It has been proposed and validated against euglycemic-hyperinsulinemic clamp.

2.6. Statistical analysis

The GH-Sub group was compared with the non-Sub group. For data analysis, SPSS (version 15.0; SPSS, Chicago, IL) was used. For all investigated parameters, group values are expressed as median and range because of nonnormal distribution. The mean of the value at time point −15 minutes and the value at time point 0 minute was defined as *baseline level*. Fisher exact test was used to evaluate significant differences in incidences. For comparison between the 2 groups, the nonparametric Mann-Whitney *U* test for unrelated measurements was used. For comparison within a group, Friedman test for related measurements was applied,

followed by Wilcoxon signed rank test. The area under the curve (AUC) was calculated by the trapezoidal rule. Waist-hip ratio was calculated by dividing waist circumference by hip circumference. A P value < .05 was considered as the nominal level of significance.

3. Results

Table 1 displays the clinical characteristics of the patients. Age and body mass index (BMI) were not significantly different between GHD-pat groups. Insulin-like growth factor–I levels were significantly higher in GH-Subs than in non-Subs (P < .001). Fat mass was significantly higher in non-Subs compared with GH-Subs (P < .01), whereas waist-hip ratio and BMD did not differ significantly between the 2 groups (Fig. 2). The percentage of patients having osteopenia was higher in the GH-Sub group compared with the non-Sub group, but more patients of the non-Sub group had significant osteoporosis. The lipid profile including total cholesterol, LDL, HDL, and triglycerides was not significantly different between the 2 GHD-pat groups.

Basal leptin was significantly higher in non-Subs compared with GH-Subs (GH-Subs, 8 μg/L [1-130]; non-Subs, 16 μg/L [3-89]; P < .05).

Table 2 shows metabolic parameters; Fig. 1, glucose and insulin levels during OGTT. Baseline glucose levels were significantly lower in GH-Subs than in non-Subs (P < .05), whereas insulin levels at baseline did not differ significantly. Glucose levels and insulin level increased significantly after glucose load in both groups (P < .001). During all further time points during the OGTT, glucose and insulin levels did not differ significantly between the 2 groups; nor did the AUC of glucose and insulin. Furthermore, C-peptide and HbA_{1c} levels did not differ significantly between GH-Subs and non-Subs (Fig. 2).

Table 2 and Fig. 2 show parameters of glucose metabolism and BMD. Insulin resistance and sensitivity estimated by HOMA-IR and ISI did not differ significantly between GH-Subs and non-Subs, nor did the β-cell function assessed by HOMA-β.

More patients of the non-Sub group than the GH-Sub group had an abnormal GT (19 [63%] of the non-Sub patients vs 9 [41%] of the GH-Sub patients), but this was not significantly different. One out of the 9 GH-Sub patients had an IFG, 8 had an IGT, and none had DM. Out of the 19

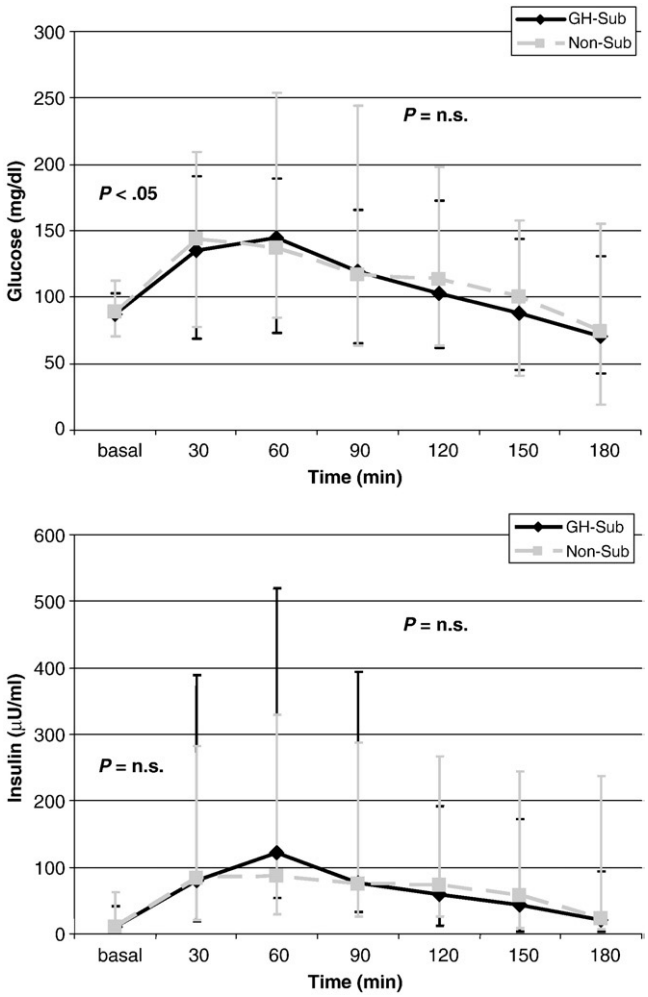

Fig. 1. Glucose levels at baseline were significantly lower for GH-Subs compared with non-Subs (P < .05). Basal insulin levels did not differ significantly between the 2 groups. During OGTT, no significant differences in glucose levels or in insulin levels were seen between GH-Subs and non-Subs. Values are given as median and range.

Table 2
Parameters of glucose metabolism and lipid profile of GHD-pat groups

	GH-Sub (n = 22)	Non-Sub (n = 30)	P value
Glucose baseline (mg/dL)	87 (71-103)	89 (71-113)	<.05
Glucose 120 min (mg/dL)	103 (62-173)	113 (64-198)	NS
Insulin baseline (μU/mL)	10 (4-42)	10 (4-63)	NS
Insulin 120 min (μU/mL)	60 (12-191)	73 (26-267)	NS
AUC glucose	18192 (11575-22209)	17811 (11647-29028)	NS
AUC insulin	10088 (4496-40787)	10478 (3934-38450)	NS
IFG (n)	1 (5%)	6 (20%)	NS
IGT (n)	8 (36%)	11 (37%)	NS
DM (n)	0	2 (7%)	NS
Patients on oral AD	0 (0%)	1 (3%)	
HbA_{1c} (%)	5.6 (4.9-6.4)	5.6 (4.6-7.2)	NS
C-peptide (ng/mL)	1.95 (0.4-4.6)	2.35 (1-4.5)	NS
HOMA-IR	2.1 (0.7-10.7)	2.2 (0.8-13.7)	NS
HOMA-β (%)	166.0 (54.5-1138.7)	168.4 (27.3-910.4)	NS
ISI	54.5 (16.5-171.7)	66.6 (10.5-165.2)	NS
Total cholesterol (mg/dL)	214 (162-295)	205 (149-309)	NS
LDL (mg/dL)	133 (85-218)	129 (65-218)	NS
HDL (mg/dL)	57 (31-84)	48 (14-93)	NS
Triglyceride (mg/dL)	123 (55-292)	134 (41-923)	NS
Patients on statins (n)	4 (18%)	3 (10%)	NS
Patients on fibrates (n)	1 (5%)	1 (3%)	NS

Data are given as median and range. AD indicates antidiabetics.

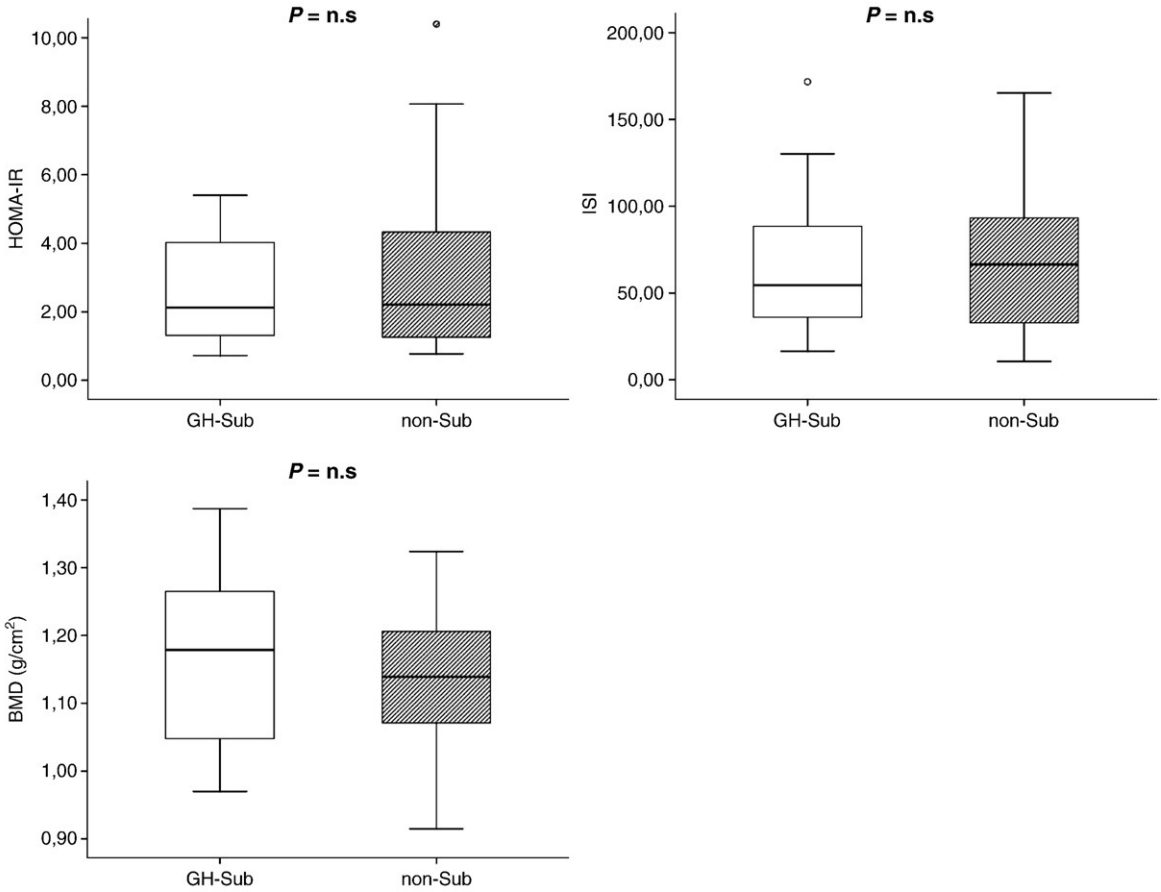

Fig. 2. Insulin resistance and insulin sensitivity estimated by HOMA-IR and ISI, respectively, were not significantly different between GH-Subs and non-Subs. Bone mineral density did also not differ significantly between both groups. Values are given in box plots showing median and range.

non-Sub patients, 6 had an IFG, 11 had an IGT, and 2 had DM (Table 2 and Fig. 3).

4. Discussion

We performed the present study comparing GHD patients with and without long-term GH substitution to further clarify the question of whether long-term GH substitution in GHD-pats improves or impairs glucose metabolism and insulin sensitivity. Our patients on GH substitution were on permanent substitution for at least 2 years (median, 10 years), whereas the other studied GHD patients did not receive GH substitution. Our main finding was that GH replacement did not cause deterioration of glucose metabolism. We even observed slightly lower fasting glucose levels in the GH-substituted group. These results are in accordance with some previously published data on GH substitution in GHD [6,11,12], which showed no significant change in glucose and insulin after GH substitution. One the other hand, a lot of data showing an impairment of glucose metabolism exist, especially during short-term GH substitution [3,17-19], but also after long-term GH substitution [21]. In most of the long-term data, an initial increase in glucose and insulin levels with an impairment of insulin sensitivity can be observed. This is followed by a decrease in glucose and insulin levels, in most cases reaching baseline levels, accompanied by an improvement in GT [14,31-33]. The deterioration of GT and insulin sensitivity at the beginning of GH substitution is, on the one hand, thought to be caused by a decline of peripheral glucose utilization [1]. On the other hand, β-cell function of GHD patients is reduced [7].

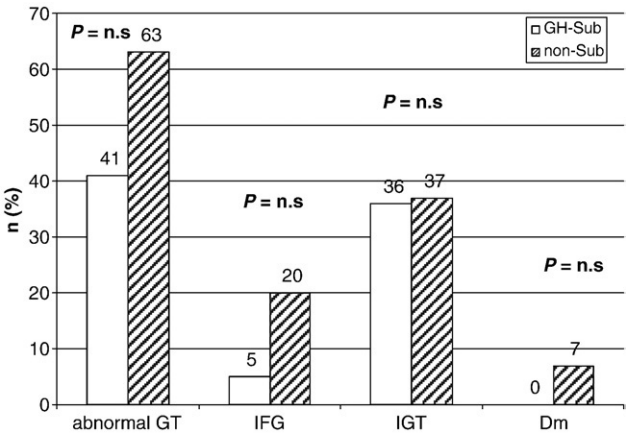

Fig. 3. Prevalence of abnormal GT, IFG, IGT, and DM for GH-Subs and non-Subs in percentage.

Furthermore, lipid oxidation is increased because of the lipolytic effect of GH [2,3]. Patients with GHD without GH substitution have increased fat mass compared with controls [5,33]. During GH substitution, fat mass can significantly be reduced [10,14,33]. Accordingly, our GH-substituted patients had significantly reduced fat mass compared with the non-substituted group. It can be suggested that insulin sensitivity in our GH-substituted patients is not significantly impaired because of GH-Sub–induced reduction in fat mass. Therefore, lipid oxidation is not increased anymore; and glucose can be normally utilized. Furthermore, with respect to our data, long-term GH substitution seems to have a rather beneficial effect on glucose metabolism, as fasting glucose levels were significantly lower in GH-substituted GHD-pats compared with non-substituted GHD-pats and less GH-substituted patients had abnormal GT. A few other studies have also revealed improving GT and insulin sensitivity after GH substitution [13-15]. A reason for the tendency of a beneficial influence of GH substitution in our patients might be the use of low-dose GH substitution. Our patients did receive GH in a median dose of 0.3 mg/d, whereas other studies used doses of more than 0.5 mg/d [4,22] or even up to 1.1 mg/d [14]. The dose of GH has been reported to influence the effect of GH on glucose and insulin [34,35]. Low-dose GH substitution seems to indeed improve or at least not to change glucose metabolism and insulin sensitivity, whereas higher doses seem to lead to an impairment [36]. An advantage of this study was the diminished influence of age on glucose metabolism and insulin sensitivity by using a cross-sectional design. In other investigations evaluating long-term GH substitution over 10 years in the same patient group, the problem arises that the studied subjects get older and BMI increases [9,37]. It is well established that BMI and fat mass increase with age accompanied by further metabolic changes [38]. This itself influences glucose metabolism and insulin sensitivity [39]. With age, the number of people with metabolic syndrome increases. As our patients did not differ significantly in BMI, this influence on glucose and insulin metabolism was excluded.

Visceral fat mass has been shown to be an independent predictor of endogenous insulin sensitivity and GT [40]. In our patients, waist-hip ratio did not differ significantly between GHD-pat groups, despite significant differences in fat mass. Therefore, in our patients, the reduction in fat mass does not seem to be mainly due to changes in visceral fat mass. Still, GT tends to improve after GH substitution. It might be speculated that the lack of significant reduction in visceral fat mass might contribute to the missing improvement of insulin sensitivity in our GH-substituted GHD patients. Still, it must be mentioned that this missing difference of waist-hip ratio might be an analytical problem because waist-hip ratio has been shown to be a poor method of assessing visceral fat mass [41,42]. Furthermore, visceral fat mass might have been reduced in our patients on GH; but the difference was just not statistically significant. Otherwise, this missing difference might be caused by using a crossover design comparing different patients at one time point. Many other cofactors might be influencing fat distribution, which diminish the effect of GH.

Most of our patients were not only deficient of the somatotropic axis; other axes were deficient as well. Therefore, it could have been that differences in hormone replacements of the other pituitary axis may have influenced glucose metabolism. However, looking at our data, the number of deficiencies and the kind of hormone replacement were similar in both groups. Furthermore, all patients were adequately substituted.

In our patients, parameters of lipid metabolism were not significantly different between groups. Slightly more GH-substituted patients, however, were on medication for lipid metabolism, which might have influenced the results. So far, most published data revealed an improvement after GH substitution. Total cholesterol and LDL are described to decrease after GH substitution, whereas HDL increased [14,21,22] or remained unchanged [31]. Triglycerides are reported either to decrease [22] or not to change after GH substitution [21]. The reason why GH-substituted patients do not seem to have improved lipid parameters is not clear. Chrisoulidou et al [11] could not find an influence of GH substitution on lipid parameters. The reason for this finding was not clear, but they speculated that the increased amount of information about cardiovascular risk and lifestyle advice might have contributed. This might also apply to our study, as all included patients visit our outpatients' clinic on a regular basis and are rather compliant. Therefore, it might be that they all take better care of themselves. Hana et al [12] also did not observe changes in lipid parameters after 1 year of GH treatment. As discussed earlier with respect to glucose metabolism, the duration of GH substitution as well as the patients' characteristics may influence lipid parameters. Results of another study in which lipid profile was improved after GH substitution in the first 3 months but, after 6 to 18 months, this improvement could not be maintained indicate that the duration of GH substitution might be a cofactor [43]. Differences in age might be one reason, as our patients were older than most of the other studied patients [14,37,44]. However, patients in the study of Hana et al [12] were also young; and still, lipid profiles did not change. Data of an international surveillance study showed the most significant improvement in lipid profiles in patients aged from 40 to 60 years [45]. Body mass index is a well-established factor of deteriorating lipid profiles [46]. However, looking at the published data, some patients' weight or BMI was reported to be higher compared with that of our patients [12,14]; some were similar [37,44]. Our patients on GH substitution had significantly lower fat mass than the nonsubstituted patients. This is in accordance with many previously published data [47,48]. We expected that higher fat mass in nonsubstituted patients would be a cofactor leading to an impaired lipid profile, but this could not be shown. It might be speculated that the dose of GH substitution may contribute to the lack of improvement as Hana et al also used a dose of 0.3 mg. Other

authors who reported an improvement of lipid profile used higher doses [14,22]. In contrast, data exist where similarly low doses of GH have been used but lipid profiles still positively changed after GH substitution [8]. Taken together, too many different aspects might influence lipid parameters in GHD patients and might have counterbalanced the effect of GH alone in our patients.

Bone mineral density measured by DXA was not significantly different between our 2 patient groups. Both groups had almost normalized median T-scores. Interestingly, more patients on GH substitution had osteopenia than patients without GH substitution. On the other hand, fewer GH substituted patients had defined osteoporosis. Although it is known that, during the first 12 to 18 months, bone remodelling takes place [49] and this time is needed to increase BMD [50], most previously published studies showed an increase in BMD not only after long-term GH substitution [25,26] but after short-term substitution as well [17,51]. However, it has also been reported that, in a group of 23 GHD-pats, BMD and bone marker initially increased after 5 years of GH substitution, but after 10 years no significant difference to baseline remained [9]. Therefore, it is suggested that, after some years of GH substitution, a plateau of BMD seems to be reached. Furthermore, patients with adult-onset GHD are widely reported to have similar BMD compared with controls [24,52,53]. This is thought to be due to the lack of a significant role of GH in the physiologic maintenance of bone mineralization in adults [24]. Contrarily, in childhood-onset GHD, BMD is lower compared with controls, which is thought to be due to a failure to achieve target genetic height and, in consequence, a mismeasurement via DXA [24,54]. Furthermore, children and adolescents who are treated with GH for GHD in childhood often do not attain their peak bone mass in their early 20s [55]. Moreover, recently, a relationship between adipokines, such as leptin and adiponectin, and bone mass has been investigated [56,57]. Leptin seems to stimulate bone growth and increase bone density, whereas adiponectin seems to be a negative regulator of bone mass by promoting bone resorption. In our patients, leptin levels were lower in GH-substituted patients, probably because of their lower fat mass. Therefore, it could have been expected that BMD was lower in this group as well. This effect might have been counterbalanced by the influence of adiponectin and GH itself. Because of the fact that our data lack a significant difference in GHD-pats dependent on GH substitution, the suggestion that GH substitution alone does not have a significant role in maintaining bone mineralization in adult-onset GHD might be confirmed. This could be because the other pituitary hormones also play an important role in bone metabolism, and they were sufficiently substituted in our patients. Nevertheless, more clarity about the clinical role of GH on bone metabolism should result from studies with clinical end points like fracture rates.

More patients in the non-Sub group received radiotherapy; and their BMI is increased, although not statistically. This might impact on the outcome of the study. Furthermore, a selection bias—patients being given GH replacement and others not being offered such treatment—cannot be excluded.

Concerning our data, it may be concluded that long-term low-dose GH substitution seems to have a slight beneficial effect on glucose metabolism. However, it does not seem to have a strong impact on other metabolic factors such as insulin sensitivity, lipid metabolism, and bone mineralization because the comparison of GHD-pats with and without GH substitution excluding BMI and age as influencing factor did not show relevant differences in these parameters.

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