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ORIGINAL ARTICLE

Expression of the growth hormone receptor isoforms and its correlation with the metabolic profile in morbidly obese subjects

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Abstract

Background and aim of the study Given the lipolytic effect of GH and its potential role in determining adipose tissue distribution, we evaluated the expression of the GH hormone receptor (GHR) isoforms in patients with morbid obesity seeking associations with metabolic parameters.

Methods 262 morbidly obese subjects (mean age 42.5 ± 11 years, 75% women) underwent PCR-genotyping of the exon 3 GHR polymorphism. In 17 of these subjects, who proved to be heterozygous for the exon 3 genotype (+3/−3), subcutaneous and visceral adipose tissue was obtained during bariatric surgery; total RNA was extracted, reversely transcribed, and the different isoforms of the GHR (exon 3 containing and lacking flGHR as well as the trGHR) were PCR-amplified using specific primers.

Results 27% were +3/+3 homozygous, 20% −3/−3 homozygous and 53% were +3/−3 heterozygous. Compared to subjects homozygous for the +3 genotype, homozygous and heterozygous carriers of the −3 genotype were significantly heavier and tended to have a higher HOMA 2-IR. Expression of the flGHR and trGHR mRNA was demonstrated in all evaluated samples of subcutaneous and visceral adipose tissue from the 17 patients. The exon 3+ isoform was expressed in all adipose tissue samples, whereas only six subjects expressed the 3− isoform as well. The only distinctive feature of these six patients was a higher HbA1c.

Conclusions The heterozygous GHR +3/−3 genotype is more prevalent in subjects with morbid obesity. Patients expressing the exon +3 and exon −3 isoforms in adipose tissue had a higher HbA1c, than those expressing only the exon −3 isoform.

Keywords Morbid obesity · GH · GH Receptor · Metabolic syndrome · Adipose tissue · Exon 3 GHR

Introduction

The human growth hormone (GH) receptor (GHR) is a member of the cytokine receptor family. Encoded by a gene located in the short arm of chromosome 5 (5p13.1), the mature protein consists of an extracellular, ligand-binding domain, a transmembrane portion involved in receptor dimerization and an intracellular portion in charge of signal transduction through the recruitment and phosphorylation of JAK2 and STAT5b [1, 2]. Several GHR mRNA variants with different 5′-untranslated regions exist in humans, which display developmental and tissue-specific profiles [1–4]. At the protein level, the GHR exists as either the full-length form (flGHR), and as two truncated isoforms, generated by alternative splicing of the immature mRNA [5, 6]. These truncated isoforms known as $trGHR_{1\text{-}277}$ and

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$trGHR_{1-279}$, interfere with the flGHR in a dominant negative manner following receptor dimerization [5, 6].

Contributing further to the heterogeneity of the GHR, there is an isoform that lacks all the aminoacids encoded by exon 3. Despite being located in the extracellular, ligand-binding domain of the GHR, the aminoacids encoded by exon 3 are not necessary for GH binding [7]. Furthermore, the +3 and −3 isoforms do not differ in regard to their in vitro binding affinity for the different GHR ligands [8]. Transfection experiments in HEK 293 cells, suggest that GH-induced signal transduction is higher through the exon 3 lacking homodimers or heterodimers than through the exon 3 containing homodimers, however, this has not been reproduced in other studies [9]. Although the exon 3 polymorphism was once thought to be due to alternative splicing of the immature mRNA [10], we now know that it is the result of an in-frame deletion occurring at the genomic level because of a recombination event of two different primate-specific retroelements [11]. Approximately 55% of the population is homozygous for the presence of exon 3 (+3/+3), 30–40% are heterozygous (+3/−3), and 10–20% are homozygous for the absence of exon 3 (−3/−3) [9, 11–13]. Some studies suggest that children with short stature of different etiologies, including girls with Turner syndrome [14], small for gestational age [14, 15], and severe GH deficiency [16] who are homozygous or heterozygous carriers of the exon 3 lacking genotype may be more sensitive to exogenous GH therapy. However, other reports in children born small for gestational age [17] and with isolated GH deficiency [18] have not found any difference in growth rate responsiveness among carriers of the different GHR genotypes. The exon 3 deleted GHR isoform has also been associated with a higher incidence of diabetes in acromegaly [13], a lower incidence of vertebral fractures in GH-deficient adults [19] and a higher occurrence of GH and IGF-1 discrepancy after treatment of acromegaly [20].

GH plays an important role in the biology of adipose tissue, where it promotes lipolysis, inhibits lipogenesis and participates in the differentiation of preadipocytes into adipocytes [21, 22]. GH deficiency is associated with an increase in visceral, and to a lesser extent subcutaneous adipose tissue and treatment with recombinant GH (rhGH) significantly reduces fat mass in these individuals [23]. However, in idiopathic obesity treatment with rhGH does not result in any significant changes in fat mass or distribution, perhaps reflecting a state of relative GH resistance [24]. Erman et al. found, that compared to lean and overweight women, obese women have a lower expression of total GHR mRNA in both visceral and subcutaneous adipose tissue and that the trGHR to flGHR ratio is increased in the subcutaneous depot [25]. Thus, it is possible that the relative expression of the different GHR isoforms is involved in determining the distribution of adipose tissue in the body. In the present study, we exon 3-genotyped a large group of subjects with morbid obesity and in some of those found to be +3/−3 heterozygous we evaluated the expression of the mRNA for the different GHR isoforms in subcutaneous and visceral adipose tissue obtained during bariatric surgery.

Materials and methods

Patients

The studied population consisted of 262 subjects with morbid obesity (BMI > 40 kg/m^2), attending the Morbid Obesity Clinic of Hospital de Especialidades, Centro Médico Nacional SXXI. All studies were performed upon enrollment to the clinic and prior to any specific intervention for their obesity, be it pharmacological or surgical. The study protocol was approved by our local scientific and ethics committees and all subjects signed the corresponding informed consent. All subjects had complete anthropometrical (weight, height, BMI, waist, and hip circumference), clinical and biochemical information (serum glucose, insulin, glycosylated hemoglobin, calculated HOMA 2-IR, and IGF-1).

Abnormalities in glucose metabolism included: diabetes mellitus, defined as the presence of fasting hyperglycemia ≥126 mg/dL and/or a glycosylated hemoglobin >6.5%; and impaired fasting glucose, defined as a fasting glucose level between 101 and 125 mg/dL [26]. Hypertension was considered to be present if the systolic or diastolic values exceeded 135 and 85 mmHg, respectively. Hypoalphalipoproteinemia was defined as a serum HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women and hypertriglyceridemia as serum triglycerides >150 mg/dL. The metabolic syndrome was defined by the presence of three or more of the following: waist circumference >90 cm in men and >80 cm in women; hypertriglyceridemia; hypoalphalipoproteinemia; systolic blood pressure >135 mmHg or diastolic blood pressure >85 mmHg, or the use of antihypertensive medications; fasting blood glucose >100 mg/dL or the use of anti-diabetic medications [27].

Hormonal and biochemical measurements

Serum insulin levels were measured by means of a chemiluminescent immunoassay with a detection limit of 0.23 μIU/mL and intra-assay and inter-assay coeffcients of variation of 3% and 4.3%, respectively, that uses the International Reference Preparation (IRP) WHO 66/304 as a calibrator (Diasorin-Liaison, Salugia, Italy). For IGF-1

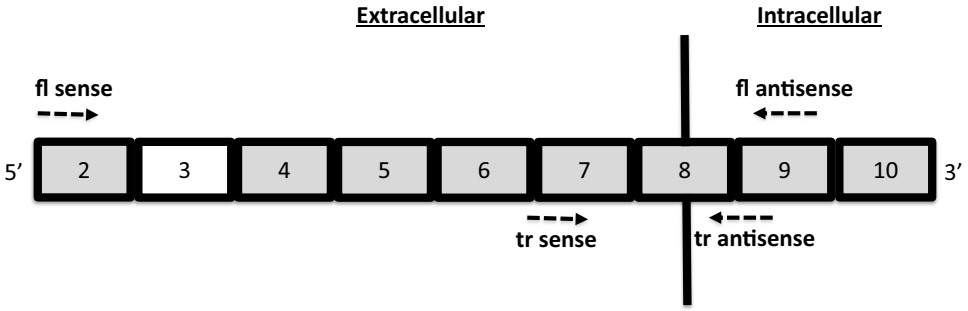

Fig. 1 Exon map of the GHR gene and primer location (upper panel). Primer sequence and expected size of PCR products

Transcript	Primers		PCR product
flGHR+3	Sense: Antisense:	5′ GGTATGGATCTCTGGCAGC 3′ 5′ GGGCAGAATCAGCATTTTAA 3′	899 bp
flGHR-3	Sense: Antisense:	5′ GGTATGGATCTCTGGCAGC 3′ 5′ GGGCAGAATCAGCATTTTAA 3′	836 bp
trGHR	Sense: Antisense:	5′ ATAAGGAATATGAAGTGAGTGTG 3′ 5′ ATCTTTGGAACTGGAACTTTGCT 3′	223 bp

measurement, serum samples were subjected to acid-ethanol extraction for binding protein separation prior to immunoassay by means of the Diasorin–Liaison chemiluminescent assay that uses the IRP WHO second 02/254 as a calibrator (Salugia, Italy); we established our own coefficients of variation (intra-assay 3%, inter-assay 4%) and normative data by analyzing 400 samples of healthy adults aged 18–60 years. Glycosylated hemoglobin (HbA1c) was determined by the Cobas AIC_3 Tina-quant assay (Cobas, Roche Diagnostics, Germany).

GHR exon 3 genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells using the QIAamp Blood Mini Kit (QIAGEN Gmbh, Manheim, Germany). Genotyping was carried out by multiplex PCR using the primers described by Pantel et al. [11]. Briefly, the exon 3 containing genotype (GHR +3, PCR product 935 bp) was amplified using the oligonucleotides G1 and G3, as antisense and sense primers, respectively, whereas the exon 3 lacking genotype (GHR −3, PCR product 532 bp) was identified using the same G1 antisense primer and the G2 sense primer (GenBank AF155912, AF210633). The PCR protocol consisted of an initial 5 min denaturing step at 95 °C, followed by 35 cycles consisting of denaturing at 95 °C for 1 min, annealing at 58 °C for 30 s and extension at 72 °C for 1.5 min. Exon 3 genotype frequencies in our morbidly obese population were compared to two historical cohorts in which we have previously evaluated such frequencies, a group of 148 patients with acromegaly (age 21–80, BMI <30 kg/m^2) and a group of 175 healthy lean controls (age 18–50, BMI 20–25 kg/m^2) [13].

Identification of GHR mRNA isoforms in adipose tissue

We evaluated the mRNA expression of the different isoforms of the GHR in subcutaneous and visceral adipose tissues obtained from 17 subjects with morbid obesity who were heterozygous for the exon 3 genotype (+3/−3). The same two bariatric surgeons performed all the procedures and the same visceral and subcutaneous depots were sampled in all patients. Adipose tissue samples were obtained at the moment of surgery, immediately immersed in RNAlater reagent (QIAGEN Inc, USA) and stored at −80 °C until used for RNA extraction which was carried out using the RNAeasy tissue Mini Kit (QIAGEN Inc, USA). After verifying RNA integrity by means of ethidium bromide-stained 1.5% agarose gel electrophoresis, 1 µg was retro transcribed using the SuperScript VILO Master Mix (Applied Biosystems, CA, USA). The resulting cDNA was used to amplify the different GHR transcripts. The PCR protocol consisted of an initial 94 °C, denaturing step lasting 12 min, followed by 40 cycles of denaturing at 94 °C for 45 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s, with a further extension step of 7 min at 72 °C. For the flGHR isoform, we used a sense primer located at the beginning of exon 2 and an antisense primer located at the beginning of exon 9, resulting in two distinct products, one containing (899 bp) and the other one lacking (836 bp) exon 3 (Fig. 1). The trGHR transcript was amplified by means of a sense primer located at the beginning of exon 7 and an antisense primer located at the end of exon 8, yielding a 223 bp product (Fig. 1). PCR products were visualized on 2%, ethidium bromide-stained agarose gel electrophoresis and their identity verified with the aid of molecular weight markers

Table 1 Growth hormone receptor (GHR) exon 3 genotype frequencies compared to two control populations

GHR exon-3 genotype	Morbid obesity (262) (%)	Acromegaly (148) (%)	Healthy controls (175) (%)	*p*
Homozygous (+3/+3)	27	45	53	<0.001* <0.001**
Homozygous (−3/−3)	20	22	17	0.48* 0.66**
Heterozygous (+3/−3)	53	32	30	<0.001* <0.001**
−3 Carriers (−3/−3) & (+3/−3)	73	54	47	<0.001* <0.001**

*Comparision between patients with morbid obesity and healthy, non-cobese adults

**Comparision between patients with morbid obesity and patients with acromegaly

and direct sequencing of representative cases. Gel images were generated by means of a Gel Doc XR+ Gel Documentation System (Bio-Rad) at the best resolution and analyzed using the Image J software, taking into account the band area and intensity of each individual product and generating optic density values which were adjusted using an amplified internal control (ribosomal protein subunit 18, RPS18).

Statistical methods

Data are presented as means ± standard deviations in the case of continuous variables with a normal distribution or median with interquartile ranges (IQR) in the case of non-normally distributed variables. The Shapiro–Wilks test was used to determine the normality of the distribution of the quantitative variables. Categorical variables are expressed as proportions and frequencies. Differences in categorical variables among the three genotype groups and subjects expressing flGHR +3 and −3 were analyzed by X^2 test. Student's *t*-test, ANOVA, Mann–Whitney-*U*-test or Kruskall–Wallis were used for continuous variables according to its distribution and number of groups compared. Multivariate logistic regression analysis was performed to explore the impact of GHR genotype on the presence of any of the components of the metabolic syndrome. The Hardy–Weinberg equilibrium was tested by the X^2 test. Statistical software package consisted of SPSS v. 21. A *p*-value of <0.05 was considered as statistically significant.

Results

Exon 3 GHR genotyping in patients with morbid obesity and its potential association with metabolic parameters

Exon 3 GHR genotyping revealed that 27% of the subjects were homozygous for the +3 genotype (+3/+3), 20% were homozygous for the −3 genotype (−3/−3), and 53% were heterozygous (+3/−3); 73% of our patients were carriers of at least one −3 allele (Table 1). The +3/−3 genotype was more frequent and the +3/+3 genotype was less frequent among this cohort of patients with morbid obesity, compared to two groups of historical controls, one consisting of 175 lean healthy adults ($p < 0.001$) and the other one consisting of 148 patients with acromegaly ($p < 0.001$) [13] (Table 1). The allelic and genotype frequencies did not deviate from the Hardy–Weinberg equilibrium.

The clinical, anthropometric, and metabolic features of the subjects with the different exon 3 GHR genotypes are analyzed comparatively in Table 2. Of the original 262 genotyped subjects, six were excluded from further analysis beause they did not have complete clinical and biochemical information available. Compared to homozygous GHR +3/+3 subjects, patients homozygous for the −3/−3 genotype were heavier and had a higher HOMA 2-IR. When we pooled together homozygous and heterozygous carriers of the −3 genotype and compared them to homozygous +3/+3 subjects only the difference in weight remained statistically significant. No significant differences were found among the three genotypes in regard to other clinical, anthropometric, or metabolic parameters. Being a carrier of any of the genotypes was not significantly associated with the presence of any of the elements of the metabolic syndrome upon multivariate analysis.

GHR mRNA expression in adipose tissue from patients with morbid obesity

The expression of the different GHR transcripts was evaluated in visceral and subcutaneous adipose tissue from 17 subjects with morbid obesity who upon genotyping were heterozygous for the exon 3 isoform (GHR +3/−3) (Figs. 2 and 3). Good-quality RNA extraction from subcutaneous adipose tissue was unsuccessful in patients 035 and 042 and from visceral adipose tissue in patient 039. Expression of the mRNA for both the full length (flGHR) and the truncated (trGHR) isoforms was demonstrated in all evaluated

Table 2 Clinical, anthropometric, and biochemical features of subjects harboring the different growth hormone receptor (GHR) exon-3 genotypes. p1+3/+3 vs. −3/−3, p2+3/+3 vs. +3/−3 plus −3/−3

	Growth hormone receptor Exon 3 genotype			p^1	p^2
	+3/+3 ($n = 69$)	−3/−3 ($n = 50$)	+3/−3 ($n = 137$)		
Age (years)	44 ± 11	41 ± 11	42.5 ± 10	0.16*	0.24*
% female	85.5	70	69	0.04**	0.008**
Weight (kg)	118.5 (106–136)	134 (117–159)	124 (113–140)	0.004***	0.01***
Body mass index (kg/m^2)	47 (42–53)	50 (44–57)	48 (43–52)	0.05***	0.38***
Waist circumference (cm)	133 (121–144)	133 (124–144)	133.5 (125–149)	0.33***	0.43***
Waist–hip ratio	0.91 (0.85–0.99)	0.91 (0.87–1.02)	0.92 (0.88–1.0)	0.53***	0.41***
Adiposity index (%)	53 ± 7	51 ± 9	51.9 ± 9	0.33*	0.20*
Systolic pressure (mmHg)	130 (120–140)	130 (120–140)	130 (120–140)	0.87***	0.15***
Diastolic pressure (mmHg)	80 (80–90)	90 (80–90)	90 (80–90)	0.13***	0.10***
Fasting glucose (mg/dL)	102 (91–125)	100 (90.5–117)	100 (90.5–113)	0.42***	0.39***
Fasting insulin (μU/mL)	22 (15–35)	30.5 (19–38)	25.5 (18–35)	0.08***	0.63***
HbA1C (%)	6.3 (5.8–7.2)	6.2 (5.8–6.6)	6.2 (5.9–6.7)	0.76***	0.71***
HOMA 2-IR	2.8 (2–4.4)	4 (2.5–4.8)	3.36 (2.4–4.4)	0.04***	0.68***
Total cholesterol (mg/dL)	183 (165–205)	182 (162–200)	182.5 (162–200)	0.80***	0.40***
Triglycerides (mg/dL)	153 (123–186)	158 (117–205)	149 (117–184)	0.72***	0.79***
Abnormalities in glucose metabolism (%)	85.5	92	88	0.27**	0.47**
Hypoalphalipoproteinemia (%)	56.5	66	61	0.29**	0.42**
Hypertriglyceridemia (%)	58	58	53	0.99**	0.57**
Hypertension (%)	68	70	61	0.82**	0.50**

*Student *t* test

**ANOVA

***Kruskal–Wallis

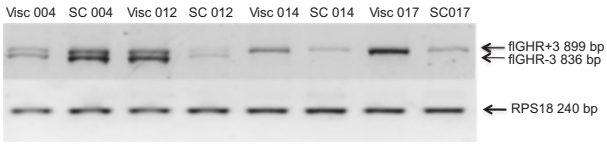

Fig. 2 Representative agarose gel electrophoresis of the full-length GHR (flGHR) amplified cDNA in visceral (Visc) and subcutaneous (SC) adipose tissue. Samples from two patients expressing both transcripts (004 and 012) and two patients expressing only the +3 isoform (014 and 017) are shown. The gene encoding for ribosomal protein subunit 18 (RPS18) was used as housekeeping gene

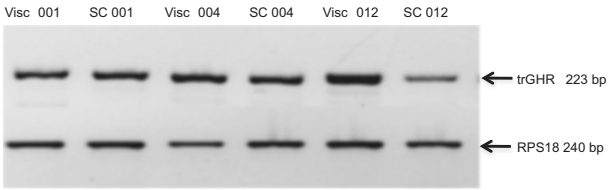

Fig. 3 Representative agarose gel electrophoresis of the truncated GHR (trGHR) amplified cDNA in visceral (Visc) and subcutaneous (SC) adipose tissue of three patients (001, 004, and 012)

samples of subcutaneous and visceral adipose tissue. In 11 patients, only the exon 3 containing flGHR was identified, whereas in six patients, both the exon 3 containing and the exon 3 lacking flGHR isoforms were found. A comparison of the clinical, anthropometric, biochemical, and metabolic features of these patients is shown in Table 3. The six patients expressing the exon 3 lacking isoform had higher glycosylated hemoglobin levels (median 6.4% [IQR 5.8–8.4]) than those expressing only the exon 3 containing isoform (median 5.3% [IQR 5–5.8]) ($p = 0.01$). Upon multivariate logistic regression analysis, the expression of either the exon 3 lacking or the exon 3 containing isoform was not associated with the presence of any of the components of the metabolic syndrome.

A semiquantitative analysis based on the density of the bands of agarose gel electrophoresis of the different PCR products revealed a tendency for a higher expression of the flGHR mRNA in visceral than in subcutaneous adipose tissue (visceral to subcutaneous ratio 1.4–27) in 10 patients; subcutaneous adipose tissue expression of this isoform predominated in two patients (visceral to subcutaneous ratio 0.170 and 0.281), and in two other patients the expression was the same in the two fat depots (visceral to subcutaneous ratio 0.88 and 1.03) (Table 4). The trGHR was expressed to the same extent (visceral to subcutaneous ratio 0.817–1.13) in visceral and subcutaneous adipose tissue in nine patients, whereas in four its expression was slightly higher in the visceral depot (visceral to subcutaneous ratio 1.289–3.019) and in one patient it was higher in the subcutaneous depot (visceral to subcutaneous ratio 0.345) (Table 4). Among the five patients expressing both, the exon 3 containing and exon 3 lacking mRNA isoforms, these two transcripts were equally expressed in visceral and subcutaneous adipose tissue in four patients, whereas the exon 3 lacking transcript slightly predominated in one patients.

Table 3 Clinical, anthropometric, and biochemical features of subjects expressing the mRNA for the full-length growth hormone receptor (flGHR), containing (+3) and lacking (−3) exon 3 in subcutaneous and/or visceral adipose tissue

	flGHR		*p*
	+3 ($n = 11$)	+3 and −3 ($n = 6$)	
Age (years)	39 (33–48)	48 (36–55)	0.21
% female	63.6	83.3	0.03
Weight (kg)	130 (119–147)	118 (103–135)	0.11
BMI (kg/m^2)	46 (41–50)	47 (46–54)	0.10
Waist circumference (cm)	132 (122–144)	127 (122–140)	0.72
Adiposity index (%)	45 (36–57.5)	48 (41–61)	0.26
Systolic pressure (mmHg)	116 (100–130)	117 (100–130)	0.83
Diastolic pressure (mmHg)	74 (60–80)	81 (80–88)	0.83
Fasting glucose (mg/dL)	99 (88–125)	110 (94–154)	0.22
Fasting insulin (μU/mL)	21 (16–31.5)	18 (12–25)	0.28
HbA1C (%)	5.3 (5–5.8)	6.4 (5.8–8.4)	0.01
HOMA 2-IR	2.6 (2–3.8)	2.9 (1.6–4.6)	0.95
Total cholesterol (mg/dL)	156 (124–186)	179 (152–196)	0.24
Triglycerides (mg/dL)	111 (90–166)	104.5 (86–190)	1.0
Abnormalities in glucose metabolism (%)	54.5	100	0.1
Hypoalphalipoproteinemia (%)	54.5	67	1.0
Hypertriglyceridemia (%)	45.5	33.3	1.0
Hypertension (%)	54.5	83.3	0.33

Discussion

GH exerts complex biological actions on protein, carbohydrate, and lipid metabolism that include a decrease in insulin sensitivity and the ensuing glucose intolerance or even hyperglycemia, protein anabolism as well as a profound lipolytic effect [21, 28]. All these actions are mediated through its interaction with its receptor, yet, few studies have explored the metabolic consequences of the different GHR isoforms. The present study constitutes the first attempt at evaluating the expression of the different GHR isoforms, both at the genomic and mRNA level in patients with morbid obesity and its potential association with components of the metabolic syndrome.

The distribution of the three different exon 3 GHR genotypes, has been rather consistent among different populations, including healthy adults and children, as well as GH-deficient children and patients with acromegaly: 45–55% are +3/+3 homozygous, 15–20% are −3/−3 homozygous, and 25–30% are +3/−3 heterozygous [9, 12, 13]. We found a significantly higher prevalence of the heterozygous +3/−3 genotype than in our two historical control populations of healthy adults and patients with acromegaly. Furthermore, over 70% of our patients were homozygous or heterozygous carriers of the GHR −3 allele. To our knowledge such a high prevalence of the −3 GHR isoform has been previously found only once in a Turkish cohort of GH-deficient children with Turner's syndrome [7].

The exon 3 lacking genotype was associated with a higher weight, as well as with a slight tendency towards a higher HOMA 2-IR, but not with any other components of the metabolic syndrome. This finding is somewhat contrary to what we have expected if we consider that the exon 3 lacking isoform has been reported to signal more efficiently than the exon 3 containing variant, and thus, should induce more lipolysis with a more favorable distribution of fat mass. On the other hand, we could speculate that such a GHR isoform, would be also more efficient in transducing the diabetogenic effect of GH and this could be part of the reason why these patients are heavier and tend to be more insulin-resistant. We could not find any experimental evidence supporting this notion although we have previously reported that diabetes mellitus is more prevalent among patients with acromegaly who are carriers of the exon 3 lacking genotype than in those who are homozygous for the exon 3 containing isoform [13], yet this finding has not been confirmed by others.

In a study of Chinese children with obesity, homozygous or heterozygous carriers of the −3 allele were found to have lower BMI, fasting insulin, triglycerides, and cholesterol levels, as well as insulin resistance indexes than GHR +3 homozygotes [29]. Furthermore, the homozygous −3/−3 genotype has been found to be protective against the development of the metabolic syndrome and diabetes [30]. Yet, a recently published report from the Swedish Obese Subjects Study that evaluated 1135 overweight and non-morbidly obese adults (mean BMI 25.2 ± 3.8), the

Table 4 Expression of the different GHR isoforms (flGHR +3, flGHR−3, and trGHR) in visceral (Visc) and subcutaneous (SC) adipose tissue in 17 patients who were heterozygous for the exon 3 genotype (+3/−3). The first six patients expressed both the +3 and the −3 isoforms in adipose tissue, whereas the remaining 11 expressed only the +3 isoform. Each individual value represents the optic density of the corresponding band on agarose gel electrophoresis, divided by the optic density of the band of the house keeping gene used as an internal control (RPS18 or ribosomal protein subunit 18S). Ratios were obtained by dividing the visceral by the subcutaneous value

Patient	flGHR+3			flGHR-3			trGHR		
	Visc	SC	Ratio	Visc	SC	Ratio	Visc	SC	Ratio
004	0.290	1.030	0.281	0.426	1.278	0.333	1.885	1.776	1.061
012	0.820	0.102	8.034	1.226	0.173	7.086	2.328	0.771	3.019
035	0.409	NA		1.192	NA		1.720	NA	
040	0.642	0.142	4.521	0.650	0.184	3.532	1.465	0.852	1.719
041	0.819	0.499	1.641	0.959	0.634	1.512	0.959	0.841	1.140
045	0.756	0.378	2.0	1.192	0.842	1.415	1.192	1.237	0.963
001	0.030	0.176	0.170	–	–	–	1.386	1.695	0.817
014	0.575	0.149	3.859	–	–	–	0.253	0.222	1.139
017	1.441	0.257	5.607	–	–	–	2.219	2.215	1.001
023	1.386	0.051	27.176	–	–	–	1.875	1.639	1.143
025	0.861	0.831	1.036	–	–	–	2.216	2.149	1.031
032	0.464	0.190	2.442	–	–	–	0.309	0.190	1.626
034	2.163	2.457	0.880	–	–	–	0.817	0.865	0.944
038	0.780	0.560	1.392	–	–	–	0.473	1.370	0.345
039	NA	1.706		–	–	–	NA	1.271	
042	1.702	NA		–	–	–	1.396	NA	
046	2.461	0.870	2.828	–	–	–	1.583	1.228	1.289

NA: No RNA available

homozygous −3/−3 genotype was associated with an increase rate of central adiposity as reflected by a higher BMI and waist-to-hip ratios [31]. This highlights the complexity of the interaction between GH and adipose tissue, whereby many other factors, such as pro-inflammatory and anti-inflammatory adipokines are likely to play a role.

Although in GH-deficient subjects, treatment with rhGH results in a significant reduction in visceral adipose tissue [23], in subjects with idiopathic obesity the effects on body composition and lipid profiles are marginal, even at supra-physiological doses [24].

It has been suggested that such a relative resistance to the lipolytic effect of exogenous GH in idiopathic obesity results from a relatively higher expression of trGHR over flGHR [25, 32]. In a group of 22 lean women who underwent adipose tissue sampling during gynecological surgery, Fisker et al. found the flGHR to predominate over the trGHR, and a positive correlation between the mRNA expression of both isoforms with intraabdominal fat [32]. In a similarly designed study, Erman et al. found a lower expression of total GHR mRNA in omental and subcutaneous adipose tissue from women with obesity than from women with normal BMI and a higher trGHR/flGHR ratio in subcutaneous fat with increasing adiposity [25]. In our study, which is the first one to evaluate the GHR in adipose tissue of subjects with morbid obesity, the expression of both, the flGHR and the trGHR mRNA expression was similar in visceral and subcutaneous adipose tissue in most patients. To what extent this contributes to body fat distribution in these individuals cannot be ascertained with certainty with the available data.

We evaluated the mRNA of the GHR in adipose tissue of 17 subjects with morbid obesity who were heterozygous for the presence of exon 3 in order to be able to assess the relative expression of the −3 and +3 transcripts. In 6 of these 17 patients, amplification of the flGHR mRNA resulted in two distinct PCR products of 899 and 836 bp, which upon sequencing proved to correspond to the exon 3 containing and exon 3 lacking isoforms, respectively. Patients expressing the exon 3 deleted isoform had higher glycosylated hemoglobin levels and tended to be more prone to abnormalities in glucose metabolism. The expression of the exon 3 containing and exon 3 lacking flGHR mRNA was similar in both fat depots. The expression of the exon 3 isoforms of the GHR in adipose tissue has been studied before in five healthy individuals by Wickelgren et al. albeit without specifying the type of adipose tissue (i.e visceral or subcutaneous). In this study, adipose tissue from three healthy male subjects expressed both the exon 3 containing and the exon 3 lacking isoforms of the GHR, whereas in two subjects (a 37-year-old female and a 1-year-old male) only the exon 3 containing isoform could be demonstrated [33].

Conclusion

We conclude that in patients with morbid obesity the frequency of the heterozygous +3/−3 genotype of the GHR is

significantly more frequent than in other populations and although carriers of the −3 allele tended to weigh more and to be more insulin resistant, no correlations could be found with other anthropometric or metabolic parameters. Both, the flGHR and trGHR isoforms are expressed in adipose tissue of patients who were genotypically heterozygous for the presence of exon 3, with only less than a third of them expressing both, the exon 3 containing and exon 3 lacking transcripts. The main limitation of our study is the small number of genotypically +3/−3 heterozygous subjects available for GHR mRNA expression analysis in adipose tissue.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures involving human paricipants were in accordance to the ethical standards of the Institutional Research Committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants.

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