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ORIGINAL ARTICLE

Prolactin and sex steroids levels in congenital lifetime isolated GH deficiency

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Abstract Growth hormone (GH) and prolactin share similarities in structure and function. We have previously shown that women with congenital isolated GH deficiency (IGHD) caused by a homozygous mutation in the GHRH receptor gene (*GHRHR*) (MUT/MUT) have a short reproductive life, with anticipated climacteric. At climacteric, they have lower prolactin levels than normal controls (N/N). Because they are able to breast feed, we hypothesized that this prolactin reduction is limited to climacteric, as result of lower estradiol exposure of the lactotrophs. The purposes of this work were to assess prolactin levels in broader age adults homozygous and heterozygous (MUT/N) for the mutation and in normal controls (N/N), and to correlate them to sex steroids levels. We enrolled 24 GH-naïve MUT/MUT (12 female), 25 MUT/N (14 female), and 25 N/N (11 female) subjects, aged 25–65 years. Anthropometric data and serum prolactin, estradiol, total testosterone, and sex hormone binding globulin (SHBG) were measured. Free testosterone was calculated. Prolactin levels were similar in the three groups. In males, testosterone and SHBG levels were higher in MUT/MUT in comparison to N/N. There was no difference in free testosterone among groups. In all 74 individuals, prolactin correlated inversely with age ($p < 0.0001$) and directly with serum estradiol ($p = 0.018$). Prolactin levels in subjects with IGHD due to a homozygous *GHRHR* mutation are similar to heterozygous and normal homozygous, but total testosterone and SHBG are higher in male MUT/MUT, with no difference in free testosterone. The reduced prolactin level is limited to climacteric period, possibly due to reduced estrogen exposure.

Keywords Prolactin · Growth hormone · Sex steroids · Homozygous · Heterozygous

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Introduction

Growth, reproduction, and lactation are integrated processes that guarantee species survival. Growth hormone (GH) and prolactin share strong similarities in structure and function. Although the main physiological role of prolactin is lactogenesis, and that of GH is postnatal growth, an overlapping of their effects may occur. GH-induced IGF-I action is critical for pubertal breast development and important for alveolar development during pregnancy, and milk production during lactation [1]. On the other hand, increased concentrations of PRL have the capacity to raise IGF-I into the normal range in individuals with GH deficiency [2].

It has been reported that long-lived GH- or IGF-I deficient rodent mutants present shortened reproductive life [3]. We have previously reported that women with severe dwarfism due to isolated GH deficiency (IGHD) caused by the homozygous c.57+1G>A mutation in the GHRH receptor gene (*GHRHR*) have reduced reproductive life due to delayed menarche, and early climacteric [4]. Climacteric women of this IGHD cohort have lower serum prolactin

levels than age-matched controls [4]. As the beginning of the climacteric is anticipated, it is possible that this prolactin reduction is just the result of lower estradiol exposure of the pituitary lactotrophs. Indeed, at younger age, these women are able to breast feed their children, and the duration of lactation is not different from normal controls [4]. This contrasts with the high frequency of lactation failure found in GHD women with prolactin insufficiency, who are long-term survivors of childhood leukemia treated with cranial radiotherapy [5]. It is possible that women with lifetime congenital IGHD may develop adaptive mechanisms to assure lactation and guaranteeing species survival. Therefore, the opportunity to study prolactin levels in a cohort with congenital lifetime IGHD, without other pituitary deficits and replacements, pituitary surgery or radiation is fundamental to clarify the relationship between GH/IGF-I axis and prolactin.

The roles of prolactin and GH in regulating metabolism are more complex. Both hormones induce pancreatic β cell proliferation and insulin production [6, 7], and at supraphysiological concentrations, both reduce insulin sensitivity (IS) [8]. Indeed, similar to GH excess, hyperprolactinemia leads to insulin resistance [9–12]. These IGHD individuals have increased IS [13], associated to high adiponectin levels [14]. As both prolactin and GH reduce adiponectin secretion [15], the high adiponectin could be also related to low prolactin levels.

Whereas individuals homozygous for the *GHRHR* mutation (MUT/MUT) have severe GHD resulting in dwarfism, heterozygous individuals (MUT/N) have a partial phenotype, without reduction in height or serum IGF-I, but with lean body mass reduction, tendency to fat body mass reduction, and increased IS [16]. The purposes of this work were to evaluate the prolactin level in MUT/MUT and MUT/N adult of both genders, and to correlate them to levels of sex steroids and to IS.

Subjects and methods

A transversal study enrolled 74 subjects invited by word of mouth and by an advertisement placed in the local Dwarfs' Association building located in Itabaianinha, in the Northeastern Brazilian state of Sergipe. They belong to a large extended kindred with high prevalence of congenital IGHD due to the c.57+1G>A *GHRHR* mutation. Inclusion criteria were age between 25 and 65 years and exclusion criteria were previous GH treatment, sex hormone replacement, or any drug that could influence prolactin levels. Genotyping was performed using predesigned TaqMan SNP Genotyping Assay C_15757069_10 (Applied Biosystems, Foster City, CA).

Height (cm) and weight (kg) data were measured and body mass index (BMI) calculated. Blood was collected after overnight fasting. In menstruating females, blood was collected in the first week after the starting of menstruation. Fluoroimmunoassays were used to measure prolactin, estradiol and total testosterone (Perkin Elmer Life and Analytical Science, Wallac Oy Turku, Finland). The sensitivities were 1.44 ng/mL, 8.17 pg/mL, and 0.09 ng/mL, respectively. Sex hormone binding globulin (SHBG) was measured by a solid-phase two-site chemiluminescent immunometric assay (IMMULITE® 2000 SHBG Los Angeles, CA 90045-6900 USA) with sensitivity of 0.02 nmol/l. The expected values are 10–57 nmol/l for males and 18–144 nmol/l for non-pregnant females. Free testosterone was calculated following the Endocrine Society recommendations [17]. IS was assessed by the homeostasis model assessment index of insulin resistance (HOMAir) with the formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5. Lower values of HOMAir indicate higher IS. Glucose was measured by the enzymatic Trinder colorimetric test. Insulin was measured by an immunofluorometric assay with an assay sensitivity of 0.5 μU/ml (PerkinElmer Life and Analytical Sciences, Turku, Finland), and intraassay and interassay variabilities were 2.5 and 3 %, respectively.

In order to verify if there is any difference in prolactin level between pre-and postmenopausal MUT/MUT, nine women age ≤ 45 years were compared with the previous data of seven climacteric MUT/MUT [4].

Variables were expressed as the mean ± SD. Analysis of variance (ANOVA) with Bonferroni post-test was used to compare the three groups. Pearson coefficient was used to verify correlation between variables. A general linear model including both ANOVA and regression, using basal prolactin as dependent variable and ANOVA with two factors (group and sex) and adjusted for age, BMI, estradiol, and testosterone, was subsequently used. Probability values of 0.05 or less were considered statistically significant.

Results

The groups were so formed: 24 MUT/MUT (12 females), 25 MUT/N (14 females), and 25 N/N (11 females). There was no difference in prolactin levels in the three groups with pooled genders: MUT/MUT: 5.98 ± 2.99, MUT/N: 7.41 ± 3.95, and N/N: 5.86 ± 2.93 ng/ml), Fig. 1 shows the Pearson coefficient inverse correlation between prolactin and age ($r = -0.397$; $p < 0.0001$), and the direct correlation between prolactin and estradiol ($r = 0.275$; $p = 0.018$) in all 74 subjects of both genders. General linear model using prolactin as a dependent variable and ANOVA

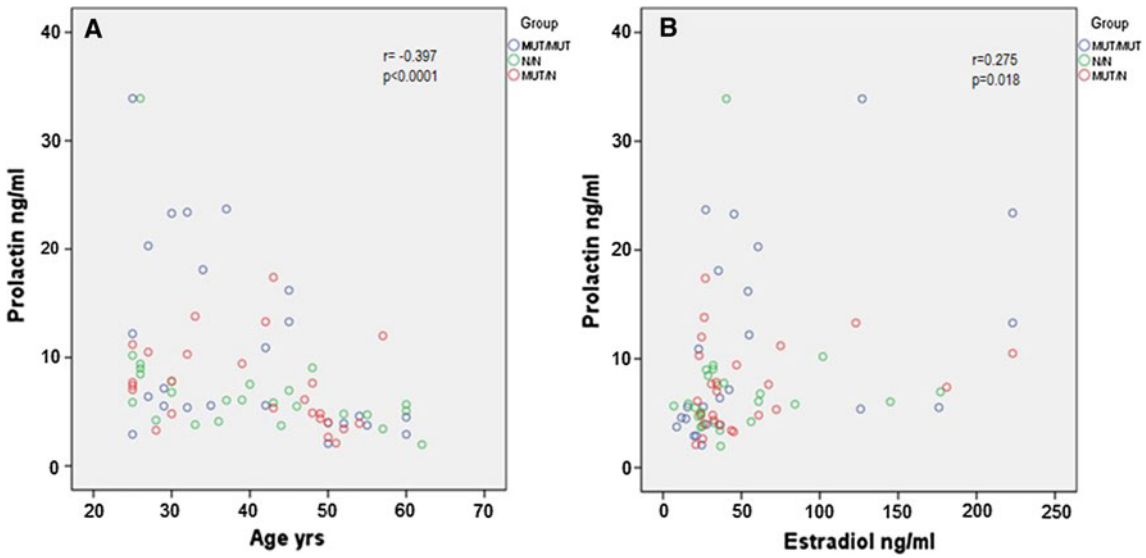

Fig. 1 Pearson coefficient correlation between prolactin and age (**a**), and prolactin and estradiol (**b**) in 74 individuals: 24 MUT/MUT subjects (12 females); 25 MUT/N subjects (14 females); and 25 N/N subjects (11 females)

with two factors (sex and group) adjusted for age and BMI was able to explain 25.8 % of the variability of prolactin, being age the only significant covariate ($p = 0.021$), with an adjusted R square of 0.081 (explaining 8.1 % of prolactin variability). HOMAir was lower in MUT/MT 0.55 (0.49) than the N/N: 0.92 (0.94), $p = 0.0031$, and MUT'N: 0.96 (0.57), $p = 0.004$ but no correlation was found between prolactin and HOMAir. Prolactin level was higher in premenopausal than postmenopausal MUT/MUT women 14.83 ± 9.96 vs. 3.85 ± 1.91 ng/ml, $p = 0.011$.

The anthropometric and hormonal data of 37 female and 37 males are shown in Tables 1 and 2, respectively. Estradiol levels were similar in MUT/MUT, MUT/N, and N/N in female and male groups, respectively. Total testosterone levels were higher in male MUT/MUT group in comparison to N/N ($p < 0.0001$). However, SHBG was also higher in male MUT/MUT in comparison to male N/N ($p = 0.002$) and male MUT/N ($p = 0.02$). As a result, there was no difference in calculated free testosterone in the groups either in males or females.

There was also a positive correlation between total testosterone and SHBG in the 37 males individuals ($r = 0.642$, $p < 0.0001$) and a negative correlation between SHGB and HOMAir (=−0.446, $p = 0.006$). No correlation was found between SHGB and HOMAir in women.

Table 1 Anthropometric and hormonal data of 37 female (25–62 years): MUT/MUT, MUT/N, and N/N groups (Mean ± SD)

Variable	N/N ($n = 11$)	MUT/MUT ($n = 12$)	MUT/N ($n = 14$)
Age (years)	43.09 ± 13.01	39.25 ± 12.23	38.57 ± 10.62
Weight (kg)	64.26 ± 12.07	38.06 ± 6.16[a]	57.5 ± 9.78[b]
Height (cm)	157.80 ± 6.50	124.37 ± 10.24[a]	156.42 ± 7.41[b]
BMI (weight/height^2)	25.85 ± 4.79	24.99 ± 4.70	23.19 ± 2.81
Estradiol (pg/ml)	68.21 ± 54.33	88.77 ± 82.45	67.58 ± 64.41
Prolactin (ng/ml)	6.35 ± 1.90	12.06 ± 9.87	7.81 ± 4.33
Testosterone (ng/ml)	0.35 ± 0.17	0.36 ± 0.23	0.40 ± 0.24
SHBG (nmol/l)	59.79 ± 31.9	74.37 ± 32.28	59.24 ± 45.54
Free Testosterone (ng/dl)	0.51 ± 0.28	0.41 ± 0.29	0.59 ± 0.52

[a] $p < 0.0001$ in comparison to N/N

[b] $p < 0.0001$ in comparison to MUT/MUT

Discussion

We had previously reported lower prolactin level in MUT/MUT climacteric women when compared to age-matched N/N women. A reduction in prolactin in IGHD subjects lacking GHRHR could be expected based on three factors: (1) the reduction of the pituitary volume [18] (although such hypoplasia is likely predominantly due to a reduction in somatotroph cells) [19]); (2) the lack of GHRH action and consequent nighttime reduced prolactin secretion [20]; and (3) (in females) the reduction of the volume of the uterus [21], the principal extra-pituitary secretion source of prolactin [22]. However, all of these three factors may also act in young adulthood reducing the prolactin levels in this period of life.

The main finding of this work is the lack of any difference in serum prolactin in MUT/MUT and MUT/N for the c.57+1G>A *GHRHR* mutation adults in both genders when a broader age range was studied. This is in agreement with the observation of normal breast feeding capacity [4],

Table 2 Anthropometric and hormonal data of 37 males (25–62 years): MUT/MUT, MUT/N, and N/N groups (Mean ± SD)

Variables	N/N ($n = 14$)	MUT/MUT ($n = 12$)	MUT/N ($n = 11$)
Age (years)	37.50 ± 11.96	39.25 ± 11.74	42.00 ± 11.39
Weight (kg)	73.89 ± 12.61	36.33 ± 5.87[a]	62.26 ± 10.56[b]
Height (cm)	164.50 ± 17.25	132.04 ± 6.98[a]	160.66 ± 8.81[b]
BMI (weight/height^2)	24.91 ± 3.06	21.17 ± 3.92	22.48 ± 2.84[c]
Estradiol (pg/ml)	31.43 ± 10.11	32.81 ± 14.35	37.31 ± 12.95
Prolactin (ng/ml)	5.86 ± 5.29	9.56 ± 7.70	6.89 ± 3.55
Testosterone (ng/ml)	5.73 ± 1.39	8.80 ± 2.53[a]	6.73 ± 1.73
SHBG(nmol/L)	28.7 ± 13.5	51.28 ± 15.97[a]	33.05 ± 16.15[d]
Free testosterone (ng/dl)	13.86 ± 4.2	15.6 ± 4.3	15.6 ± 5.6

[a] $p < 0.0001$ in comparison to N/N

[b] $p < 0.0001$ in comparison to MUT/MUT

[c] $p < 0.05$ in comparison to N/N

[d] $p < 0.05$ in comparison to MUT/MUT

and in contrast with the high frequency of lactation failure found in GHD women with prolactin insufficiency, long-term survivors of childhood leukemia treated with cranial radiotherapy [5]. Prolactin levels are normal and IGF-I reduction is more severe in our IGHD women than in leukemia survivors, in agreement with the hypothesis that it was lack of prolactin—and not GHD—responsible for these women's inability to breastfeed [5]. Accordingly, mice with targeted ablation of the GHRH gene have normal pituitary prolactin content and do not have problems with lactation [23]. Furthermore, we have previously shown that GH releasing peptide type 2 (GHRP-2), a GH secretagogue analog to the natural ligand Ghrelin, elicits a normal increase in prolactin levels in these IGHD individuals [24]. Therefore, we conclude that prolactin secretion is normal in congenital IGHD individuals with a *GHRHR* mutation.

Prolactin level was higher in the pre- than in postmenopausal MUT/MUT women [4]. Therefore, prolactin level reduction in MUT/MUT IGHD women is exclusively a climacteric finding. While there is no consensus in the literature about basal prolactin levels in climacteric [25–27], a recent analysis of the full 24-h profile clearly showed a dramatic drop in prolactin secretion in women after menopause [28]. We found a significant negative correlation between prolactin and age, and a positive correlation between basal prolactin and estradiol levels, suggesting that prolactin reduction in MUT/MUT climacteric women reflects anticipation of estradiol reduction seen at menopause [29].

Total testosterone is higher in MUT/MUT men compared to N/N. However, this finding results from increased SHBG, with no difference in calculated free testosterone. Interestingly, the "Practical guidelines of Testosterone Therapy in Adult Men with Androgen Deficiency Syndromes: An Endocrine Society Clinical Practice Guideline," omits GH deficiency as a cause of SHBG increase, despite listing acromegaly as a cause of SHBG reduction [17]. SHBG and IR can be linked by genetic or acquired (nutrition or body composition changes) factors, involving hepatocyte nuclear factor-4 alpha, the key regulator of *SHBG* transcription in the liver, but possibly other factors like PPAR gamma [30]. As low serum SHBG is a sensitive biomarker of insulin resistance, the high SHBG in males MUT/MUT may be caused by the increased IS [13].

In conclusion, serum prolactin levels in adult individuals who are homozygous and heterozygous for an inactivating *GHRHR* mutation are normal. The lower level of prolactin previously reported in climacteric IGHD women may reflect the short reproductive life with late menarche and earlier reduction in estradiol exposure of the pituitary lactotrophs.

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Ethical standards The Institutional Review Board of the Federal University of Sergipe approved the protocol. Written informed consent was obtained from all subjects.

Conflict 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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