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EFEITOS DA GHRELINA SOBRE A SECREÇÃO DE GH, ACTH E CORTISOL NA DOENÇA DE CUSHING ANTES E APÓS O TRATAMENTO COM CETOCONAZOL

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INTRODUÇÃO

INTRODUÇÃO

O controle da secreção do hormônio de crescimento (GH) pelos somatotrofos da adenohipófise é determinado por uma complexa interação entre dois peptídeos hipotalâmicos: o hormônio liberador de GH (GHRH), que estimula tanto a síntese como a secreção de GH, e a somatostatina (SRIF), que tem um efeito inibitório sobre a liberação de GH (Dieguez *et al.*, 1988). Além do GHRH e da SRIF, diversos outros fatores modulam a secreção de GH, atuando diretamente sobre a hipófise ou sobre a liberação destes dois peptídeos hipotalâmicos (Lengyel *et al.*, 1992).

Em 1977, antes da descoberta do GHRH, Bowers e col. desenvolveram pequenos peptídeos sintéticos a partir da molécula de met-encefalina que eram capazes de liberar GH (Bowers *et al.*, 1980). Posteriormente, em 1984, tal descoberta levou à síntese de peptídeos mais potentes, incluindo um hexapeptídeo denominado GHRP-6 (growth hormone-releasing peptide-6) (Bowers *et al.*, 1984). Este peptídeo promove a liberação de GH por mecanismos não totalmente esclarecidos, porém diferentes dos utilizados pelo GHRH (Korbonits & Grossman *et al.*, 1995). Estudos realizados com estes secretagogos de GH (GHS) nas últimas décadas reforçaram a hipótese de existir um papel fisiológico de tais compostos na regulação da secreção de GH (Dieguez & Casanueva, 2000). A presença de receptores específicos para os GHS, tanto no hipotálamo quanto na hipófise, apontava para a existência de um peptídeo endógeno semelhante, que, porém, ainda não havia sido identificado (Codd *et al*., 1989; Blake & Smith, 1991; Goth *et al.*, 1992). A clonagem do receptor “órfão” dos GHS, em 1996, comprovou a hipótese da existência de um terceiro sistema de controle da secreção de GH (Howard *et al.*, 1996). Este receptor pertence ao grupo de receptores ligados à proteína G, é altamente conservado entre as espécies, e é expresso na adenohipófise, hipotálamo e outras áreas do sistema nervoso central (Howard *et al.*, 1996). Finalmente, em 1999, Kojima e col. clonaram o ligante endógeno dos GHS, que foi isolado no estômago, e denominado ghrelina. A estrutura deste peptídeo, que é acilado, é completamente diferente dos peptídeos conhecidos e

também da estrutura química dos GHS (Kojima *et al.*, 1999). A modificação n-octanoil do resíduo serina na posição 3 da molécula é fundamental para a atividade biológica do peptídeo (Kojima *et al.*, 1999; Bednarek *et al.*, 2000). A ghrelina está presente em altas concentrações no trato gastrointestinal, principalmente no estômago (Kojima *et al.*, 1999; Date *et al.*, 2000a) e, em menores concentrações, no sistema nervoso central, principalmente no núcleo arqueado (Kojima *et al.*, 1999; Shuto *et al.*, 2001).

A ghrelina promove a liberação de GH tanto *in vivo* como *in vitro*, em animais e no homem, de modo dose-dependente (Kojima *et al.*, 1999; Takaya *et al.*, 2000; Date *et al.*, 2000b; Peino *et al.*, 2000). Em humanos é o mais potente estímulo para a secreção de GH, levando à maior liberação de GH que o GHRP-6 e o GHRH em doses equimolares (Arvat *et al.*, 2001). Da mesma forma que os GHS, a ghrelina estimula a secreção de GH através de mecanismos hipofisários e hipotalâmicos (Korbonits *et al.*, 2004). A ghrelina e os GHS ativam os GHS-R em cultura de células hipofisárias *in vitro* (Kojima *et al.* 1999), porém sua atividade *in vivo* é maior (Arvat *et al*., 2001), sugerindo que seu principal sítio de ação seja hipotalâmico. Além disso, os efeitos desses peptídeos estão reduzidos na desconexão hipotálamo-hipofisária (Popovic *et al*., 1995; Popovic *et al*., 2003). Foi demonstrado que a integridade da via do GHRH é fundamental para a ação da ghrelina e dos GHS (Dickson *et al.*, 1995; Pandya *et al.*, 1998; Maheshwari *et al.*, 1999; Tannenbaum *et al*., 2001; Tannenbaum *et al*., 2003). Além disso, a ghrelina e os GHS podem ativar os GHS-R expressos em ¼ dos neurônios produtores de GHRH no núcleo arqueado (Tannenbaum *et al.*, 2001). Foi proposto um modelo de ação da ghrelina que envolve, além da liberação hipotalâmica de GHRH, a amplificação do efeito do GHRH no somatotrofo, e também o antagonismo funcional da somatostatina (Tannenbaum *et al*., 2003). A ação da ghrelina e dos GHS no somatotrofo se dá através da ativação do sistema da proteína quinase C, com a elevação de diacil glicerol, inositol trifosfato e de cálcio intracelular (Howard *et al.*, 1996; Chen *et al*., 1996), enquanto que o GHRH estimula a liberação de AMPc intracelular, ativando a via da proteína quinase A (Goth *et al*., 1992).

Recentemente foi demonstrado, *in vitro*, que a ghrelina também é capaz de estimular a liberação de AMPc sem a presença do GHRH e ativar os sistemas de influxo de cálcio extracelular (Malagon et al., 2003), e também de estimular a via da MAP (mitogen-activated protein) quinase (Kineman et al., 2007), ações estas mais amplas que as dos GHS, o que poderia explicar sua maior potência. A descoberta da ghrelina veio comprovar a existência de uma terceira via de regulação da secreção de GH. Entretanto, não se conhece ainda o papel desta via na fisiologia e fisiopatologia da secreção desse hormônio (Lengyel, 2006).

A ghrelina e os GHS também são capazes de estimular a liberação de ACTH e de cortisol em indivíduos normais, com um efeito mais potente da ghrelina (Takaya *et al*., 2000; Arvat *et al*., 2001). A ação desses peptídeos é exclusivamente hipotalâmica, já que não aumentam a liberação de ACTH em fragmentos de hipófise *in vitro* (Cheng et al, 1993; Kojima et al., 1999), e os corticotrofos normais não expressam GHS-R (Korbonits *et al.*, 2001). Na desconexão hipotálamo-hipofisária o efeito da ghrelina e dos GHS sobre o ACTH é abolido (Popovic *et al*., 1995; Popovic *et al*., 2003). Além disso, foi demonstrado que o GHRP-6 estimula a liberação de arginina-vasopressina (AVP) em fragmentos hipotalâmicos de ratos *in vitro* (Korbonits *et al.*, 1999b), enquanto que a ghrelina aumenta a liberação de AVP, CRH e NPY (Wren *et al.*, 2002), com um efeito predominante na secreção de AVP (Mozid *et al.*, 2003). Embora controverso, os GHS e a ghrelina estimulam a secreção de ACTH em humanos provavelmente através do aumento da liberação hipotalâmica de AVP (Korbonits *et al*., 1999a; Coiro *et al*., 2005)

A administração de ghrelina também leva ao aumento dos níveis de glicose em indivíduos normais (Broglio *et al*., 2001), provavelmente por estimular diretamente a liberação de glicose hepática (Gauna *et al*., 2005).

1) Secreção de GH, ACTH e cortisol na doença de Cushing (DC)

Os glicocorticóides exercem um importante papel na regulação da secreção de GH. O retardo de crescimento e a diminuição dos níveis de GH causados pela exposição crônica a quantidades suprafisiológicas desses esteróides têm sido amplamente observados há mais de 20 anos (Voutilainen *et al.*,1985; Dieguez *et al.*,1988; Wajchenberg *et al.*, 1996).

A secreção de GH está inibida no hipercortisolismo crônico e as respostas do GH a diversos estímulos, como ghrelina, GHRP-6 e GHRH estão diminuídas na DC (Hotta *et al.*, 1988; Borges *et al.*, 1997; Leal-Cerro *et al.*, 2002b; Giordano *et al.*, 2005; Correa-Silva *et al.*, 2006). Os mecanismos envolvidos no efeito inibitório do hipercortisolismo crônico sobre a liberação de GH não estão totalmente esclarecidos. A hipersecreção de SRIF parece improvável, pois seu bloqueio pela piridostigmina não reestabelece a resposta do GH ao GHRH no hipercortisolismo endógeno (Borges *et al*.1993; Leal-Cerro *et al.*, 1990). Além disso, a supressão da SRIF endógena, após altas doses de SRIF exógena, não aumenta a secreção de GH na DC (Leal-Cerro *et al.*, 2002a). A diminuição da liberação hipotalâmica de GHRH pode estar envolvida, uma vez que a administração repetida (“priming”) de GHRH aumenta a resposta do GH a esse peptídeo (Leal-Cerro *et al*., 1993). Como não ocorre restauração total da resposta do GH, provavelmente existem outros fatores envolvidos. O mecanismo mais provável parece ser a inibição direta dos somatotrofos pelo hipercortisolismo (Leal-Cerro *et al.*, 1998; Leal-Cerro *et al*., 2002a).

Na DC as respostas do ACTH e do cortisol à administração de ghrelina e de GHRP-6 estão aumentadas, quando comparadas às de indivíduos normais (Leal-Cerro *et al.*, 2002b; Giordano *et al.*, 2005; Correa-Silva *et al*., 2006). Isso pode ocorrer devido a uma ação direta desses peptídeos nos GHS-R presentes no adenoma corticotrófico

(Korbonits *et al.*, 1998)., embora um efeito hipotalâmico também tenha sido sugerido na DC (Arvat *et al.*, 1999; Oliveira *et al.*, 2003).

2) Efeitos do tratamento do hipercortisolismo na secreção de GH e de ACTH na DC

Existem dados na literatura sugerindo que intervenções agudas, tais como a inibição da síntese de ácidos graxos livres (Leal- Cerro *et al.*, 1997) e a restrição calórica de curta duração (Leal-Cerro *et al.*, 1998), são capazes de aumentar a liberação de GH em pacientes com DC. Entretanto, a recuperação da secreção de GH na DC após a remissão do hipercortisolismo, através de cirurgia transesfenoidal ou radioterapia, é bastante controversa (Tyrrell *et al.*, 1977; Kuwayama *et al.*, 1981; Burke *et al.*, 1990; Magiakou *et al.*, 1994; Hughes *et al.*, 1999; Veldman *et al.*, 2000; Tzanela *et al.*, 2004; Pecori Giraldi *et al.*, 2007). Isto poderia ocorrer devido à indução de lesão somatotrófica por essas modalidades de tratamento.

O cetoconazol é um inibidor da esteroidogênese adrenal que tem sido usado no tratamento da síndrome de Cushing nas últimas duas décadas. Esse composto é capaz de normalizar os níveis de cortisol em aproximadamente 70% destes pacientes, e raramente causa lesão hepática (Engelhardt *et al.*, 1994).

Não existem dados na literatura sobre o efeito do tratamento com cetoconazol na secreção de GH em pacientes com DC. Os estudos que avaliaram a secreção de ACTH basal (Loli *et al.*, 1986; Boscaro *et al.*, 1987; McCance *et al.*, 1987; Terzolo *et al.*, 1988; Sonino *et al.*, 1991) e a estimulada pelo CRH (Loli *et al.*, 1986; Boscaro *et al.*, 1987) durante o tratamento com cetoconazol apresentam resultados conflitantes.

OBJETIVOS

ESTUDO 1

a) Avaliar as respostas do GH à administração de ghrelina, GHRP-6 e GHRH na DC.

b) Comparar as respostas do ACTH e do cortisol à ghrelina e ao GHRP-6 na DC.

c) Avaliar as respostas da glicose à ghrelina e ao GHRP-6 na DC.

ESTUDO 2

Avaliar o efeito da diminuição dos níveis circulantes de glicocorticóides em pacientes com DC, através do tratamento com cetoconazol por 3 e 6 meses:

a) nas respostas do GH à ghrelina, ao GHRP-6 e ao GHRH.
b) na liberação de ACTH e de cortisol após ghrelina e GHRP-6.
c) nas respostas da glicose à ghrelina e ao GHRP-6.

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Decreased GH secretion and enhanced ACTH and cortisol release after ghrelin administration in Cushing's disease: Comparison with GH-releasing peptide-6 (GHRP-6) and GHRH

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Decreased GH secretion and enhanced ACTH and cortisol release after ghrelin administration in Cushing's disease: Comparison with GH-releasing peptide-6 (GHRP-6) and GHRH

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Abstract GH responsiveness to GH secretagogues (GHS) is blunted in Cushing's disease (CD), while ACTH/cortisol responses are enhanced, by mechanisms still unclear. Ghrelin, the endogenous ligand for GHS-receptors (GHS-R), increases GH, ACTH, cortisol and glucose levels in humans. This study evaluated the GH, ACTH, cortisol and glucose-releasing effects of ghrelin in CD in comparison with GHRP-6. GHRH-induced GH release was also studied. Ten patients with CD (BMI 26.9 ± 1.0 kg/m^2) and ten controls (BMI 24.4 ± 1.1 kg/m^2) received ghrelin (1 μg/kg), GHRP-6 (1 μg/kg) and GHRH (100 μg) separately. GH, ACTH, cortisol and glucose levels were measured. In CD ghrelin-induced GH (μg/L; mean $\pm$ SE) release (peak: 7.2 ± 3.0) was higher than seen with GHRP-6 (2.7 ± 1.0) and GHRH (0.7 ± 0.2), but lower than in controls (ghrelin: 58.3 ± 12.1; GHRP-6: 22.9 ± 4.8; GHRH: 11.3 ± 3.7). In controls ACTH (pg/mL) release after ghrelin (79.2 ± 26.8) was higher than after GHRP-6 (23.6 ± 5.7). In CD these responses (ghrelin: 192 ± 43; GHRP-6: 185 ± 56) were similar, and enhanced compared to controls. The same was observed with cortisol. Glucose levels failed to increase after ghrelin in CD, differently than in controls. Our data suggests that hypothalamic and pituitary pathways of GH release activated by ghrelin, GHRP-6 and GHRH are deranged in chronic hypercortisolism. The increased ACTH/cortisol responses to ghrelin and GHRP-6 in CD could be mediated by overexpression of GHS-R in ACTH-secreting adenomas. Hypercortisolism apparently impairs the ability of ghrelin to increase glucose levels.

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Introduction

GH secretion is classically modulated by an interplay between GHRH and SRIF. However, several studies have suggested that GH secretagogues (GHS) might also have a role in this process, acting at both hypothalamic and pituitary receptors [1–3]. Ghrelin, the recently discovered endogenous ligand of this receptor, is present both in the stomach and in the hypothalamus, mainly in the arcute nucleus [4–6]. However, this acylated peptide has a different chemical structure than GHS [4]. This peptide can cross the blood-brain barrier [7] and is able to induce GH release in a potent manner in animals and humans after i.v injection [8–10]. It also increases circulating ACTH and cortisol levels [10]. The main site of action of ghrelin and GHS is the hypothalamus, as there is a major decrease in their GH-releasing ability in hypothalamic-pituitary disconnection, which also abolishes the ACTH and cortisol rise [11, 12]. However, ghrelin and GHS also act at pituitary level to increase GH release, by different receptors and intracellular mechanism than those activated by GHRH [4, 13, 14]. A model for ghrelin/GHS action has been proposed, which would involve an activation of GHRH neurons in the arcuate nucleus, with increased GHRH release, amplification of GHRH effects at the somatotroph and functional antagonism of SRIF [15, 16]. Ghrelin is also able to increase glucose levels in humans, probably by a direct hepatic action, which is followed by a decrease in circulating insulin [17, 18].

It is well known that endogenous glucocorticoid excess impairs GH secretion [19]. It has been previously shown that GH responses to several stimuli, including GHRH, are

severely blunted [20, 21]. We and others have demonstrated that the GH response to GHRP-6 is decreased in endogenous hypercortisolism [22, 23]. The mechanisms involved in the inhibitory effect of long-term endogenous glucocorticoid excess on GH secretion are as yet unclear. High glucocorticoid levels could interfere with hypothalamic and/or pituitary GH-releasing pathways [19]. However, it has been suggested that an increase in hypothalamic somatostatin release is unlikely to have a major role [19]. A decrease in hypothalamic GHRH secretion [24] and a direct effect of glucocorticoids at the somatotroph [25] could be involved.

It has also been shown that patients with Cushing's disease (CD) have an increased ACTH and cortisol responsiveness to hexarelin compared to normal subjects [26]. This could be due to a direct effect of this peptide on GHS-R in the corticotroph adenoma [27], while in normal subjects a hypothalamic site of action has been suggested [28].

As ghrelin and GHS have different chemical structures and as several receptors for these peptides might exist [4, 5, 29] the aims of this work were to evaluate the GH, ACTH, cortisol and glucose-releasing effects of ghrelin in CD and compare them to GHRP-6. GHRH-induced GH release was also studied, as this peptide stimulates GH via different mechanisms than those used by GHS.

Material and methods

Subjects

Ten patients with CD (8 women and 2 men) were studied (9 micro and 1 macroadenoma). Their mean age was 33.2 ± 3.6 years (range: 18–56) and their mean body mass index (BMI) was 26.9 ± 1.0 kg/m^2 (range: 20.6–31.6). All patients, except one, were previously untreated. One patient with a microadenoma had been submitted to transphenoidal surgery and had recurrence of the disease after one year of clinical and laboratory remission. Diagnosis of CD was established on the basis of clinical features and standard hormonal criteria, including increased free urinary cortisol excretion, lack of suppression of serum cortisol after low-dose dexamethasone test (1 mg orally overnight), normal or high basal plasma ACTH and serum cortisol levels at 08:00 h, and positive DDAVP test. Magnetic resonance imaging (MRI) of the sellar region showed a pituitary adenoma in all patients. The diagnosis of ACTH-secreting tumor was confirmed after surgery by positive ACTH immunostaining of the excised pituitary adenoma. All patients had normal renal function and four had secondary diabetes mellitus treated with diet, metformin or insulin.

Ten normal subjects (4 women and 6 men) with a mean age of 33.5 ± 3.3 years (range: 20–47) and matched for BMI (24.4 ± 1.1 kg/m^2; range: 19.5–30.8) were also studied. They were free of any medication at the time of the study protocol. The women were tested in the early follicular phase of their menstrual cycle.

Study protocol

The experimental protocol was approved by the ethics committee of Universidade Federal de São Paulo, and all subjects gave prior informed consent.

Each subject was submitted to three tests, in random order, with a minimum interval of 48 hrs between them, either receiving ghrelin (Neosystem, Strasbourg, France), GHRP-6 (Bachem, San Carlos, USA) or GHRH (1–29) NH2 (Clinalfa, Läufelfingen, Switzerland). All tests were performed after an overnight fast, and the subjects remained recumbent throughout. At 08:00 h an indwelling catheter was inserted into an antecubital vein and was kept patent by a slow saline infusion. The tests started 45 min later. After the first blood sample, each subject received ghrelin at a dose of 1 μg/kg iv, GHRP-6 at the same dose or GHRH at a dose of 100 μg iv. Blood samples were obtained every 15 min until 120 min for hormonal determination. Glucose levels were measured every 30 min.

Assays

Serum GH was measured in duplicate by an immunofluorometric assay (Wallac, Turku, Finland). The sensitivity of the method is 0.01 μg/L, with mean inter- and intra-assay coefficients of variation (CV) of 7.0% and 6.7% respectively. An immunochemiluminometric assay (Diagnostic Prod. Corporation, Los Angeles, USA) was used to measure plasma ACTH. The sensitivity of the method is 5 pg/mL, with mean inter- and intra-assay CV of 3.6% and 2.8% respectively. Serum cortisol levels were measured in duplicate by an imunofluorometric assay (Wallac, Turku, Finland), with sensitivity of 0.2 μg/dL, and mean inter and intra-assay CV of 8.2% and 6.2% respectively. Glucose levels were determined by the hexokinase method.

Statistical analysis

Friedman's analysis of variance was performed to compare GH, cortisol and ACTH levels after the injection of each peptide and to compare GH responses in the same group. The Wilcoxon signed rank test was used for comparisons of ACTH and cortisol values within the same group. The Mann-Whitney rank sum test was performed for comparisons between two different groups. The area under the curve (AUC) was calculated by trapezoidal integration. The Spearman correlation coefficient was calculated when appropriate. Undetectable GH (μg/L) and ACTH (pg/mL) values were considered to be equal to 0.01 and 5.0 respectively,

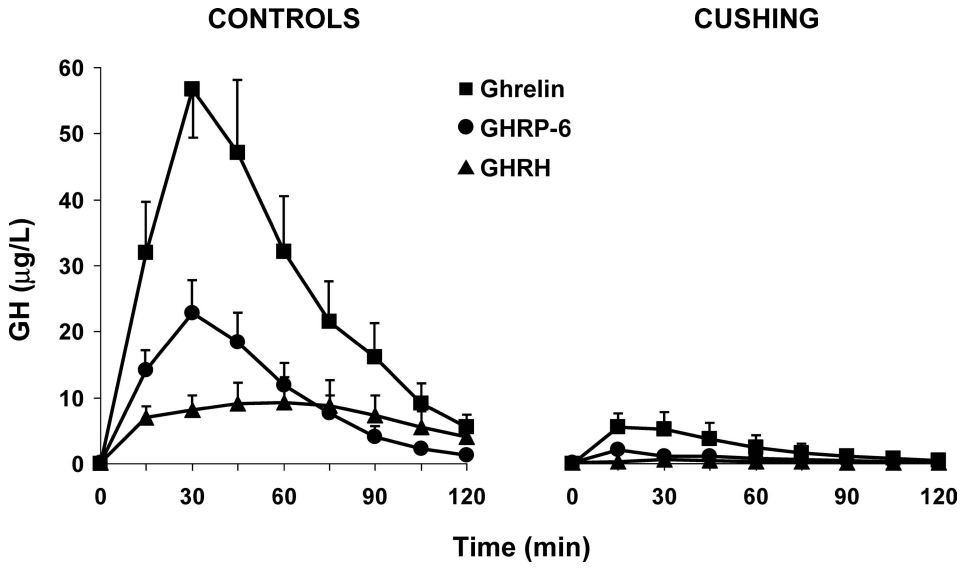

Fig. 1 Mean GH values after ghrelin, GHRP-6 and GHRH administration in Cushing's disease ($n = 10$) and in controls ($n = 10$); mean ± SE

for statistical purposes. $P < 0.05$ was considered statistically significant. Results are reported as mean ± SE.

Results

In normal subjects mean peak GH (μg/L; mean ± SE) and AUC (μg/L.120 min) values after ghrelin administration were 58.3 ± 12.1 and 3268.0 ± 773.0, respectively. This response was higher than that obtained after GHRP-6 (peak: 22.9 ± 4.8; AUC: 1234.0 ± 302.0) and GHRH injection (11.3 ± 3.7; 863.0 ± 297.0). GH responsiveness to GHRP-6 and GHRH did not differ significantly (Fig. 1).

In patients with CD ghrelin induced a higher GH response (peak: 7.2 ± 3.0; AUC: 310.0 ± 169.0) than seen with GHRP-6 (2.7 ± 1.0; 106.0 ± 48.0) and GHRH (0.7 ± 0.2; 42.0 ± 12.0). GH responsiveness to GHRH was lower than that of GHRP-6 in terms of peak GH values (Fig. 1).

When patients with CD were compared to controls, a significant decrease in GH responses to ghrelin, GHRP-6 and GHRH were observed in hypercortisolemic patients (Fig. 2). There was a negative correlation between basal serum cortisol values and peak GH values after ghrelin ($r = -0.624$, $P = 0.048$) and GHRP-6 injection ($r = -0.697$, $P = 0.021$) in patients with CD. No correlation was found between BMI or age and GH responses in these patients.

In the control group mean peak ACTH (pg/mL) and AUC (pg/mL.90 min) values were significantly higher after ghrelin injection (79.2 ± 26.8; 4342 ± 1532) compared to GHRP-6 (23.6 ± 5.7; 1399 ± 155). The same pattern was observed for peak cortisol (μg/dL) and AUC (μg/dL.90 min) values after ghrelin (peak: 17.3 ± 1.3; AUC: 1298 ± 97) and GHRP-6 (13.3 ± 0.9; 922 ± 72) (Fig. 3).

Patients with CD had similar ACTH responses to ghrelin (192 ± 43; 12750 ± 2784) and GHRP-6 (185 ± 56; 12086 ± 3430). Cortisol responses to ghrelin (36.3 ± 5.3; 2827 ± 406) and GHRP-6 (33.3 ± 4.7; 2504 ± 351) did not differ significantly.

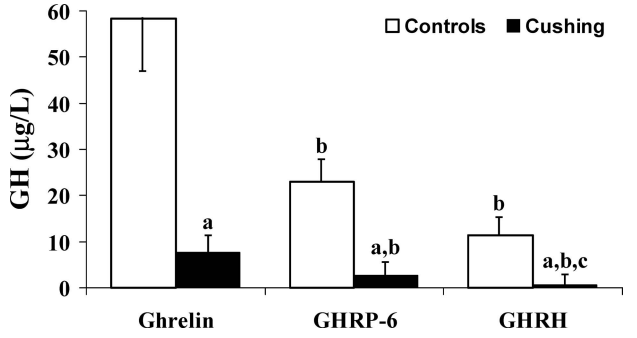

Fig. 2 Peak GH values after ghrelin, GHRP-6 and GHRH administration in Cushing's disease ($n = 10$) and in controls ($n = 10$) (mean ± SE; a, $P < 0{,}05$ vs. controls; b, $P < 0{,}05$ vs. ghrelin; c, $P < 0{,}05$ vs. GHRP-6)

When patients with CD were compared to controls, higher ACTH and cortisol values both after ghrelin and GHRP-6 injection were observed in hypercortisolemic patients. No correlations were found between ACTH responses and basal cortisol levels in CD.

In normal subjects there was a significant rise in glucose levels (mg/dL) 30 min after ghrelin administration (basal: 83 ± 2; 30 min: 90 ± 2) and these values remained elevated until 90 min. Patients with CD did not increase glucose levels significantly after ghrelin injection (Table 1), even when the data was analysed excluding the diabetic patients (data not shown). No significant changes in glucose values were seen after GHRP-6 administration both in controls and in CD.

There were no differences in age and BMI between the two groups.

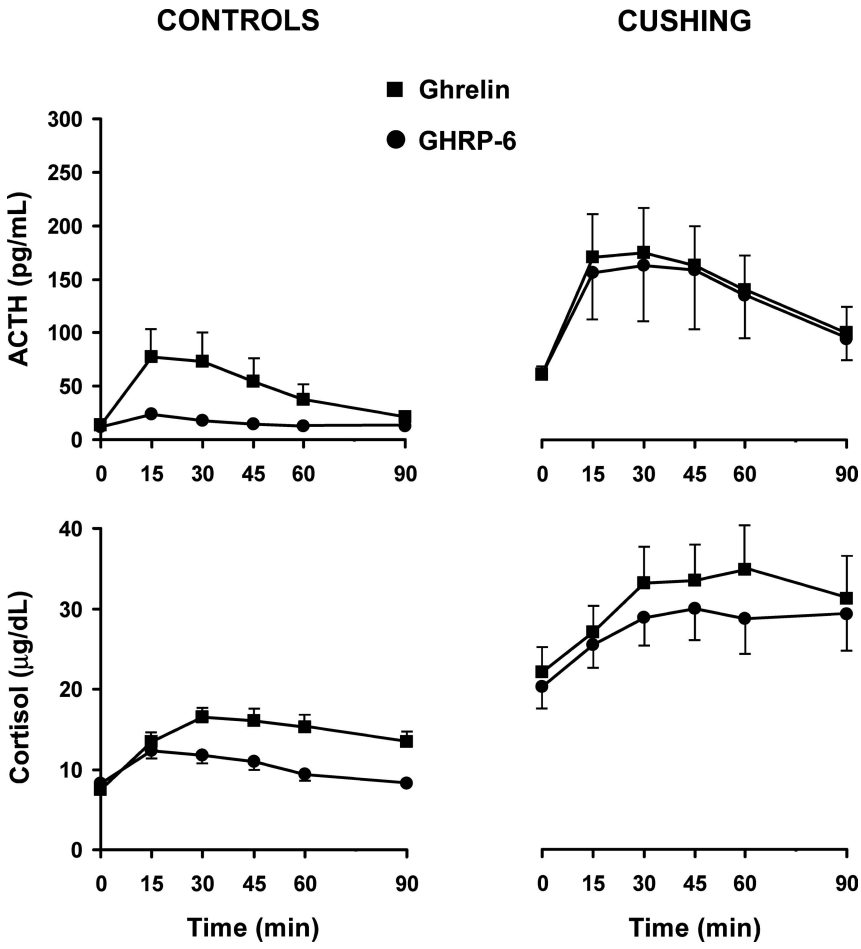

Fig. 3 Mean ACTH and cortisol values after ghrelin and GHRP-6 administration in Cushing's disease ($n = 10$) and in controls ($n = 10$); mean ± SE

Table 1 Mean glucose levels after ghrelin administration in Cushing's disease ($n = 9$) and in controls ($n = 10$); mean ± SE

	Glucose levels (mg/dL) Time (min)					
Group	0	30	60	90	120	P
Cushing	112 ± 17	116 ± 15	117 ± 15	118 ± 15	118 ± 16	NS
Controls	83 ± 2	90 ± 2	87 ± 2	87 ± 2	88 ± 2	<0.01

Side effects

Hunger sensation was reported in five patients with CD and six controls after ghrelin administration. Transient facial flushing after ghrelin was observed in two CD, while with GHRP-6 and GHRH this was seen in four patients and two controls. Nausea was seen after both ghrelin and GHRP-6 in one control.

Discussion

In our patients with CD GH responses to ghrelin, GHRP-6 and GHRH were blunted. The magnitude of GH release after ghrelin was higher compared to the other stimuli, which coud be due to the greater potency of this peptide. It is also possible that ghrelin-activated GH-releasing pathways are less deranged by high glucocorticoid levels. We and others have previously shown that GHRP-6 and GHRH-induced GH release is inhibited in endogenous hypercortisolism [22, 23]. Moreover, our data confirms two recent reports showing that the GH response to ghrelin is decreased in patients with CD [30, 31].

Obesity is associated with blunted GH responses to ghrelin, GHRP-6 and GHRH [32–34]. A decreased GH response to ghrelin was previously found in obese [30] and overweight [31] hypercortisolemic patients compared to lean normal subjects. A relationship between obesity and blunted GH response to ghrelin could not be excluded in these patients [30]. In our study patients had a mean BMI of 26.9 ± 1.1 kg/m^2 and were matched to the control group. Moreover, we did not find a correlation between GH responses to ghrelin and BMI.

Therefore, our data suggests that it is unlikely that obesity could have a major role in the decreased GH response to ghrelin in CD.

The mechanisms involved in the inhibition of GH release in endogenous hypercortisolism remain unclear. Ghrelin and GHS act at both hypothalamic and pituitary level to modulate GH secretion [2, 4]. The main site of action is the hypothalamus as there is lack of significant GH release after the administration of these compounds in hypothalamic-pituitary disconnection [11, 12]. These peptides activates GHS-R, which have been located in GHRH neurons in the arcuate nucleus [16]. It has also been shown that an intact GHRH system is necessary for their GH-releasing effect to occur [16, 35].

Interestingly, it has been previously suggested that glucocorticoid excess inhibits hypothalamic GHRH neurons [30]. This could eventually explain our findings, as chronic GHRH deficiency blunts the GH responses to acute injections of ghrelin/GHS and GHRH [11, 12, 36]. Another possibility would be a direct pituitary effect of glucocorticoids. GHS/ghrelin and GHRH bind to different pituitary receptors and activate different intracellular transduction pathways at the somatotroph [37]. GHRH stimulates intracellular cyclic AMP and protein kinase A mechanisms [13], while ghrelin and GHRP-6 activate protein kinase C signal transduction, via inositol triphosphate [2, 14]. Although controversial in animals [38, 39], it has been shown that glucocorticoids downregulate human GHS-R [40]. Therefore, these steroids could eventually interfere with ghrelin/GHS-stimulated transduction mechanisms at the somatotroph, with a possible additional effect on GHS-R located in GHRH-releasing neurons in the hypothalamus [16]. Although further studies are necessary, these hypotheses could perhaps explain why chronic glucocorticoid excess inhibits GH-releasing pathways activated by both ghrelin/GHS and GHRH.

In controls ghrelin was able to induce a higher ACTH and cortisol release than seen with GHRP-6 injection, which is similar to that reported with hexarelin [10]. It has been previously shown that the ACTH-releasing effect of these peptides occur at hypothalamic level, as it is lost after hypothalamic-pituitary disconnection [11, 12]. Ghrelin is able to stimulate both AVP and CRH release from rat hypothalamic fragments *in vitro* [41, 42]. Although controversial, in normal subjects the ACTH-releasing effect of hexarelin and ghrelin could be due to an increase in hypothalamic AVP secretion [3, 28, 43].

In our patients with CD the ACTH/cortisol responses after the injection of ghrelin and GHRP-6 were higher than those obtained in controls, confirming earlier reports [26, 30, 31, 44]. Our results also show that the higher ACTH/cortisol-releasing potency of ghrelin compared to GHRP-6 seen in the control group is lost in CD, as in these patients these responses were similar. Therefore, in endogenous hypercortisolism ghrelin and GHRP-6 stimulate ACTH/cortisol release differently than in controls. Although in normal corticotrophs GHS-R are apparently lacking [15], there is increased expression of these receptors in ACTH-producing tumors [27, 45]. The enhanced ACTH/cortisol release seen in CD could be due to a direct effect of these peptides on GHS-R in the tumor. It is likely that these tumoral receptors are quite resistant to the normal inhibitory effect of high circulating glucocorticoids. However, other mechanisms might be involved in this process, as previously suggested [44, 46].

Ghrelin administration caused a significant increase in glucose levels in normal subjects, which was similar in magnitude to that described previously [17]. This was not seen with GHRP-6 injection, as reported earlier [47]. However, in our patients with CD this increase in glucose values after ghrelin was not observed, differently than reported by Giordano et al. [31]. The reasons for these discrepant findings are unclear. Even when our data was analysed excluding the diabetic subjects, hypercortisolemic patients failed to increase glucose values after ghrelin administration. It has been recently shown that the increase in circulating glucose after ghrelin injection is non-GH dependent as it is maintained in GH-deficient patients [48]. It has also been demonstrated that ghrelin stimulates glucose output from porcine hepatocytes in culture [18]. Our data could, therefore, suggest that high circulating glucocorticoids impair the hepatic action of ghrelin to increase glucose levels.

In summary, in patients with CD there is a decrease in ghrelin-induced GH release and this response is higher than seen with GHRP-6 and GHRH. This suggests that hypothalamic and pituitary pathways of GH release activated by these peptides are deranged in endogenous hypercortisolism. The ACTH/cortisol releasing ability of both ghrelin and GHRP-6 are enhanced in CD, which could be mediated by overexpression of GHS-R in ACTH-secreting adenomas. Our data also suggests that high circulating glucocorticoids impair the ability of ghrelin to increase glucose levels. Further studies are necessary to elucidate the mechanisms involved in these altered responses.

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Increased GH and ACTH responses to ghrelin after 6 months of ketoconazole-induced fall of cortisol levels in patients with Cushing's disease: comparisons with GH-releasing peptide-6 (GHRP-6) and GHRH

Estudo 2

Increased GH and ACTH responses to ghrelin after 6 months of ketoconazole-induced fall of cortisol levels in patients with Cushing's disease: comparisons with GH-releasing peptide-6 (GHRP-6) and GHRH

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Abstract

Context: In Cushing's disease (CD), GH release after ghrelin, GHRP-6 and GHRH is blunted and ACTH/cortisol responses to ghrelin and GHRP-6 are exaggerated. Recovery of GH secretion after remission of hypercortisolism is controversial. The effect of clinical treatment with ketoconazole, a steroidogenesis inhibitor, on GH secretion in CD is unknown, while ACTH results are controversial. *Objective:* To compare ghrelin- and GHRP-6-induced GH and ACTH/cortisol release before and after ketoconazole treatment in patients with CD. GHRH-induced GH secretion and glucose response to ghrelin were also studied. *Design/Patients:* 8 untreated patients with CD (BMI: 28.5 $\pm$ 0.8kg/m^2) were evaluated before and after 3 and 6 months of ketoconazole treatment and compared to 11 controls (BMI: 25.0 $\pm$ 0.8kg/m^2). *Results:* After ketoconazole use mean urinary free cortisol (UFC) decreased significantly (before: 222.4 ± 35.0 µg/24h; 3rdmonth: 61.6 ± 10.1; 6thmonth: 39.1 ± 10.9), reaching normal or near-normal values in 6 cases at the 6th month. There was a significant increase in ghrelin-induced GH release after 6 months of treatment (peak before: 6.8 ± 2.3 µg/L; 6thmonth: 16.0 ± 3.6), but GH values were still lower than those of controls (54.1 ± 11.2). GHRP-6-induced GH secretion increased, although not significantly, while GH responsiveness to GHRH and IGF-1 levels were unchanged. Ghrelin- and GHRP-6-stimulated peak cortisol levels decreased during ketoconazole use (ghrelin and GHRP-6 before: 42.9 ± 5.0 µg/dL and 37.9 ± 4.8; 3rdmonth: 23.1 ± 2.5 and 27.8 ± 3.3; 6thmonth: 25.0 ± 2.7 and 20.7 ± 2.6). An increase in peak ACTH values after ghrelin was observed in the 6th month of treatment (before: 272 ± 70 pg/mL; 6thmonth: 509 ± 51). After 6 months of treatment ghrelin-induced glucose increase was seen after 30 minutes, similarly to controls, while at diagnosis this occurred at 120 minutes. *Conclusions:* GH responsiveness to ghrelin increase significantly after 6 months of ketoconazole in CD, although become lower than those of controls. This could suggest that relatively short-term periods of normal or near-normal cortisol values are able to improve glucocorticoid-induced GH suppression in CD. GH-releasing mechanisms stimulated by ghrelin/GHS could be more sensitive to the decrease in circulating cortisol levels, as no changes in GHRH-induced GH release were observed. The enhanced ACTH responses to ghrelin after ketoconazole in CD could be due to activation of the hypothalamic-pituitary-adrenal (HPA) axis and/or to an increase in GHS-R expression in the corticotrophic adenoma, consequent to reductions in circulating glucocorticoids. Hypercortisolism may alter the normal pattern of glucose release after ghrelin.

Introduction

GH secretion is classically regulated by GHRH and SRIF, but the GH secretagogues (GHS) might also have a role in this process, acting at both hypothalamic and pituitary receptors (Bowers *et al.*, 1984; Howard *et al.*, 1996; Arvat *et al.*, 1997). Ghrelin, the endogenous ligand of the GHS receptors (GHS-R), is present in the stomach and in the hypothalamus, mainly in the arcuate nucleus (Gnanapavan *et al.*, 2002). The active peptide, which is acylated, has a different chemical structure than GHS (Kojima *et al.*, 1999). Ghrelin and GHRP-6, a GHS, are able to induce GH, ACTH and cortisol release (Arvat *et al.*, 2001), and their main site of action is the hypothalamus, as these effects are reduced or abolished in hypothalamic-pituitary disconnection (Popovic *et al.*,1995; Popovic *et al.*, 2003). The proposed model for ghrelin/GHS action on GH secretion probably involves activation of GHRH neurons in the arcuate nucleus, with increased GHRH release, amplification of GHRH effects at the somatotroph and functional antagonism of SRIF (Smith *et al.*, 1997; Tannenbaum *et al.*, 2003). GHS/ghrelin and GHRH bind to different receptors and activate different intracellular mechanisms at the somatotroph. GHRH stimulates cyclic AMP and protein kinase A pathways (Goth *et al.*, 1992) while ghrelin and GHRP-6 activate protein kinase C signal transduction, via inositol triphosfate (Howard *et al.*, 1996; Chen *et al.*, 1996). Ghrelin is also able to increase glucose levels (Broglio *et al.*, 2001).

GH release is impaired in chronic hypercortisolism. It has been shown that GH responsiveness to several stimuli, including ghrelin, GHRP-6 and GHRH are blunted in patients with Cushing's disease (CD) (Hotta *et al.*, 1988; Borges *et al.*, 1997; Leal-Cerro *et al.*, 2002b; Giordano *et al.*, 2005; Correa-Silva *et al.*, 2006). The mechanisms involved in the inhibitory effect of chronic endogenous glucocorticoid excess on GH secretion are not clear. An increase in SRIF release from the hypothalamus is unlikely to have a major role (Leal-Cerro *et al.*, 2002a). A decrease in hypothalamic GHRH secretion (Leal-Cerro *et al.*, 1993) and a direct effect of glucocorticoids at the somatotroph (Leal-Cerro *et al.*, 1998) might be involved.

In CD there is an increase in ACTH and cortisol responsiveness to ghrelin and GHRP-6 compared to normal subjects (Correa-Silva *et al.*, 2006). This could be due to a direct effect of these peptides on GHS-R in the corticotroph adenoma (Korbonits *et al.*, 1998), while in normal subjects (Korbonits *et al.*, 1999a), and perhaps also in CD, a hypothalamic site of action has been suggested (Arvat *et al.*, 1999; Oliveira *et al.*, 2003).

There is data in the literature suggesting that GH secretory ability can be acutely restored in these patients (Leal- Cerro *et al.*, 1997, Leal-Cerro *et al.*, 1998). However, recovery of GH secretion in CD after remission of hypercortisolism with transphenoidal surgery (TS) or radiotherapy (RT) is quite controversial (Table 1) (Tyrrell *et al.*, 1977; Kuwayama *et al.*, 1981; Burke *et al.*, 1990; Magiakou *et al.*, 1994; Hughes *et al.*, 1999; Veldman *et al.*, 2000; Tzanela *et al.*, 2004; PecoriGiraldi *et al.*, 2007), which could be due, at least in part, to treatment-induced somatotroph damage. GH recovery after bilateral adrenalectomy is variable (Tyrrell *et al.*, 1977; Whitehead *et al.*, 1990) (Table 1).

Ketoconazole is a steroidogenesis inhibitor that has been used for the treatment of Cushing's syndrome in the last two decades. This compound is able to normalize cortisol levels in 70% of these patients (Engelhardt *et al.*, 1994), and rarely causes hepatic damage (Sonino *et al.*, 1991).

There is no data in the literature about the effect of ketoconazole treatment on GH secretion in patients with CD. For basal and CRH-stimulated ACTH release, conflicting results have been reported during ketoconazole use (Loli *et al.*, 1986; McCance *et al.*, 1987; Boscaro *et al.*, 1987; Terzolo *et al.*, 1988; Sonino *et al.*, 1991).

Therefore, the aim of this study was to evaluate GH, ACTH and cortisol responses to ghrelin and GHRP-6 before and after 3 and 6 months of ketoconazole treatment in untreated patients with CD. GHRH-induced GH release was additionally studied, as this peptide stimulates GH secretion via different mechanisms than those of ghrelin and GHRP-6. Glucose values after ghrelin were also investigated.

Table 1: Analysis of recovery of GH secretion (GH responses to different stimuli) after treatment of CD: *A)* early (less than 6 months) and *B)* long-term evaluation (more than 6 months).
(TS:transphenoidal surgery; RT:radiotherapy; adr: adrenalectomy).

***A)* Early Evaluation:**

Author	GH response normalization	Time after TS
Tyrrell *et al* , 1977	3/4 (75%)	2-5 months
Kuwayama *et al* , 1981	4/9 (44%)	3-6 months
Burke *et al* , 1990	12/37 (32%)	4-8 weeks
Magiakou *et al* , 1994	2/7 (29%)	6 months
Tzanella *et al* , 2004	2/4 (50%)	6 months

Mean normalization: **38%**

***B)* Long-term Evaluation:**

Author	GH response normalization	Time/Type of Treatment
Tyrrell *et al* , 1977	9/13 (69%)	until 18 years / adr
Whitehead et al, 1990	8/8 (100%)	5-34 years / adr
Magiakou *et al* , 1994	0/7 (0%)	1 year / TS
Hughes et al, 1999	7/9 (41%)	after 2 years / TS
Carroll *et al* , 2004	2/13 (15%)	9-108 months/ TS-RT
Tzanella *et al* , 2004	3/4 (75%)	18 months / TS
Giraldi *et al* , 2007	12/34 (35%)	after 2 years / TS

Mean normalization: **47%**

Material and methods

Subjects

Eight female patients with CD were studied. Six patients had microadenomas while 2 patients had macroadenomas, with maximal tumor diameters of 11 and 13 mm. Their mean age (± SE) was 33.8 ± 3.1 years (range: 19-41) and their mean body mass index (BMI) was 28.5 ± 0.8 kg/m^2 (range: 26.2-32.1). All patients were previously untreated. Diagnosis of CD was established on the basis of clinical features and standard hormonal criteria, including increased free urinary cortisol excretion, lack of suppression of serum cortisol after low-dose dexamethasone test (1mg orally overnight), normal or high basal plasma ACTH and serum cortisol levels at 0800 h, and positive DDAVP test. Magnetic resonance imaging (MRI) of the sellar region showed a pituitary adenoma in all patients. After the end of the experimental protocol, all patients were submitted to transphenoidal surgery and the diagnosis of ACTH-secreting tumor was confirmed by positive ACTH immunostaining of the excised pituitary adenoma. None of the patients was on replacement therapy for hypopituitarism. All patients had normal renal function and four had secondary diabetes mellitus at the time of the diagnosis. One patient was treated with diet, two with metformin and one with metformin and insulin.

Eleven normal subjects (4 women and 7 men) with a mean age of 32.1 ± 2.5 years (range: 20-47) and mean BMI of 25.0 ± 0.8 kg/m^2 (range: 20.9-30.8) were also studied. They were free of any medication at the time of the study protocol. The women were tested in the early follicular phase of their menstrual cycle.

Study protocol

The experimental protocol was approved by the ethics committee of Universidade Federal de São Paulo, and all subjects gave prior informed consent.

Each subject was submitted to three tests, in random order, with a minimum

interval of 48 hours between them, either receiving acylated ghrelin (Neosystem, Strasbourg, France), GHRP-6 (Bachem, San Carlos, USA) or GHRH (1-29)NH2 (Clinalfa, Läufelfingen, Switzerland). Patients with CD were studied before and after three and six months of treatment with ketoconazole. Ketoconazole was given at a dose of 400 mg/day initially, administered two or three times a day. The response to the drug was evaluated monthly by measurements of urinary free cortisol and the dose of ketoconazole was titrated accordingly (400-1200 mg/day).

All tests were performed after an overnight fast, and the subjects remained recumbent throughout. Patients were advised to take ketoconazole before the tests at the same time as usual (0600h). At 0800h an indwelling catheter was inserted into an antecubital vein and was kept patent by a slow saline infusion. The tests started 45 minutes later. After the first blood sample, each subject received ghrelin at a dose of 1 μg/kg i.v, GHRP-6 at the same dose or GHRH at a dose of 100 μg i.v. Blood samples were obtained every 15 minutes until 120 minutes for hormonal and biochemical determinations.

Assays

Serum GH was measured in duplicate by an immunofluorometric assay (Wallac, Turku, Finland). The sensitivity of the method is 0.01 μg/L, with mean inter- and intra-assay coefficients of variation (CV) of 7% and 6.7%, respectively. An immunochemiluminometric assay (DPC, Los Angeles, USA) was used to measure plasma ACTH. The sensitivity of the method is 5 pg/mL, with mean inter- and intra-assay CV of 3.6% and 2.8%, respectively. Serum cortisol levels were measured in duplicate by a fluoroimmunoassay (Wallac, Turku, Finland), with sensitivity of 0.2 μg/dL, and mean inter and intra-assay CV of 8.2% and 6.2%, respectively. IGF-I levels were determined by an immunochemiluminometric assay (DPC, Los Angeles, USA) with sensivity of 20 ng/mL and mean inter and intra-assay CV of 6.5% and 3.8%,

respectively. Urinary free cortisol (UFC) was measured by liquid chromatography and tandem mass spectrometry (Fleury Laboratory, São Paulo, Brazil; Vieira JG *et al*., 2005), with sensitivity of less than 1µg/L, and mean inter- and intra-assay CV of 7.7% and 4.4%, respectively. Reference values for UFC were defined as values between 3 and 43 µg/24h. Glucose levels were determined by the hexokinase method.

Statistical analysis

Repeated measures ANOVA or Friedman's analysis of variance were performed when appropriate to compare GH, cortisol, ACTH and glucose levels after the injection of each peptide. These tests were also used to compare data in the same group before and after 3 and 6 months of treatment. Paired *t* test or Wilcoxon signed rank test was used to compare data within the same group. Unpaired *t* test or Mann Whitney rank sum test were performed to compare data between patients and controls. Mean basal levels were calculated using all individual values obtained before the injection of each peptide. The area under the curve (AUC) was calculated by trapezoidal integration. Delta (Δ) values (subtracting baseline or during treatment) were also calculated when appropriate. Spearman's correlation coefficients were calculated when appropriate. Undetectable GH (µg/L) and ACTH (pg/mL) values were considered to be equal to 0.01 and 5 respectively, for statistical purposes. $P<0.05$ was considered statistically significant. Results are reported as mean ± SE.

Results

Basal values and clinical data (Table 2)

There was no difference in age between patients and controls. Before treatment patients with CD had significantly higher BMI values compared to the control group. There was a trend to a decrease in mean BMI during treatment (P=0.08) and after 6 months BMI values were statistically similar to those of controls. Basal GH and IGF-I levels in CD were not different than those of controls initially, and these values did not change significantly during treatment. At diagnosis patients with CD had higher basal cortisol levels compared to controls. These values decreased significantly during ketoconazole use, but were still higher than those of controls at the 3rd and 6th month. Basal ACTH values in CD were always higher than those observed in the control group and increased significantly after 6 months of treatment. In CD, mean fasting glucose levels were higher than those of controls and decreased significantly during treatment, becoming similar to the control group at the 6th month. All diabetic patients improved their glycemic control with ketoconazole treatment, with normalization of glucose levels in one patient. Insulin was withdrawn in another patient.

UFC values (Figure 1)

Patients with CD had mean UFC (μg/24h) of 222.4 ± 34.5 at the time of the diagnosis. After 3 and 6 months of ketoconazole treatment mean UFC decreased significantly to 61.6 ± 10.1 (mean reduction: 70%) and 39.1 ± 10.9 (mean reduction: 80%), respectively. All patients showed a decrease in UFC values after 3 months of treatment, and after 6 months 4 patients had their values within the normal range while 2 had UFC levels near the upper-limit of normality. Only one patient did not show normalization of UFC values throughout the whole period, despite a major decrease both at the 3 rd (74%) and at the 6 th (76%) month of treatment. Another patient had an "escape" at the 6 th month, after normalization in the 3 rd month. None of the patients

had UFC values below the normal range throughout the treatment.

GH responses (Figure 2 and Figure 3)

In control subjects peak GH (μg/L) and AUC (μg/L.120 min) values after ghrelin (54.1 ± 11.2; 3123 ± 707) were higher than those of GHRP-6 (25.7 ± 4.5; 1396 ± 284) and GHRH (11.7 ± 3.3; 857 ± 267). The same pattern was observed in patients with CD before treatment. These patients had significantly lower peak GH and AUC values after ghrelin (6.8 ± 2.3; 238 ± 88), GHRP-6 (2.8 ± 0.8; 107 ± 37) and GHRH (1.1 ± 0.2; 64 ± 14) compared to the control group. After 3 months of ketoconazole use, GH responses to ghrelin (peak: 7.9 ± 2.2; AUC: 273 ± 83), GHRP-6 (4.1 ± 1.0; AUC: 149 ± 30) and GHRH (1.2 ± 0.3; 73 ± 18) did not change significantly. After 6 months of treatment, there was a significant increase in GH response to ghrelin in CD (peak: 16.0 ± 3.6; AUC: 602 ± 175) when compared to those before treatment and also after 3 months of ketoconazole use. However, GH responsiveness to ghrelin in CD remained lower than in controls. GH response to GHRP-6 (peak: 5.1 ± 1.5; AUC: 194 ± 71) also increased, but did not reach statistical significance. Individual analysis showed that all patients, except one, had an increase in GH responsiveness to ghrelin and this was also seen in the majority after GHRP-6 stimulation. In 3 patients peak GH values were within the range observed in the control group, while in one patient GH levels were just below the lower limit of controls for both ghrelin and GHRP-6. These patients already showed an increase in GH responsiveness at the 3rd month. The only patient who failed to increase GH values after ghrelin had lack of normalization of UFC levels during the whole study period. When this patient was excluded from the statistical analysis, no significant differences were observed. GHRH-induced GH release (1.2 ± 0.4; 69 ± 21) did not change after 6 months of treatment and only 2 patients reached values just above the lower limit of controls.

No correlations were found between Δ BMI, Δ UFC, Δ basal cortisol, Δ ACTH or Δ fasting glucose and Δ GH peak and AUC after 6 months of treatment.

Cortisol responses (Figure 4)

In CD peak cortisol (μg/dL) and AUC values (μg/dL.90 min) after ghrelin (peak: 42.9 ± 5.0; AUC: 3236 ± 394) and GHRP-6 (peak: 37.9 ± 4.8; AUC: 2893 ± 344) before treatment were higher than those obtained in controls (ghrelin peak: 16.6 ± 1.3; AUC: 1230 ± 98; GHRP-6 peak: 13.4 ± 0.8; AUC: 938 ± 63). In both groups cortisol release was higher after ghrelin compared to GHRP-6. After 3 months of ketoconazole use, peak cortisol and AUC values decreased significantly after ghrelin (peak: 23.1 ± 2.5; AUC: 1820 ± 220) and GHRP-6 (peak: 27.8 ± 3.3; AUC: 2078 ± 214) administration. No further significant decrease was observed after 6 months of treatment after both ghrelin (peak: 25.0 ± 2.7; AUC:1941 ± 210) and GHRP-6 (peak: 20.7 ± 2.6; AUC: 1577 ± 175). Stimulated cortisol values in CD (peak and AUC) at 3 and 6 months were still higher than those observed in the control group for both peptides. When we analyzed the Δ AUC levels, the significant decrease in cortisol responses to ghrelin and GHRP-6 was still observed, but cortisol responsiveness to both peptides became similar to controls already at the 3rd month of treatment (data not shown).

ACTH responses (Figure 5)

At diagnosis patients with CD had higher peak ACTH (pg/mL) and AUC values (pg/mL .90 min) after ghrelin (peak: 272 ± 70; AUC: 15239 ± 3348) and GHRP-6 (peak: 276 ± 78; AUC: 16678 ± 4806) compared to controls (ghrelin peak: 62.6 ± 11.7; AUC: 3181 ± 517; GHRP-6 peak: 31.1 ± 6.4; AUC: 1690 ± 217). In patients with CD ghrelin-induced ACTH release was similar to that of GHRP-6, while in controls the former was higher. Ghrelin-induced ACTH release increased after 3 (peak: 384 ± 70; AUC: 24129 ± 4500) and 6 months of treatment (peak: 509 ± 51; AUC: 29850 ± 2555), although only the latter reached statistical significance. ACTH responsiveness to GHRP-6 was also enhanced during the 3rd (peak: 372 ± 75; AUC: 22316 ± 3830) and the 6th month (peak: 357 ± 55; AUC: 23493 ± 3976) of treatment, but not significantly. Even the patient who did not normalize UFC levels had an increase in basal and stimulated

ACTH values throughout the study period. When we analyzed the Δ AUC values after ghrelin in CD (before: 10619 ± 3365; 3rd month: 17887 ± 4355; 6th month: 21418 ± 3784) a trend to increase was observed (P=0.08), while no differences were seen after GHRP-6 (before: 12172 ± 4394; 3rd month: 16535 ± 4101; 6th month: 16017 ± 4441). For both peptides these values were always higher than those of controls (ghrelin: 1827 ± 412; GHRP-6: 492 ± 194). No correlations were found between cortisol and ACTH responses to ghrelin or GHRP-6. Also, there were no correlations between Δ UFC or Δ basal cortisol during treatment and Δ ACTH after both peptides at the 6th month of treatment.

Glucose responses (Table 3)

In patients with CD before treatment, there was a progressive rise in glucose levels after ghrelin, with a trend to higher values observed at 120 min (P=0.051) and not at 30 min, as seen in controls. After 6 months of ketoconazole, glucose levels increased 30 min after ghrelin administration, similarly to controls.

Mean glucose values during ghrelin, GHRP-6 and GHRH tests were similar at all time points of the protocol, both before and during treatment, except at the 6th month, when mean glucose levels after ghrelin were higher compared to GHRP-6 and GHRH tests (data not shown).

Side effects

Hunger sensation, nausea, sleepiness and facial flushing were reported occasionally after ghrelin administration. Three patients had a transient and mild increase in alanine transaminase (ALT) after the first months of ketoconazole administration.

Table 2: Basal values and clinical data of patients with CD (n=8) before and after 3 and 6 months of ketoconazole treatment and of controls (n=11) (a, P<0.05 *vs* controls; b, P<0.05 *vs* before treatment)

	Controls	CD		
		before	3rd month	6th month
Age (years)	32.1 ± 2.5	33.8 ± 3.1		
BMI (kg/m^2)	25.0 ± 0.8	28.5 ± 0.8 [a]	27.6 ± 0.7 [a]	27.2 ± 0.8
GH (µg/L)	0.1 ± 0.07	0.3 ± 0.1	0.2 ± 0.03	0.3 ± 0.05
IGF-I (ng/mL)	177.2 ± 12.6	232.4 ± 41.6	225.5 ± 23.1	220.5 ± 30.4
Cortisol (µg/dL)	7.9 ± 0.7	22.2 ± 2.5 [a]	16.8 ± 1.6 [a,b]	14.6 ± 1.6 [a,b]
ACTH (pg/mL)	14.2 ± 2.1	50.7 ± 7.1 [a]	66.8 ± 6.6 [a]	88.4 ± 13.1 [a,b]
Glucose (mg/dL)	84.6 ± 2.2	113.1 ± 10.4 [a]	101.0 ± 6.1 [a,b]	92.8 ± 5.8 [b]

Figure 1: Urinary free cortisol (UFC) values in patients with CD (n=8) before and after 3 and 6 months of ketoconazole treatment (the broken lines represent the normal range: 3-43 µg/24h)

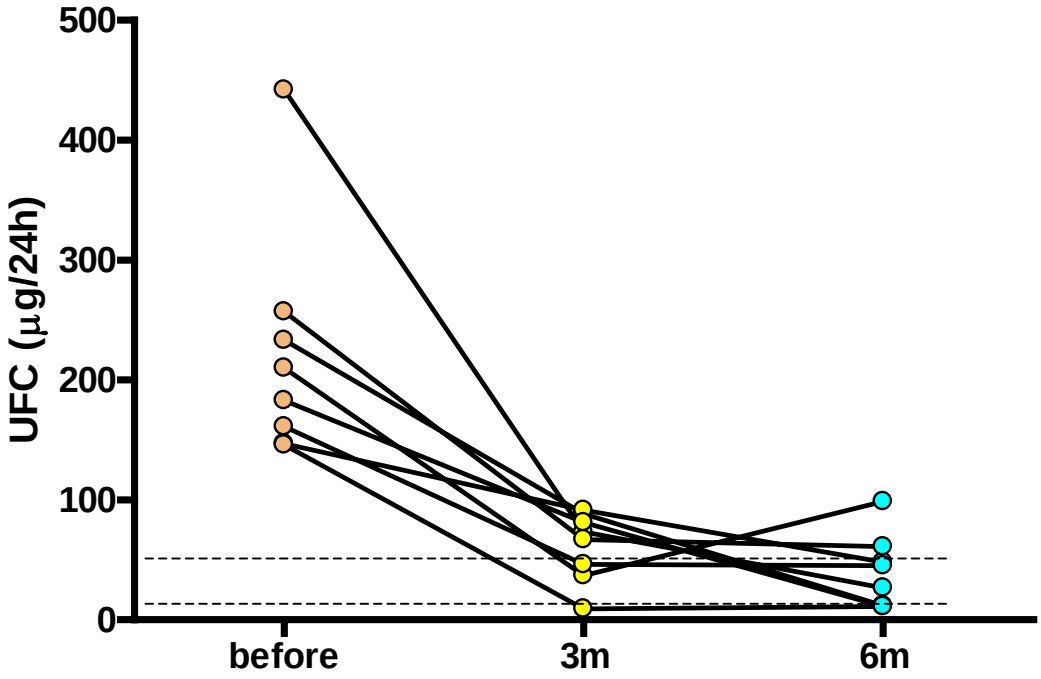

Figure 2: Individual (A) and mean GH values (B) after ghrelin administration in CD (n=8) before and after 3 and 6 months of ketoconazole treatment and in controls (n=11) (mean ± SE; a, P<0.05 *vs* controls)

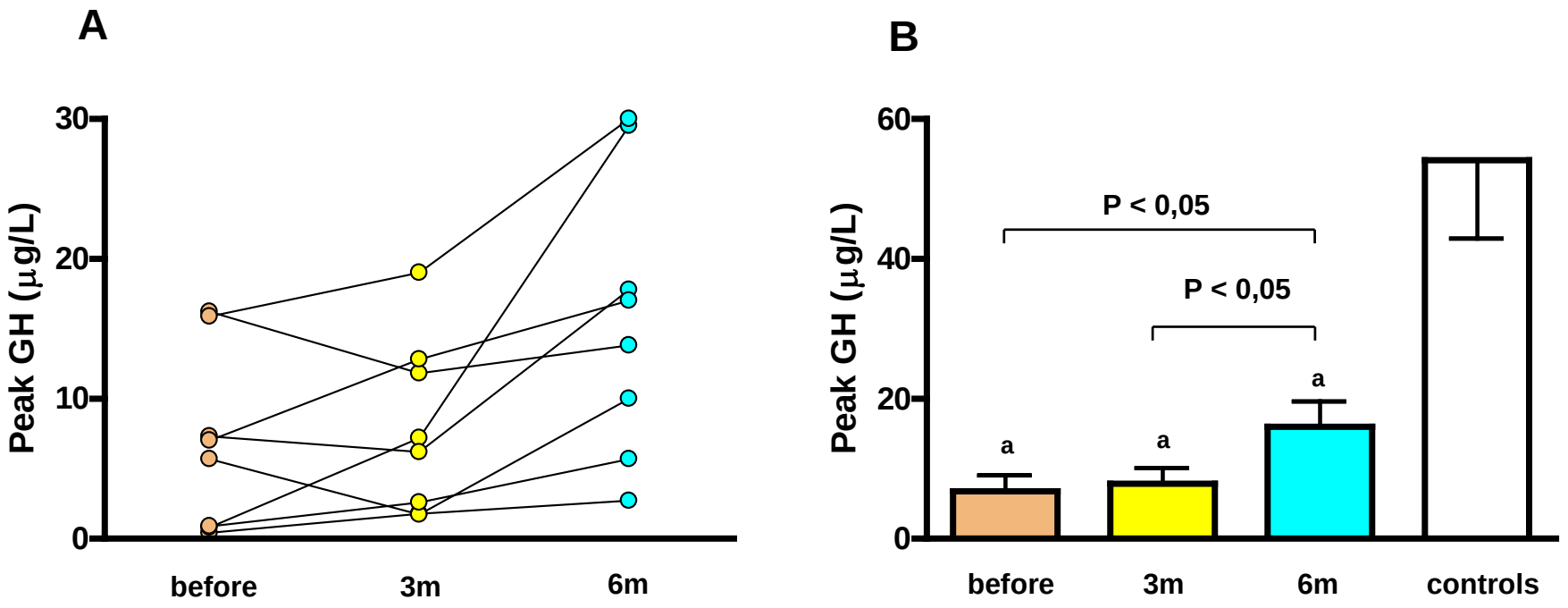

Figure 3: Mean GH values after GHRP-6 and GHRH administration in CD (n=8) before and after 3 and 6 months of treatment and in controls (n=11) (mean ± SE; a, P<0,05 *vs* controls)

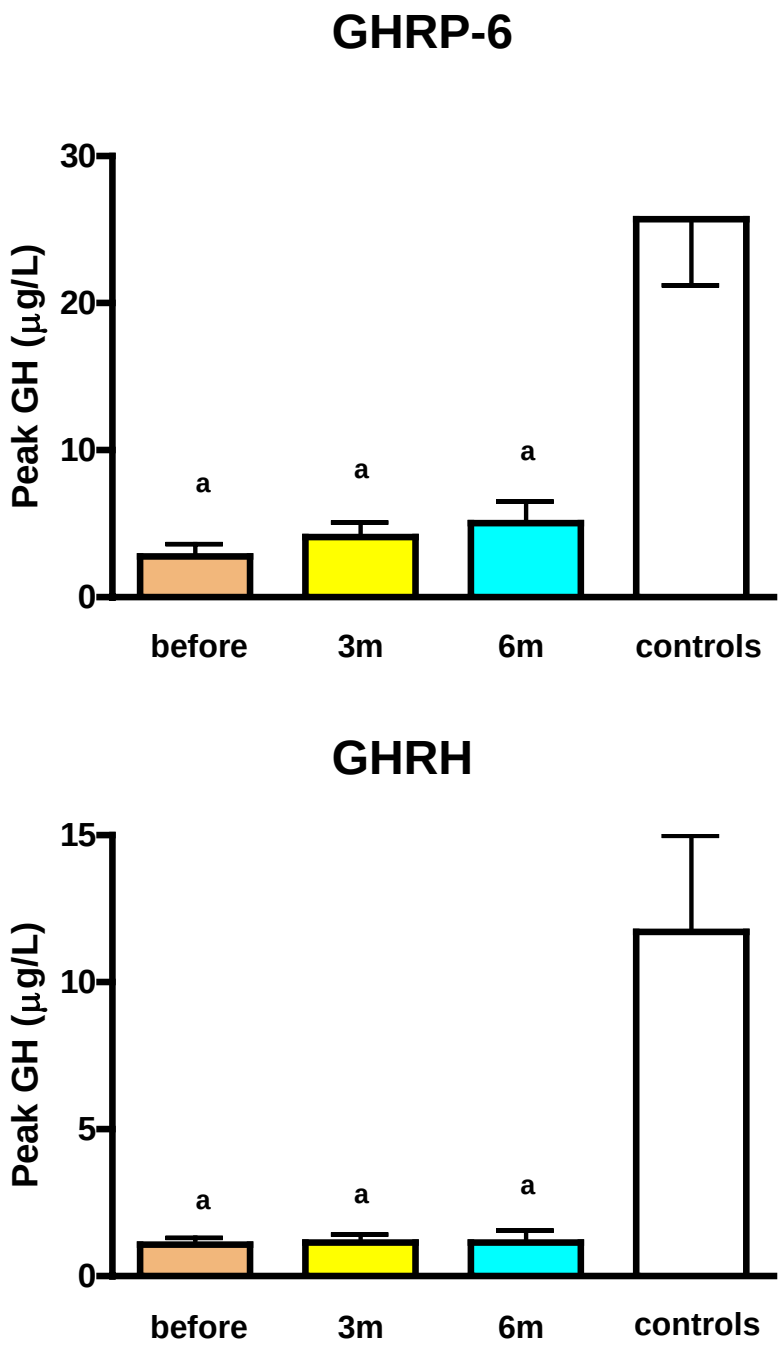

Figure 4: Mean cortisol values after ghrelin and GHRP-6 administration in CD (n=8) before and after 3 and 6 months of ketoconazole treatment and in controls (n=11) (mean ± SE; a, P<0.05 *vs* controls)

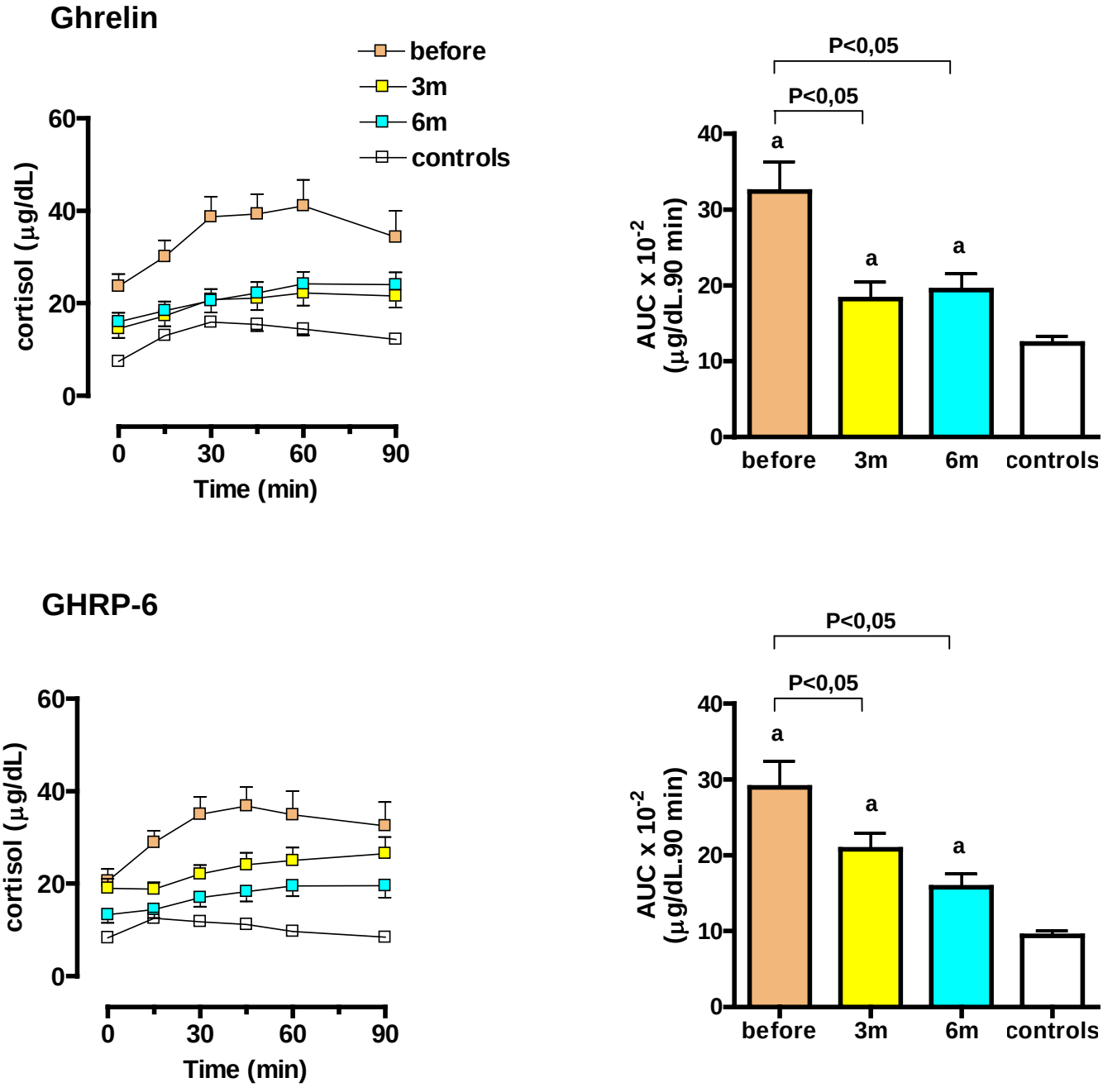

Figure 5: Mean ACTH values after ghrelin and GHRP-6 administration in CD (n=8) before and after 3 and 6 months of ketoconazole treatment and in controls (n=11) (mean ± SE; a, P<0.05 *vs* controls)

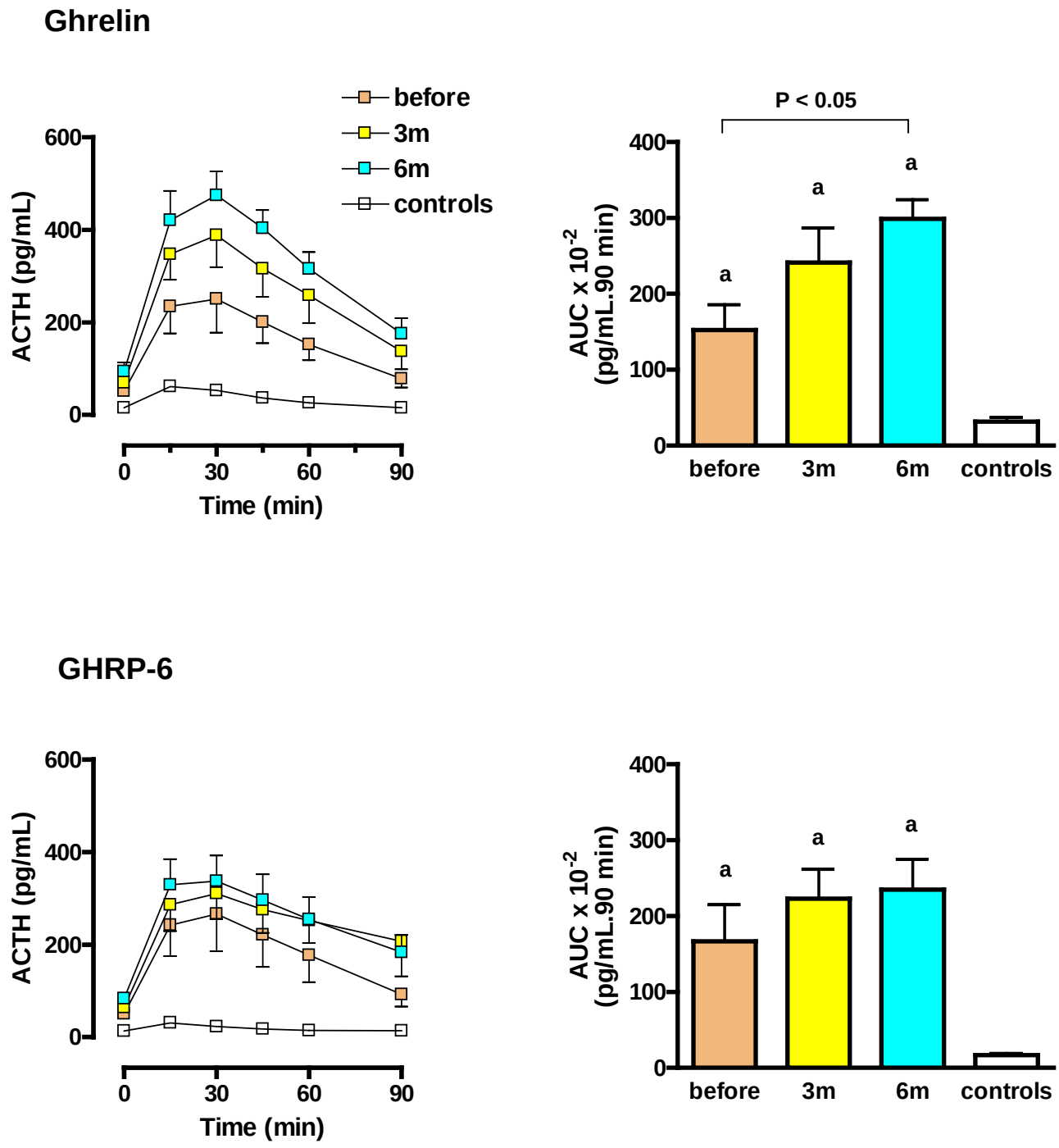

Table 3: Mean glucose levels after ghrelin administration in patients with CD (n=8) before and after 3 and 6 months of ketoconazole treatment and in controls (n=11) (*, P<0.05 *vs* 0 min; **, P=0.051 *vs* 0 min)

	Glucose levels (mg/dL)				
	Time (min)				
Group	**0**	**30**	**60**	**90**	**120**
Controls	84 ± 2	91 ± 2 *	87 ± 2 *	87 ± 2 *	89 ± 2 *
CD before	116 ± 12	120 ± 11	125 ± 12	125 ± 11	127 ± 13 **
3rd month	101 ± 7	106 ± 7	105 ± 7	103 ± 8	104 ± 8
6th month	94 ± 6	102 ± 7 *	102 ± 7 *	100 ± 8 *	101 ± 7 *

Discussion

There are controversial results about the recovery of GH secretion in patients with CD previously submitted to surgery and/or radiotherapy (Tyrrell *et al.*, 1977; Kuwayama *et al.*, 1981; Burke *et al.*, 1990; Magiakou *et al.*, 1994; Hughes *et al.*, 1999; Veldman *et al.*, 2000; Carroll *et al.*, 2004; Tzanela *et al.*, 2004; Pecori Giraldi *et al.*, 2007), which could be due to GH deficiency caused by pituitary damage. GH recovery after bilateral adrenalectomy is also variable (Tyrrell *et al.*, 1977; Whitehead *et al.*, 1990). We, therefore, evaluated GH secretion in CD after pharmacological reduction or correction of hypercortisolism in patients not submitted to any pituitary intervention. Moreover, as most of our patients had microadenomas, with only 2 harboring small macroadenomas, they were likely to have an intact GH axis. It has been previously shown that acute clinical interventions such as inhibition of free fatty acid synthesis (Leal-Cerro *et al.*, 1997) and short term dietary restriction (Leal-Cerro *et al.*, 1998) are able to increase GH secretion in CD. However, we did not observe significant changes in GH release after ghrelin, GHRP-6 and GHRH after 1 month of ketoconazole use in patients with CD, despite major reductions in UFC values (Correa-Silva *et al*., 2007).

In our present study, patients with CD had blunted GH responses to ghrelin, GHRP-6 and GHRH before ketoconazole treatment, as reported previously by us and others (Hotta *et al.*, 1988; Borges *et al.*, 1997; Leal-Cerro *et al.*, 2002b; Giordano *et al.*, 2005; Correa-Silva *et al.*, 2006). GH responsiveness to ghrelin was higher, similarly to controls, which could be due to the higher potency of this peptide compared to GHRP-6 and GHRH (Arvat *et al.*, 2001).

After 3 months of treatment no significant changes in mean GH values after ghrelin, GHRP-6 and GHRH were seen. Despite major decreases in UFC levels since the 3rd month, GH responsiveness to ghrelin only increased significantly after 6 months of ketoconazole treatment, although it remained lower than seen in the control group. At this time point 6 patients had normal or near-normal UFC values. GHRP-6-induced

GH release also increased, but not significantly, while no changes in GH responses to GHRH were observed. Individual analysis showed that 50% of our patients had peak GH values after ghrelin/GHRP-6 within or near the range of controls after 6 months of treatment, while only 2 reached this range after GHRH stimulation. Most of these patients already had increases in GH values at the 3rd month. The only patient who did not increase GH values after ghrelin failed to achieve normal UFC during the entire study period. However, no correlation was observed between UFC or serum cortisol levels and GH responsiveness to ghrelin, which has already been shown by some authors with other tests (Tzanela *et al.*, 2004; Pecori Giraldi *et al.*, 2007). These results are in agreement with one report of early normalization of GH secretion after adrenalectomy in CD (Tyrrell *et al.*, 1977). Also, they are similar to those obtained by Tzanela *et al* who showed recovery of GH secretion after pyridostigmine+GHRH and/or ITT in 50% (2/4) of patients with CD, who had intact pituitary function, and who were in remission 6 months after TS. However, in most studies lack of recovery or much lower GH recovery rates have been described in the first 6 months after TS (Kuwayama *et al.*, 1981; Burke *et al.*, 1990; Magiakou *et al.*, 1994). Our results suggest that, if there is no pituitary damage, even relatively short-term periods of normal or near-normal cortisol values are able to improve glucocorticoid-induced GH suppression in CD. Moreover, this is apparently unrelated to a decrease in circulating IGF-I, as no significant changes in IGF-I levels were observed during the study period, supporting previous data in patients with CD after TS (Magiakou *et al.*, 1994; Tzanela *et al.*, 2004).

Obesity and hyperglycemia, two features of hypercortisolism, are associated with blunted GH responses to ghrelin, GHRP-6 and GHRH (Micic *et al.*, 1999; Broglio *et al.*, 2002; Alvarez-Castro *et al.*, 2004) and could be contributing factors to the partial recovery of GH axis in some of our patients. However, we have previously shown that these blunted GH responses also occur in non-obese/overweight patients compared to BMI-matched controls (Correa-Silva *et al.*, 2006). Moreover, although our patients with CD had slightly higher BMI values initially, these values became similar to controls after

6 months of ketoconazole use, and no correlation was found between GH responses to ghrelin and BMI in CD, as described previously with ghrelin (Correa-Silva *et al.*, 2006) and other tests (Tzanela *et al.*, 2004; PecoriGiraldi *et al.*, 2007). Despite a significant decrease in fasting glucose levels during treatment, no correlation was found between glucose values and GH responsiveness to ghrelin. Therefore, the partial recovery of GH axis in some of our patients is unlikely to be due to these factors.

Periods of subtle hypercortisolism, due to the pharmacological profile of ketoconazole, cannot be totally excluded, and could have contributed to the partial improvement of the somatotrophic axis in some of our patients. Conversely, although short periods of hypocortisolism could have ocurred, in our study UFC values always remained above the lower limit of normality in all subjects. Moreover, it has been previously shown that short-term glucocorticoid deficiency does not alter GH responsiveness to GHRP-6 in normal subjects and in patients with Addison's disease (Pinto *et al.*, 1999).

The possible mechanisms of decreased GH secretion and also of recovery of somatotroph responsiveness in CD remain unknown. It has been previously suggested that glucocorticoid excess inhibits hypothalamic GHRH release (Leal-Cerro *et al.*, 1993), which could lead to chronic GHRH deficiency. Although controversial in animals (Tamura *et al*., 2000; Luque *et al*., 2006), this steroid also downregulates human GHS-R (Pettersen *et al.*, 2001). Therefore, glucocorticoids could eventually interfere with ghrelin/GHS-stimulated transduction mechanisms at the somatotroph, with a possible additional effect on GHS-R located in GHRH-releasing neurons at the hypothalamus (Tannenbaum *et al*, 2003). As GHS/ghrelin and GHRH activate different pathways of GH release, our data could eventually suggest that ghrelin/GHS-modulated mechanisms are more sensitive to the decrease in circulating glucocorticoid levels than those of GHRH. Moreover, this could be more pronounced for ghrelin than GHRP-6, as ghrelin stimulates multiple intracellular mechanisms in the somatotroph, as described

recently (Malagon *et al.*, 2003; Kineman *et al.*, 2007). However, additional studies are necessary to further confirm these hypotheses.

At diagnosis our patients with CD showed exaggerated cortisol and ACTH responses to ghrelin and GHRP-6 compared to controls, as previously demonstrated (Leal-Cerro *et al.*, 2002b; Giordano *et al.*, 2005; Correa-Silva *et al.*, 2006). ACTH responsiveness to both peptides was similar in CD, while in controls the ACTH-releasing potency of ghrelin was higher, suggesting different stimulating mechanisms. Cortisol response to ghrelin was slightly higher compared to GHRP-6 both in controls and in CD. We have previously shown that cortisol release after ghrelin and GHRP-6 is similar in CD (Correa-Silva *et al.*, 2006). The reason for this different finding is not apparent, but it could be eventually due to an additional direct adrenal effect of ghrelin, although this is controversial (Arvat *et al.*, 2001, Korbonits *et al.*, 2004).

In normal subjects the ACTH-releasing action of ghrelin and GHRP-6 occur at hypothalamic level (Popovic *et al.*, 1995, Popovic *et al.*, 2003). GHRP-6 stimulates AVP secretion from rat hypothalamic fragments *in vitro* (Korbonits *et al.*, 1999b), while ghrelin enhances AVP, CRH and NPY release (Wren *et al.*, 2002), but a predominant effect on AVP has been demonstrated (Mozid *et al.*, 2003). Although controversial, hexarelin and ghrelin increase ACTH secretion in humans probably through AVP pathways (Korbonits *et al.*, 1999a; Coiro *et al.*, 2005). The mechanisms of ACTH release by ghrelin/GHS in CD remain unknown. It has been shown that GHS-R are overexpressed in ACTH-producing tumors (Korbonits *et al.*, 1998; Korbonits *et al.*, 2001), in contrast to normal corticotrophs, which lack GHS-R (Korbonits *et al.*, 2001). The enhanced ACTH responses to ghrelin and GHRP-6 in CD could, therefore, be due to a direct effect of these peptides on tumoral GHS-R (Korbonits *et al.*, 1998). Interestingly, ACTH release after ghrelin stimulation is lower than after CRH in ACTH-secreting pituitary adenomas *in vitro* (Giraldi *et al.*, 2007). As hexarelin-induced ACTH release in patients with CD is 7-fold higher than after CRH (Ghigo *et al.*, 1997), this

could suggest that GHS have an additional hypothalamic effect to stimulate ACTH release in CD.

During ketoconazole use, there was a fall in basal circulating cortisol levels. After 3 and 6 months of treatment cortisol responses to ghrelin and GHRP-6 decreased significantly and already became similar to the control group, when analysed as Δ AUC. This has also been demonstrated for CRH-stimulated cortisol release in CD after use of ketoconazole (Loli *et al.*, 1986; Boscaro *et al.*, 1987) and shows the potent inhibition of steroid synthesis by this compound.

In our patients basal ACTH levels increased in parallel to the fall in circulating cortisol during ketoconazole treatment, which could be due to activation of normal corticotrophs which were earlier suppressed. In normal subjects an increase in basal ACTH levels has also been shown during chronic use of this compound (Deuschle *et al.*, 2003), while in CD results are controversial, with unchanged (Loli *et al.*, 1986; McCance *et al.*, 1987; Sonino *et al.*, 1991), increased (Boscaro *et al.*, 1987) and even decreased (Terzolo *et al.*, 1988) values. A direct inhibitory effect of ketoconazole on pituitary cells has been suggested (Stalla *et al.*, 1989), but seems unlikely (Burrin *et al.*, 1986; Sonino *et al.*, 1991).

After 3 months of ketoconazole use mean ACTH values after ghrelin and GHRP-6 increased, although not significantly. After 6 months of treatment there was a significant enhancement of peak and AUC values after ghrelin. A trend to increase ($P=0.08$) was observed even when the data was analysed as Δ AUC, suggesting that ACTH responsiveness to ghrelin is enhanced during ketoconazole use. Although there was an increase in GHRP-6 induced ACTH release, it did not reach statistical significance. Therefore, it is possible that ghrelin has an additional ACTH-releasing action in these circumstances. In the literature there are few and controversial data about CRH-stimulated ACTH release during ketoconazole use in CD (Loli *et al.*, 1986; Boscaro *et al.*, 1987). An increase in ACTH response to this peptide has been

described after 4 to 6 weeks of treatment (Boscaro *et al.*, 1987), although no changes were also observed after 3 to 7 months of ketoconazole use (Loli *et al.*, 1986).

There are several hypotheses to explain the increase in ACTH values in CD during ketoconazole treatment. Prolonged and consistent inhibition of cortisol synthesis could be associated with increased ACTH secretion, due to activation of the HPA axis (Boscaro *et al.*, 1987). It has also been shown that ghrelin is produced by tumoral corticotroph cells and can directly induce ACTH secretion by an autocrine/paracrine effect (Martinez-Fuentes *et al.*, 2006). Whether this mechanism is initially operating and is modulated by a decrease in cortisol levels, and whether it can be further enhanced by exogenous ghrelin administration is presently unknown. Interestingly, glucocorticoids reduce human GHS-R expression (Pettersenn *et al.*, 2001). It is possible, therefore, that the fall in circulating cortisol levels induced by ketoconazole could increase GHS-R expression at the corticotroph adenoma and/or at hypothalamic level. Further studies are necessary to elucidate these possibilities.

In normal subjects a significant increase in glucose levels was observed 30 minutes after ghrelin administration, as described previously (Broglio *et al.*, 2001; Correa-Silva *et al.*, 2006). At diagnosis, patients with CD showed a progressive rise in glucose levels after ghrelin injection, with a trend to higher values after 120 minutes. In an earlier study we have also observed a progressive, but non-significant, increase in glucose values after ghrelin administration in CD (Correa-Silva *et al.*, 2006), differently than reported by Giordano et al (Giordano *et al*., 2005). The reason for this late increase in glucose levels, which is different from the early response seen in controls, is not apparent (Broglio *et al.*, 2001, Correa-Silva *et al.*, 2006). After 6 months of ketoconazole treatment, maximal glucose responses to ghrelin were seen at 30 minutes, as observed in the control group. It has been demonstrated that ghrelin stimulates glucose output from porcine hepatocytes in culture (Gauna *et al.*, 2005). Therefore, it is possible that hypercortisolism may alter the hepatic action of ghrelin, and that reduction of circulating cortisol can restore its normal effect.

In summary, our results show that mean GH responsiveness to ghrelin increases, although does not normalize, after 6 months of ketoconazole treatment in CD. In 50% of the patients GH values reach the range of controls. This could suggest that relatively short-term periods of normocortisolism or near-normal cortisol levels are able to improve glucocorticoid-induced GH suppression in CD. GH-releasing mechanisms stimulated by ghrelin/GHS could be more sensitive to the decrease in circulating cortisol levels, as no changes in GHRH-induced GH release were observed. The enhanced ACTH responses to ghrelin after ketoconazole in CD could be due to activation of HPA axis or to an increase in GHS-R expression at the corticotroph adenoma, consequent to reductions in circulating glucocorticoids. Hypercortisolism may alter the normal pattern of glucose release after ghrelin. Further studies are necessary to clarify these hypotheses.

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SUMÁRIO E CONCLUSÕES

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ESTUDO 1

Nosso estudo demonstrou que a resposta do GH à ghrelina está diminuída na doença de Cushing e é maior que a obtida após a administração de GHRP-6 e GHRH. Isto sugere que no hipercortisolismo ocorre um comprometimento das vias hipotalâmicas e hipofisárias de liberação de GH estimuladas por esses peptídeos.

A ghrelina e o GHRP-6 promovem uma resposta exagerada de ACTH e de cortisol em pacientes com doença de Cushing. Isto poderia ser mediado pela expressão aumentada dos GHS-R nos adenomas corticotróficos.

Além disso, o hipercortisolismo altera a liberação de glicose induzida pela ghrelina.

ESTUDO 2

Nossos dados mostraram que na doença de Cushing a resposta do GH à ghrelina aumenta de forma significativa após 6 meses de tratamento com cetoconazol, apesar de ainda ser menor que a observada nos controles. Isso sugere que a normalização dos níveis de cortisol ou níveis próximos do normal por períodos de tempo relativamente curtos podem aumentar a secreção de GH previamente suprimida pelo hipercortisolismo. Os mecanismos de liberação de GH estimulados pela ghrelina e pelos GHS podem ser mais sensíveis à diminuição dos níveis de glicocorticóides circulantes, uma vez que não ocorre modificação na secreção de GH induzida pelo GHRH durante o tratamento.

A liberação de ACTH estimulada pela ghrelina aumenta após 6 meses de uso de cetoconazol. Isto poderia ocorrer pela ativação do eixo hipotálamo-hipófise-adrenal ou pelo aumento da expressão dos GHS-R no adenoma produtor de ACTH, conseqüentes à redução dos níveis de cortisol.

Além disso, o tratamento do hipercortisolismo restaura o padrão normal de resposta da glicose à ghrelina.

ANEXOS – Estudo 1

TABELA 1: Dados clínicos do grupo controle

Indiv.	Sexo	Idade (anos)	IMC[1] (kg/m2)
1	M	28	25,7
2	M	28	23,3
3	M	30	22,4
4	F	47	30,8
5	M	30	25,5
6	M	47	28,1
7	M	36	19,5
8	F	34	20,9
9	F	35	23,7
10	F	20	24,3
média		33,5	24,4
DP		8,4	2,7
EP		3,3	1,1

1- Índice de massa corpórea

TABELA 2: Dados clínicos dos pacientes com doença de Cushing

Indiv.	Sexo	Idade (anos)	IMC (kg/m^2)	cortisol urinário (%LSN [4])	HAS[2]	DM[3]	tumor
1	F	35	31,6	90	S	S	micro
2	M	25	25,2	221	N	N	micro
3	F	27	23,9	487	S	N	micro
4	M	19	26,3	118	S	N	micro
5	F	56	27,5	98	S	N	micro
6	F	37	27,8	134	S	N	micro
7	F	38	26,4	764	S	S	macro
8	F	38	31,3	81	S	N	micro
9	F	39	28,4	183	S	S	micro
10	F	18	20,6	1284	S	S	micro
média		33,2	26,9	346			
DP		11,3	3,3	396			
EP		3,6	1	125			

2- Hipertensão arterial sistêmica

3- Diabetes Mellitus

4- % limite superior da normalidade

TABELA 3: Valores individuais e níveis médios de GH, pico e área sob a curva (AUC: μg/L.120 min) após administração de ghrelina em controles

	GH (μg/L)										
	Tempo (minutos)										
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**pico**	**AUC**
1	0,03	70,4	110,4	80,8	57,3	52,7	40,8	25,4	14,2	110,4	6674
2	0,02	37,5	95,8	90,4	57,3	33,1	41,1	23,9	15,3	95,8	5802
3	0,2	35,2	43,5	29,0	19,3	14,4	8,6	4,8	3,0	43,5	2346
4	0,04	9,9	17,8	12,0	6,1	3,5	2,2	1,3	0,7	17,8	798
5	0,02	17,5	25,1	18,5	10,4	8,5	6,0	4,2	2,7	25,1	1373
6	0,02	24,5	31,6	23,8	16,7	7,7	5,4	2,7	1,7	31,6	1699
7	0,2	7,3	50,8	65,8	45,8	25,4	13,5	6,9	3,7	65,8	3262
8	0,08	16,8	41,9	32,4	21,2	13,0	6,4	5,2	2,3	41,9	2071
9	0,07	23,0	27,9	17,0	7,0	3,7	2,5	1,0	0,6	27,9	1237
10	0,06	78,1	123,5	102,3	80,8	53,1	34,1	16,9	11,6	123,5	7419
média	0,07	32,0	56,8	47,2	32,2	21,5	16,1	9,2	5,6	58,3	3268
DP	0,07	24,3	38,4	34,1	26,1	19,0	16,0	9,3	5,8	38,4	2445
EP	0,02	7,7	12,1	10,8	8,3	6,0	5,1	2,9	1,8	12,1	773

TABELA 4: Valores individuais e níveis médios de GH, pico e área sob a curva (AUC: μg/L.120 min) após administração de GHRP-6 em controles

	GH (μg/L)										
	Tempo (minutos)										
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**pico**	**AUC**
1	0,4	23,8	35,5	25,8	17,9	11,9	7,1	3,8	2,3	35,5	1907
2	0,04	12,1	29,1	26,3	15,8	8,9	4,7	2,6	1,4	29,1	1503
3	0,08	19,5	24,7	17,9	9,2	4,8	2,4	1,5	1,2	24,7	1210
4	0,1	1,0	4,2	3,0	1,6	0,8	0,4	0,2	0,1	4,2	170
5	0,01	13,3	17,7	15,9	11,8	6,7	2,4	1,2	0,8	17,7	1041
6	0,2	8,8	10,9	7,3	4,2	2,3	1,4	0,7	0,5	10,9	539
7	0,1	11,6	14,2	8,4	4,2	2,4	1,0	0,5	0,3	14,2	638
8	0,7	11,0	25,2	19,3	12,7	6,9	3,7	2,1	1,1	25,2	1227
9	0,2	6,2	10,6	9,6	4,3	3,0	2,3	2,0	1,4	10,6	582
10	0,2	34,6	56,5	51,5	37,3	28,6	15,4	8,8	4,3	56,5	3524
média	0,2	14,2	22,9	18,4	11,9	7,6	4,1	2,3	1,3	22,9	1234
DP	0,2	9,6	15,2	14,0	10,5	8,1	4,4	2,5	1,2	15,2	955
EP	0,07	3,0	4,8	4,4	3,3	2,6	1,4	0,8	0,4	4,8	302

TABELA 5: Valores individuais e níveis médios de GH, pico e área sob a curva (AUC: μg/L.120 min) após administração de GHRH em controles

	GH (μg/L)										
	Tempo (minutos)										
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**pico**	**AUC**
1	0,03	15,8	19,3	28,8	37,6	38,5	30,8	18,7	11,0	38,5	2925
2	0,03	8,3	9,2	6,8	7,5	7,3	5,7	3,8	2,4	9,2	747
3	0,03	6,2	5,0	4,0	3,5	2,7	2,2	1,4	1,1	6,2	383
4	0,08	0,5	1,1	0,7	0,7	1,8	1,9	1,5	1,9	1,9	138
5	0,008	3,9	4,8	5,4	4,4	3,2	1,9	1,2	0,6	5,4	377
6	0,08	0,8	0,7	1,8	1,4	1,2	2,1	2,7	2,8	2,8	182
7	0,04	3,4	3,6	2,2	2,3	1,8	1,2	0,8	0,8	3,6	236
8	0,06	7,5	8,1	6,6	4,8	2,4	1,4	0,8	0,4	8,1	477
9	0,1	16,0	21,5	24,8	22,6	19,8	13,7	18,2	13,8	24,8	2153
10	0,6	8,0	8,1	11,0	8,2	9,4	12,5	7,1	5,3	12,5	1009
média	0,1	7,0	8,1	9,2	9,3	8,8	7,3	5,6	4,0	11,3	863
DP	0,2	5,4	7,1	9,8	11,8	11,9	9,5	7,0	4,7	11,6	940
EP	0,06	1,7	2,2	3,1	3,7	3,8	3,0	2,2	1,5	3,7	297

TABELA 6: Valores individuais e níveis médios de GH, pico e área sob a curva (AUC:μg/L.120 min) após administração de ghrelina em pacientes com doença de Cushing

	GH (μg/L)										
	Tempo (minutos)										
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**pico**	**AUC**
1	0,1	4,3	2,6	1,0	0,5	0,3	0,2	0,1	0,07	4,3	136
2	0,07	11,3	27,6	25,8	18,3	13,4	9,7	6,4	4,3	27,6	1720
3	0,02	0,3	0,5	0,3	0,1	0,07	0,03	0,03	0,02	0,5	20
4	0,04	7,4	3,5	1,5	0,7	0,3	0,2	0,2	0,08	7,4	208
5	0,2	21,9	12,5	5,6	3,2	1,8	0,5	0,4	0,5	21,9	694
6	0,02	0,4	0,2	0,04	0,04	0,03	0,02	0,03	0,02	0,4	13
7	0,04	0,3	0,8	0,6	0,4	0,2	0,1	0,08	0,04	0,8	38
8	0,2	5,7	2,1	0,8	0,4	0,2	0,1	0,08	0,08	5,7	143
9	0,02	0,9	0,5	0,2	0,1	0,08	0,04	0,03	0,03	0,9	28
10	0,04	2,7	1,5	0,7	0,3	0,1	0,1	0,9	0,07	2,7	95
média	0,08	5,5	5,2	3,7	2,4	1,6	1,1	0,8	0,5	7,2	310
DP	0,07	6,8	8,7	7,9	5,7	4,2	3,0	2,0	1,3	9,6	535
EP	0,02	2,2	2,7	2,5	1,8	1,3	1,0	0,6	0,4	3,0	169

TABELA 7: Valores individuais e níveis médios de GH, pico e área sob a curva (AUC:μg/L.120 min) após administração de GHRP-6 em pacientes com doença de Cushing

	GH (μg/L)										
	Tempo (minutos)										
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**pico**	**AUC**
1	0,2	1,5	0,8	0,4	0,2	0,2	0,1	0,07	0,1	1,5	51
2	0,08	2,0	3,8	6,7	6,4	5,1	3,7	2,9	2,6	6,7	479
3	0,04	0,1	0,07	0,04	0,04	0,04	0,02	0,02	0,02	0,1	5
4	0,06	8,9	3,4	1,5	0,6	0,4	0,3	0,2	0,2	8,9	232
5	0,3	5,2	2,0	0,9	0,4	0,3	0,2	0,2	0,2	5,2	142
6	0,04	0,08	0,04	0,03	0,03	0,02	0,01	0,03	0,03	0,08	4
7	0,08	1,3	0,5	0,3	0,2	0,08	0,04	0,04	0,04	1,3	38
8	0,2	3,1	1,2	0,6	0,4	0,2	0,3	0,2	0,2	3,1	93
9	0,04	0,2	0,1	0,08	0,04	0,03	0,03	0,03	0,03	0,2	8
10	0,01	0,04	0,03	0,03	0,03	0,03	0,03	0,02	0,02	0,04	3
média	0,1	2,2	1,2	1,1	0,8	0,6	0,5	0,4	0,3	2,7	106
DP	0,09	2,9	1,4	2,0	2,0	1,6	1,1	0,9	0,8	3,2	151
EP	0,03	0,9	0,4	0,6	0,6	0,5	0,4	0,3	0,3	1,0	48

TABELA 8: Valores individuais e níveis médios de GH, pico e área sob a curva (AUC:μg/L.120 min) após administração de GHRH em pacientes com doença de Cushing

	GH (μg/L)										
	Tempo (minutos)										
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**pico**	**AUC**
1	0,8	1,3	1,0	0,8	0,4	0,2	0,2	0,3	0,2	1,3	71
2	0,04	1,0	1,3	1,2	1,2	0,7	0,5	0,4	0,4	1,3	99
3	0,02	0,03	0,03	0,03	0,04	0,03	0,02	0,02	0,02	0,04	3
4	0,08	0,5	0,3	0,3	0,5	0,5	0,2	0,1	0,1	0,5	37
5	0,2	0,4	0,4	0,4	0,3	0,3	0,2	0,2	0,3	0,4	37
6	0,04	0,2	0,2	0,2	0,1	0,08	0,08	0,04	0,04	0,2	14
7	0,03	0,3	0,8	0,6	0,3	0,2	0,1	0,1	0,08	0,8	37
8	0,1	0,4	1,9	1,5	1,1	0,8	0,5	0,3	0,2	1,9	100
9	0,03	0,1	0,2	0,08	0,08	0,08	0,04	0,04	0,08	0,2	10
10	0,02	0,07	0,1	0,07	0,07	0,07	0,07	0,04	0,04	0,1	8
média	0,1	0,4	0,6	0,5	0,4	0,3	0,2	0,2	0,1	0,7	42
DP	0,2	0,4	0,6	0,5	0,4	0,3	0,2	0,1	0,1	0,6	37
EP	0,08	0,1	0,2	0,2	0,1	0,09	0,06	0,04	0,04	0,2	12

TABELA 9:Valores individuais e níveis médios de cortisol, pico e área sob a curva (AUC:μg/dL.90 min) após administração de ghrelina em controles

	cortisol (μg/dL) Tempo (min)							
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**	**PICO**	**AUC**
1	8,4	16,3	23,8	23,2	22,2	19,1	23,8	1799
2	9,6	12,2	15,2	14,9	14,1	13,6	15,2	1258
3	7,3	10,2	16,4	18,1	17,9	12,7	18,1	1319
4	5,9	14,2	18,5	18,3	15,7	13,5	18,5	1365
5	9,2	15,2	17,7	20,1	19,6	17,3	20,1	1565
6	5,9	13,6	15,6	13,7	13,0	10,8	15,6	1142
7	4,5	11,9	16,0	15,9	17,2	17,2	17,2	1336
8	9,6	10,6	12,5	10,2	9,4	8,5	12,5	911
9	6,3	8,6	10,7	9,3	7,6	7,5	10,7	760
10	7,5	21,5	18,8	17,7	16,7	14,8	21,5	1524
média	7,5	13,4	16,5	16,1	15,3	13,5	17,3	1298
DP	1,9	3,7	3,6	4,3	4,5	3,8	4,0	306
EP	0,6	1,2	1,1	1,4	1,4	1,2	1,3	97

TABELA 10:Valores individuais e níveis médios de cortisol, pico e área sob a curva (AUC:μg/dL.90 min) e após administração de GHRP-6 em controles

	cortisol (μg/dL) Tempo (min)							
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**	**PICO**	**AUC**
1	10,9	13,3	16,2	16,3	12,7	10,6	16,3	1214
2	14,3	15,6	15,7	16,8	13,6	13,0	16,8	1330
3	12,9	12,5	11,6	10,6	9,6	7,5	12,9	946
4	6,2	8,7	10,4	9,3	8,3	6,5	10,4	757
5	8,2	12,6	11,7	10,3	9,5	8,0	12,6	914
6	9,0	15,7	11,4	9,7	9,2	8,1	15,7	948
7	6,0	14,0	11,4	8,7	5,8	4,3	14,0	749
8	5,1	10,3	8,8	6,6	5,9	6,9	10,3	660
9	3,0	9,0	7,1	6,3	7,7	7,8	9,0	649
10	7,4	11,2	12,9	15,3	12,0	9,1	15,3	1053
média	8,3	12,3	11,7	11,0	9,4	8,2	13,3	922
DP	3,5	2,5	2,8	3,8	2,7	2,4	2,8	229
EP	1,1	0,8	0,9	1,2	0,8	0,7	0,9	72

TABELA 11: Valores individuais e níveis médios de cortisol, pico e área sob a curva (AUC:μg/dL.90 min) após administração de ghrelina em pacientes com doença de Cushing

	cortisol (μg/dL) Tempo (min)							
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**	**PICO**	**AUC**
1	6,6	14,0	20,0	18,9	20,9	16,3	20,9	1558
2	17,5	21,2	22,7	20,6	19,1	20,8	22,7	1841
3	25,5	30,8	33,0	36,6	34,3	31,6	36,6	2943
4	10,6	11,1	12,7	12,6	11,6	10,5	12,7	1044
5	16,2	21,7	27,4	28,4	30,5	31,9	31,9	2449
6	23,9	31,7	42,1	46,1	49,1	39,0	49,1	3668
7	37,0	44,1	62,4	58,0	68,8	69,2	69,2	5330
8	20,0	25,0	30,4	28,8	25,0	21,0	30,4	2291
9	29,2	30,8	36,0	35,8	37,4	30,1	37,4	3051
10	34,9	40,1	45,2	48,9	52,1	43,0	52,1	4092
média	22,1	27,1	33,2	33,5	34,9	31,3	36,3	2827
DP	9,9	10,6	14,3	14,4	17,5	16,7	16,8	1284
EP	3,1	3,3	4,5	4,6	5,5	5,3	5,3	406

TABELA 12: Valores individuais e níveis médios de cortisol, pico e área sob a curva (AUC:μg/dL.90 min) e após administração de GHRP-6 em pacientes com doença de Cushing

	cortisol (μg/dL) Tempo (min)							
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**	**PICO**	**AUC**
1	2,6	7,7	11,2	9,1	7,6	6,7	11,2	711
2	21,4	23,4	26,7	24,2	22,8	21,3	26,7	2108
3	31,8	37,2	36,7	32,9	32,6	27,6	37,2	2988
4	10,9	12,5	12,4	12,9	12,6	12,1	12,9	1114
5	17,2	25,5	31,1	32,7	31,2	36,3	36,3	2715
6	22,6	31,0	38,2	43,2	47,9	46,7	47,9	3634
7	26,6	38,4	46,8	52,0	54,7	58,7	58,7	4369
8	16,7	20,9	22,6	19,6	18,1	21,3	22,6	1799
9	34,1	36,6	38,9	32,7	30,4	30,1	38,9	3014
10	18,8	21,4	24,4	40,4	30,2	31,7	40,4	2589
média	20,3	25,5	28,9	30,0	28,8	29,3	33,3	2504
DP	9,4	10,5	11,7	13,6	14,6	15,5	15,0	1111
EP	3,0	3,3	3,7	4,3	4,6	4,9	4,7	351

TABELA 13: Valores individuais e níveis médios de ACTH, pico, área sob a curva (AUC:pg/mL.90 min) após administração de ghrelina em controles

	ACTH (pg/mL) Tempo (min)							
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**	**PICO**	**AUC**
1	7,6	66,1	42,4	30,2	25,0	12,4	66,1	2886
2	33,4	67,1	58,9	47,4	38,1	25,4	67,1	4090
3	5,0	21,4	32,4	25,1	16,5	5,0	32,4	1667
4	23,7	144,0	129,0	77,7	50,0	31,5	144,0	7035
5	9,5	60,0	57,5	33,4	26,3	13,5	60,0	3128
6	11,5	50,9	44,0	31,4	22,2	13,4	50,9	2681
7	11,5	290,0	298,0	247,0	157,0	74,9	298,0	17267
8	15,7	21,9	19,6	16,5	12,8	11,9	21,9	1454
9	10,0	19,2	21,4	16,2	14,0	11,0	21,4	1407
10	11,1	30,3	28,3	21,5	16,5	9,6	30,3	1800
média	13,9	77,1	73,2	54,6	37,8	20,9	79,2	4342
DP	8,5	83,5	85,1	70,0	43,5	20,5	84,8	4845
EP	2,7	26,4	27,0	22,1	13,7	6,5	26,8	1532

TABELA 14: Valores individuais e níveis médios de ACTH, pico, área sob a curva (AUC:pg/mL.90 min) após administração de GHRP-6 em controles

	ACTH (pg/mL) Tempo (min)							
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**	**PICO**	**AUC**
1	6,9	49,4	29,4	19,9	15,3	9,6	49,4	2021
2	21,8	27,3	22,0	18,9	17,1	14,9	27,3	1795
3	10,4	10,8	8,5	7,0	7,8	7,6	10,8	762
4	19,0	33,0	27,0	20,0	16,0	21,0	33,0	2018
5	6,7	13,6	12,8	10,4	7,7	6,5	13,6	873
6	14,6	26,9	20,5	17,3	12,8	12,9	26,9	1562
7	7,5	10,0	7,7	7,0	7,0	6,5	10,0	682
8	13,1	21,0	16,5	14,0	13,2	19,0	21,0	1453
9	7,0	20,0	15,0	14,5	16,4	19,0	20,0	1449
10	8,1	24,0	18,3	15,0	13,0	11,0	24,0	1378
média	11,7	23,6	17,8	14,4	12,6	12,8	23,6	1399
DP	5,3	11,8	7,2	4,9	3,8	5,5	11,8	489
EP	1,7	3,7	2,3	1,6	1,2	1,7	3,7	155

TABELA 15: Valores individuais e níveis médios de ACTH, pico, área sob a curva (AUC:pg/mL.90 min) após administração de ghrelina em pacientes com doença de Cushing

	ACTH (pg/mL) Tempo (min)							
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**	**PICO**	**AUC**
1	26,7	109,0	118,0	95,0	74,0	40,0	118,0	7295
2	88,7	120,0	101,0	81,9	76,4	76,7	120,0	8078
3	44,6	91,6	100,0	114,0	89,7	58,1	114,0	7808
4	76,1	78,3	71,7	56,8	64,2	65,2	78,3	6095
5	72,9	481,0	463,0	371,0	336,0	278,0	481,0	32002
6	75,6	300,0	372,0	328,0	235,0	116,0	372,0	22595
7	67,7	169,0	192,0	281,0	277,0	187,0	281,0	19175
8	72,7	140,0	132,0	114,0	101,0	72,1	140,0	9689
9	54,6	126,0	116,0	98,8	76,8	57,7	126,0	8115
10	26,4	94,0	87,5	93,4	73,3	44,9	94,0	6644
média	60,6	170,9	175,3	163,4	140,3	99,6	192,4	12750
DP	21,6	126,1	133,4	115,8	101,6	76,2	137,5	8802
EP	6,8	39,9	42,2	36,6	32,1	24,1	43,5	2784

TABELA 16: Valores individuais e níveis médios de ACTH, pico, área sob a curva (AUC:pg/mL.90 min) após administração de GHRP-6 em pacientes com doença de Cushing

	ACTH (pg/mL) Tempo (min)							
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**	**PICO**	**AUC**
1	25,9	47,3	41,9	34,8	27,9	29,4	47,3	3123
2	75,8	111,0	89,7	82,4	77,0	68,6	111,0	7577
3	65,1	117,0	61,1	87,0	67,4	47,4	117,0	6692
4	63,3	62,7	66,6	63,1	65,0	57,7	66,6	5689
5	53,6	288,0	219,0	179,0	164,0	215,0	288,0	17607
6	86,0	438,0	606,0	567,0	470,0	136,0	606,0	37425
7	74,0	245,0	304,0	331,0	278,0	208,0	331,0	23130
8	58,9	77,2	78,4	78,6	68,0	58,8	79,0	6367
9	54,6	121,0	106,0	83,4	69,0	55,5	121,0	7451
10	53,4	56,9	56,6	78,2	67,9	66,6	78,2	5803
média	61,1	156,4	162,9	158,5	135,4	94,3	184,5	12086
DP	16,4	127,5	176,6	166,9	137,9	67,6	176,1	10847
EP	5,2	40,3	55,8	52,8	43,6	21,4	55,7	3430

TABELA 17: Valores individuais e níveis médios de glicose após a administração de ghrelina em controles

	glicose (mg/dL)				
	Tempo (minutos)				
Indiv.	**0**	**30**	**60**	**90**	**120**
1	85	99	89	90	92
2	81	86	83	75	85
3	84	91	94	90	93
4	81	87	81	83	83
5	82	86	85	89	92
6	102	105	103	102	100
7	80	88	84	87	86
8	73	77	75	75	75
9	84	90	89	86	86
10	81	90	85	90	87
média	83	90	87	87	88
DP	7	8	8	8	7
EP	2	2	2	3	2

TABELA 18: Valores individuais e níveis médios de glicose após a administração de GHRP-6 em controles

	glicose (mg/dL)				
	Tempo (minutos)				
Indiv.	**0**	**30**	**60**	**90**	**120**
1	86	91	90	86	100
2	88	86	82	85	84
3	88	93	93	97	93
4	77	84	82	79	82
5	85	89	88	91	93
6	104	107	104	105	106
7	93	93	93	92	95
8	75	73	75	77	78
9	82	82	83	83	84
10	86	88	85	85	84
média	86	89	88	88	88
DP	8	9	8	9	9
EP	3	3	3	3	3

TABELA 19: Valores individuais e níveis médios de glicose após a administração de ghrelina nos pacientes com doença de Cushing

	glicose (mg/dL)				
	Tempo (minutos)				
Indiv.	**0**	**30**	**60**	**90**	**120**
1	215	203	205	206	216
2	78	78	81	80	82
3	105	104	107	111	110
4	59	79	78	75	77
5	82	88	88	89	92
6	87	97	97	102	86
7	177	177	175	174	172
8	88	99	100	92	93
9	118	117	124	132	135
10	-	-	-	-	-
média	112	116	117	118	118
DP	51	44	44	45	48
EP	17	15	15	15	16

TABELA 20: Valores individuais e níveis médios de glicose após a administração de GHRP-6 em pacientes com doença de Cushing

	glicose (mg/dL)				
	Tempo (minutos)				
Indiv.	**0**	**30**	**60**	**90**	**120**
1	83	66	53	48	48
2	79	79	82	82	79
3	102	99	101	104	102
4	71	76	75	70	75
5	86	90	80	86	92
6	84	75	91	91	93
7	85	83	85	86	84
8	88	91	90	91	88
9	167	171	175	176	168
10	-	-	-	-	-
média	94	92	92	93	92
DP	29	31	34	35	32
EP	10	10	11	12	11

ANEXOS – Estudo 2

TABELA 1: Dados clínicos do grupo controle

Indiv.	Sexo	Idade (anos)	IMC[1] (kg/m^2)
1	M	28	25,7
2	M	28	23,3
3	M	30	22,4
4	F	47	30,8
5	M	30	25,5
6	M	47	28,1
8	F	34	20,9
9	F	35	23,7
10	F	20	24,3
11	M	26	24,9
12	M	28	25,9
média		32,1	25,0
DP		8,4	2,7
EP		2,5	0,8

1 - índice de massa corpórea

TABELA 2: Valores individuais e níveis médios das dosagens basais do grupo controle

Indiv.	GH (µg/L)	IGF-I (ng/mL)	cortisol (µg/dL)	ACTH (pg/mL)	glicose (mg/dL)
1	0,2	257	9,7	7,3	85,5
2	0,03	131	12	27,6	84,5
3	0,1	158	10,1	7,7	86,0
4	0,07	127	6,1	21,4	79,0
5	0,01	171	8,7	8,1	83,5
6	0,1	155	7,5	13,1	103,0
8	0,3	152	7,4	14,4	74,0
9	0,1	184	4,7	8,5	83,0
10	0,3	244	7,5	9,6	83,5
11	0,2	194	8,4	22,2	88,5
12	0,9	176	5,1	16,6	80,0
média	0,2	177	7,9	14,2	84,6
DP	0,2	42	2,2	7,0	7,3
EP	0,08	12,7	0,7	2,1	2,2

TABELA 3: Dados clínicos dos pacientes com doença de Cushing antes do tratamento com cetoconazol

Indiv.	Sexo	Idade (anos)	IMC (kg/m^2)	HAS [2]	DM [3]	tumor
6	F	37	27,8	S	N	micro
7	F	38	26,4	S	S	macro
8	F	38	31,3	S	N	micro
9	F	39	28,4	S	S	micro
11	F	41	32,1	N	S	micro
12	F	38	28,7	S	S	macro
13	F	20	26,2	N	N	micro
14	F	19	27,1	N	N	micro
média		33,8	28,5			
DP		8,9	2,2			
EP		3,1	0,8			

1- hipertensão arterial sistêmica

2- diabetes mellitus

TABELA 4: Valores individuais e médios de IMC dos pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	IMC (kg/m^2)		
	pré	**3 meses**	**6 meses**
6	27,8	26,3	27,1
7	26,4	26,3	24,8
8	31,3	30,3	30,5
9	28,4	27,9	27,7
11	32,1	29,7	26,7
12	28,7	28,5	28,3
13	26,2	24,7	23,4
14	27,1	27,3	28,9
média	28,5	27,6	27,2
DP	2,2	1,9	2,3
EP	0,8	0,7	0,8

TABELA 5 : Valores individuais e médios de GH basal dos pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	GH (µg/L)		
	pré	3 meses	6 meses
6	0,03	0,05	0,09
7	0,05	0,06	0,5
8	0,2	0,2	0,4
9	0,03	0,2	0,2
11	0,2	0,3	0,4
12	0,6	0,3	0,3
13	0,1	0,1	0,2
14	0,9	0,2	0,1
média	0,3	0,2	0,3
DP	0,3	0,1	0,2
EP	0,1	0,03	0,05

TABELA 6: Valores individuais e médios de IGF-I basal dos pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	IGF-I (ng/mL)			
	pré	3 meses	6 meses	referência
6	104	133	128	**(106-277)**
7	118	234	317	**(106-277)**
8	300	306	240	**(106-277)**
9	190	196	114	**(106-277)**
11	159	179	204	**(98-261)**
12	195	182	172	**(106-277)**
13	410	254	226	**(122-384)**
14	383	320	360	**138-442)**
média	232,4	225,5	220,1	
DP	117,7	65,2	85,9	
EP	41,6	23,1	30,4	

TABELA 7: Valores individuais e médios de cortisol basal dos pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	cortisol (µg/dL)		
	pré	3 meses	6 meses
6	23,3	17,2	18,7
7	31,8	22,0	15,4
8	18,4	17,3	20,5
9	31,7	15,8	15,8
11	20,8	16,5	12,3
12	21,2	22,8	14,8
13	19,6	8,5	5,7
14	10,8	14,1	13,8
média	22,2	16,8	14,6
DP	6,9	4,5	4,5
EP	2,5	1,6	1,6

TABELA 8: Valores individuais e médios de ACTH basal dos pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	ACTH (pg/mL)		
	pré	3 meses	6 meses
6	80,8	48,5	78,5
7	70,9	90,5	80,0
8	65,8	91,5	178,5
9	54,6	82,5	72,5
11	28,0	58,5	88,0
12	30,2	65,1	71,8
13	34,5	47,2	74,5
14	41,0	50,7	63,2
média	50,7	66,8	88,4
DP	20,2	18,8	37,1
EP	7,1	6,6	13,1

TABELA 9: Valores individuais e níveis médios de glicemia de jejum basal dos pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	glicemia (mg/dL)		
	pré	3 meses	6 meses
6	85,5	95,5	76,5
7	131,0	118,0	97,5
8	88,0	93,5	90,5
9	142,5	113,0	109,0
11	134,0	94,5	93,0
12	152,0	128,5	121,0
13	89,0	88,0	73,0
14	83,0	77,0	82,0
média	113,1	101,0	92,8
DP	29,3	17,2	16,3
EP	10,4	6,1	5,8

TABELA 10: Valores individuais e médios de cortisol livre urinário dos pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	cortisol urinário (µg/24h)		
	pré	3 meses	6 meses
6	233,0	88,3	11,6
7	442,0	73,2	26,5
8	210,0	36,9	98,5
9	147,0	91,4	48,0
11	161,0	46,0	45,0
12	257,0	66,8	61,0
13	183,0	81,0	11,0
14	146,0	9,0	11,2
média	222,4	61,6	39,1
DP	97,5	28,7	30,7
EP	34,5	10,1	10,9

referência: 3-43 µg/24h

TABELA 11: Valores individuais e níveis médios de GH após a administração de ghrelina em controles

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
1	0,03	70,4	110,4	80,8	57,3	52,7	40,8	25,4	14,2
2	0,02	37,5	95,8	90,4	57,3	33,1	41,1	23,9	15,3
3	0,2	35,2	43,5	29,0	19,3	14,4	8,6	4,8	3,0
4	0,04	9,9	17,8	12,0	6,1	3,5	2,2	1,3	0,7
5	0,02	17,5	25,1	18,5	10,4	8,5	6,0	4,2	2,7
6	0,02	24,5	31,6	23,8	16,7	7,7	5,4	2,7	1,7
8	0,08	16,8	41,9	32,4	21,2	13,0	6,4	5,2	2,3
9	0,07	23,0	27,9	17,0	7,0	3,7	2,5	1,0	0,6
10	0,06	78,1	123,5	102,3	80,8	53,1	34,1	16,9	11,6
11	0,3	29,1	39,6	30,6	22,0	12,8	7,3	4,1	2,2
12	0,8	33,4	38,5	31,8	25,5	21,8	16,0	11,3	7,0
média	0,1	34,1	54,1	42,6	29,4	20,4	15,5	9,2	5,6
DP	0,2	21,6	37,1	32,2	24,5	18,1	15,4	8,9	5,5
EP	0,07	6,5	11,2	9,7	7,4	5,5	4,6	2,7	1,7

TABELA 12: Valores individuais e níveis médios de GH após a administração de GHRP-6 em controles

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
1	0,4	23,8	35,5	25,8	17,9	11,9	7,1	3,8	2,3
2	0,04	12,1	29,1	26,3	15,8	8,9	4,7	2,6	1,4
3	0,08	19,5	24,7	17,9	9,2	4,8	2,4	1,5	1,2
4	0,1	1,0	4,2	3,0	1,6	0,8	0,4	0,2	0,1
5	0,01	13,3	17,7	15,9	11,8	6,7	2,4	1,2	0,8
6	0,2	8,8	10,9	7,3	4,2	2,3	1,4	0,7	0,5
8	0,7	11,0	25,2	19,3	12,7	6,9	3,7	2,1	1,1
9	0,2	6,2	10,6	9,6	4,3	3,0	2,3	2,0	1,4
10	0,2	34,6	56,5	51,5	37,3	28,6	15,4	8,8	4,3
11	0,08	16,7	29,3	17,9	9,7	5,8	2,8	1,6	0,9
12	1,7	39,2	32,2	26,9	22,1	16,6	11,4	7,7	4,4
média	0,3	16,9	25,1	20,1	13,3	8,8	4,9	2,9	1,7
DP	0,5	11,7	14,4	13,0	10,1	8,0	4,6	2,8	1,4
EP	0,1	3,5	4,4	3,9	3,0	2,4	1,4	0,8	0,4

TABELA 13: Valores individuais e níveis médios de GH após a administração de GHRH em controles

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
1	0,03	15,8	19,3	28,8	37,6	38,5	30,8	18,7	11,0
2	0,03	8,3	9,2	6,8	7,5	7,3	5,7	3,8	2,4
3	0,03	6,2	5,0	4,0	3,5	2,7	2,2	1,4	1,1
4	0,08	0,5	1,1	0,7	0,7	1,8	1,9	1,5	1,9
5	0,008	3,9	4,8	5,4	4,4	3,2	1,9	1,2	0,6
6	0,08	0,8	0,7	1,8	1,4	1,2	2,1	2,7	2,8
8	0,06	7,5	8,1	6,6	4,8	2,4	1,4	0,8	0,4
9	0,1	16,0	21,5	24,8	22,6	19,8	13,7	18,2	13,8
10	0,6	8,0	8,1	11,0	8,2	9,4	12,5	7,1	5,3
11	0,08	5,8	5,7	6,6	3,9	2,2	1,3	0,8	1,4
12	0,3	12,8	9,8	5,5	5,0	3,3	2,3	2,2	1,6
média	0,1	7,8	8,5	9,3	9,1	8,3	6,9	5,3	3,8
DP	0,2	5,3	6,6	9,1	11,2	11,4	9,1	6,7	4,5
EP	0,05	1,6	2,0	2,8	3,4	3,4	2,7	2,0	1,4

TABELA 14:Valores individuais e níveis médios de GH após a administração de ghrelina em pacientes com doença de Cushing antes do tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,02	0,4	0,2	0,04	0,04	0,03	0,02	0,03	0,02
7	0,04	0,3	0,8	0,6	0,4	0,2	0,1	0,08	0,04
8	0,2	5,7	2,1	0,8	0,4	0,2	0,1	0,08	0,08
9	0,02	0,9	0,5	0,2	0,1	0,08	0,04	0,03	0,03
11	0,08	7,3	4,5	2,2	1,2	0,7	0,3	0,2	0,1
12	0,4	16,2	7,1	3,1	1,4	0,8	0,4	0,3	0,2
13	0,2	14,6	15,9	9,0	4,8	2,5	1,3	0,7	0,5
14	0,3	7,0	5,3	2,0	1,2	0,7	0,3	0,2	0,1
média	0,2	6,6	4,6	2,2	1,2	0,7	0,3	0,2	0,1
DP	0,1	6,2	5,2	2,9	1,6	0,8	0,4	0,2	0,2
EP	0,05	2,2	1,8	1,0	0,5	0,3	0,1	0,08	0,06

TABELA 15: Valores individuais e níveis médios de GH após a administração de ghrelina em pacientes com doença de Cushing após 3 meses de tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,08	1,8	0,9	0,6	0,2	0,1	0,08	0,2	0,03
7	0,04	7,2	5,6	1,9	0,9	0,5	0,2	0,2	0,1
8	0,08	1,7	1,3	0,6	0,4	0,2	0,2	0,1	0,08
9	0,08	1,7	2,6	1,1	0,5	0,2	0,1	0,08	0,04
11	0,2	2,5	6,2	2,7	1,2	0,6	0,3	0,2	0,1
12	0,2	11,8	4,5	3,1	1,0	0,5	0,3	0,2	0,2
13	0,08	10,8	19,0	10,4	5,4	2,8	1,3	0,8	0,5
14	0,2	12,8	8,3	3,8	1,5	0,8	0,4	0,2	0,2
média	0,1	6,3	6,1	3	1,4	0,7	0,4	0,2	0,2
DP	0,07	4,9	5,8	3,2	1,7	0,9	0,4	0,2	0,2
EP	0,02	1,7	2,1	1,1	0,6	0,3	0,1	0,08	0,05

TABELA 16: Valores individuais e níveis médios de GH após a administração de ghrelina em pacientes com doença de Cushing após 6 meses de tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,1	2,7	1,3	0,6	0,3	0,2	0,1	0,08	0,08
7	0,4	30,1	20,4	9,2	4,3	2,3	1,1	0,8	0,7
8	0,9	10,0	4,5	2,4	1,4	0,8	0,4	0,6	0,6
9	0,2	5,7	3,2	1,7	0,7	0,3	0,2	0,1	0,1
11	0,5	17,8	12,5	5,4	2,5	1,2	0,6	0,3	0,2
12	0,3	13,8	5,2	2,2	1,2	0,7	0,3	0,2	0,2
13	0,2	16,0	30,7	27,7	14,8	8,6	4,1	2,0	1,0
14	0,1	16,2	17,0	7,0	2,8	1,1	0,6	0,3	0,2
média	0,3	14,0	11,9	7,0	3,5	1,9	0,9	0,5	0,4
DP	0,3	8,4	10,3	8,9	4,7	2,8	1,3	0,6	0,3
EP	0,09	3,0	3,6	3,1	1,7	1,0	0,5	0,2	0,1

TABELA 17: Valores individuais e níveis médios de GH após a administração de GHRP-6 em pacientes com doençade Cushing antes do tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,04	0,08	0,04	0,03	0,03	0,02	0,01	0,03	0,03
7	0,08	1,3	0,5	0,3	0,2	0,08	0,04	0,04	0,04
8	0,2	3,1	1,2	0,6	0,4	0,2	0,3	0,2	0,2
9	0,04	0,2	0,1	0,08	0,04	0,03	0,03	0,03	0,03
11	0,08	1,5	0,7	0,3	0,2	0,2	0,08	0,08	0,08
12	0,6	6,4	3,0	1,2	0,6	0,4	0,2	0,2	0,2
13	0,08	4,1	5,5	3,6	2,8	1,6	1,0	0,9	0,9
14	0,3	4,1	3,3	2,1	1,1	0,5	0,3	0,2	0,2
média	0,2	2,6	1,8	1	0,7	0,4	0,2	0,2	0,2
DP	0,2	2,2	2	1,3	0,9	0,5	0,3	0,3	0,3
EP	0,07	0,8	0,7	0,4	0,3	0,2	0,1	0,1	0,1

TABELA 18: Valores individuais e níveis médios de GH após a administração de GHRP-6 em pacientes com doença de Cushing após 3 meses de tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,03	0,2	0,1	0,03	0,04	0,03	0,01	0,02	0,02
7	0,03	2,3	4,9	3,0	1,3	0,7	0,3	0,2	0,1
8	0,08	0,7	1,1	0,9	0,7	0,6	0,4	0,3	0,2
9	0,2	3,4	1,4	0,1	0,3	0,2	0,1	0,06	0,04
11	0,4	7,0	4,1	1,6	0,7	0,3	0,2	0,2	0,1
12	0,4	8,6	3,4	1,5	0,8	0,5	0,3	0,2	0,1
13	0,2	2,2	2,8	2,4	1,7	1,2	0,6	0,3	0,2
14	0,2	4,3	4,7	2,7	1,5	0,7	0,3	0,2	0,2
média	0,2	3,6	2,8	1,5	0,9	0,5	0,3	0,2	0,1
DP	0,1	2,9	1,8	1,1	0,6	0,4	0,2	0,1	0,07
EP	0,05	1,0	0,6	0,4	0,2	0,1	0,06	0,04	0,03

TABELA 19: Valores individuais e níveis médios de GH após a administração de GHRP-6 em pacientes com doença de Cushing após 6 meses de tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,08	0,7	0,3	0,2	0,08	0,08	0,04	0,08	0,03
7	0,2	10,7	6,0	2,7	1,5	1,0	0,5	0,3	0,2
8	0,1	2,2	1,3	0,5	0,3	0,2	0,2	0,1	0,08
9	0,2	2,3	0,8	0,3	0,2	0,1	0,08	0,04	0,04
11	0,3	4,0	2,0	1,0	0,5	0,3	0,2	0,1	0,08
12	0,2	3,7	1,9	1,0	0,6	0,3	0,2	0,1	0,1
13	0,2	6,7	12,2	9,9	7,1	3,2	1,4	0,8	0,4
14	0,3	4,6	3,8	2,3	0,7	0,4	0,2	0,1	0,1
média	0,2	4,4	3,5	2,2	1,4	0,7	0,4	0,2	0,1
DP	0,08	3,1	4	3,2	2,4	1,1	0,4	0,3	0,1
EP	0,03	1,1	1,4	1,1	0,8	0,4	0,2	0,09	0,04

TABELA 20: Valores individuais e níveis médios de GH após a administração de GHRH em pacientes com doença de Cushing antes do tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,04	0,2	0,2	0,2	0,1	0,08	0,08	0,04	0,04
7	0,03	0,3	0,8	0,6	0,3	0,2	0,1	0,1	0,08
8	0,1	0,4	1,9	1,5	1,1	0,8	0,5	0,3	0,2
9	0,03	0,1	0,2	0,08	0,08	0,08	0,04	0,04	0,08
11	0,3	1,4	1,3	1,0	0,5	0,3	0,3	0,2	0,3
12	0,8	0,8	1,0	0,9	0,7	0,6	0,4	0,3	0,3
13	0,08	1,5	1,4	1,2	1,0	0,7	0,5	0,4	0,3
14	2,2	2,0	1,1	0,6	0,3	0,2	0,2	0,2	0,3
média	0,4	0,8	1,0	0,8	0,5	0,4	0,3	0,2	0,2
DP	0,8	0,7	0,6	0,5	0,4	0,3	0,2	0,1	0,1
EP	0,3	0,3	0,2	0,2	0,1	0,1	0,07	0,05	0,04

TABELA 21: Valores individuais e níveis médios de GH após a administração de GHRH em pacientes com doença de Cushing após 3 meses de tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,04	0,2	0,2	0,2	0,1	0,08	0,08	0,08	0,08
7	0,1	0,2	0,4	0,2	0,2	0,08	0,08	0,08	0,2
8	0,3	1,6	1,8	1,5	1,3	1,2	0,6	0,3	0,2
9	0,2	0,4	1,6	1,3	0,5	0,2	0,1	0,08	0,08
11	0,2	0,8	0,7	0,4	0,5	0,6	0,9	0,7	0,5
12	0,3	0,4	0,5	0,3	0,2	0,3	0,4	0,6	0,8
13	0,08	0,5	0,4	0,3	0,5	0,7	1,0	0,7	0,7
14	0,08	0,9	2,2	2,5	2,3	1,6	0,8	0,4	0,3
média	0,2	0,6	1,0	0,8	0,7	0,6	0,5	0,4	0,4
DP	0,1	0,5	0,8	0,8	0,7	0,6	0,4	0,3	0,3
EP	0,04	0,2	0,3	0,3	0,3	0,2	0,1	0,1	0,1

TABELA 22: Valores individuais e níveis médios de GH após a administração de GHRH em pacientes com doença de Cushing após 6 meses de tratamento com cetoconazol

	GH (µg/L)								
	Tempo (minutos)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
6	0,08	0,3	0,3	0,2	0,2	0,08	0,08	0,04	0,08
7	0,8	3,5	2,7	1,5	0,7	0,5	0,3	0,2	0,2
8	0,08	0,3	0,4	0,3	0,2	0,2	0,1	0,2	0,3
9	0,3	0,4	0,3	0,2	0,2	0,2	0,2	0,5	1,0
11	0,3	0,9	0,9	0,6	0,4	0,4	0,3	0,4	0,5
12	0,4	0,4	0,4	0,4	0,3	0,3	0,3	0,2	0,2
13	0,2	1,1	1,8	2,2	2,2	1,9	1,3	0,7	0,5
14	0,04	0,3	0,3	0,3	0,5	0,4	0,4	0,3	0,3
média	0,3	0,9	0,9	0,7	0,6	0,5	0,4	0,3	0,4
DP	0,2	1,1	0,9	0,7	0,7	0,6	0,4	0,2	0,3
EP	0,09	0,4	0,3	0,3	0,2	0,2	0,1	0,07	0,1

TABELA 23: Resposta de GH expressa em pico e AUC (área sob a curva) após a administração de ghrelina, GHRP-6 e GHRH em controles

Indiv.	PICO (µg/L)			AUC (µg/L.120 min)		
	ghrelina	GHRP-6	GHRH	ghrelina	GHRP-6	GHRH
1	110,4	35,5	38,5	6674	1907	2925
2	95,8	29,1	9,2	5802	1503	747
3	43,5	24,7	6,2	2346	1210	383
4	17,8	4,2	1,9	798	170	138
5	25,1	17,7	5,4	1373	1041	377
6	31,6	10,9	2,8	1699	539	182
8	41,9	25,2	8,1	2071	1227	477
9	27,9	10,6	24,8	1237	582	2153
10	123,5	56,5	12,5	7419	3524	1009
11	39,6	29,3	6,6	2201	1264	406
12	38,5	39,2	12,8	2733	2387	628
média	54,1	25,7	11,7	3123	1396	857
DP	37,1	14,9	10,9	2345	943	884
EP	11,2	4,5	3,3	707	284	267

TABELA 24: Resposta do GH expressa em pico e AUC (área sob a curva) após a administração de ghrelina em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	PICO (µg/L)			AUC (µg/L.120 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	0,4	1,8	2,7	13	59	81
7	0,8	7,2	30,1	38	249	1031
8	5,7	1,7	10,0	143	69	313
9	0,9	2,6	5,7	28	95	181
11	7,3	6,2	17,8	247	208	610
12	16,2	11,8	13,8	444	324	358
13	15,9	19,0	30,7	737	762	1568
14	7,0	12,8	17,0	254	420	677
média	6,8	7,9	16,0	238	273	602
DP	6,4	6,2	10,3	249	235	495
EP	2,3	2,2	3,6	88	83	175

TABELA 25: Resposta do GH expressa em pico e AUC (área sob a curva) após a administração de GHRP-6 em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	PICO (µg/L)			AUC (µg/L.120 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	0,08	0,2	0,7	4,0	7,0	23
7	1,3	4,9	10,7	38	191	343
8	3,1	1,1	2,2	93	73	73
9	0,2	3,4	2,3	8,0	85	59
11	1,5	7,0	4,0	47	215	124
12	6,4	8,6	3,7	186	233	119
13	5,5	2,8	12,2	300	171	624
14	4,1	4,7	4,6	178	219	185
média	2,8	4,1	5,1	107	149	194
DP	2,4	2,8	4,2	105	83	200
EP	0,8	1,0	1,5	37	30	71

TABELA 26: Resposta do GH expressa em pico e AUC (área sob a curva) após a administração de GHRH em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	PICO (µg/L)			AUC (µg/L.120 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	0,2	0,2	0,3	14	15	19
7	0,8	0,4	3,5	37	21	149
8	1,9	1,8	0,4	100	128	28
9	0,2	1,6	1,0	10	65	40
11	1,4	0,9	0,9	80	74	65
12	1,0	0,8	0,4	79	49	39
13	1,5	1,0	2,2	103	67	173
14	2,0	2,5	0,5	88	163	40
média	1,1	1,2	1,2	64	73	69
DP	0,7	0,8	1,1	38	51	59
EP	0,2	0,3	0,4	14	18	21

TABELA 27: Valores individuais e níveis médios de cortisol após a administração de ghrelina em controles

	cortisol (μg/dL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
1	8,4	16,3	23,8	23,2	22,2	19,1
2	9,6	12,2	15,2	14,9	14,1	13,6
3	7,3	10,2	16,4	18,1	17,9	12,7
4	5,9	14,2	18,5	18,3	15,7	13,5
5	9,2	15,2	17,7	20,1	19,6	17,3
6	5,9	13,6	15,6	13,7	13,0	10,8
8	9,6	10,6	12,5	10,2	9,4	8,5
9	6,3	8,6	10,7	9,3	7,6	7,5
10	7,5	21,5	18,8	17,7	16,7	14,8
11	6,9	11,3	14,9	14,2	11,4	8,4
12	5,3	9,1	11,0	9,4	11,2	7,8
média	7,4	13,0	15,9	15,4	14,4	12,2
DP	1,6	3,8	3,8	4,6	4,5	3,9
EP	0,5	1,1	1,1	1,4	1,3	1,2

TABELA 28: Valores individuais e níveis médios de cortisol após a administração de GHRP-6 em controles

	cortisol (μg/dL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
1	10,9	13,3	16,2	16,3	12,7	10,6
2	14,3	15,6	15,7	16,8	13,6	13,0
3	12,9	12,5	11,6	10,6	9,6	7,5
4	6,2	8,7	10,4	9,3	8,3	6,5
5	8,2	12,6	11,7	10,3	9,5	8,0
6	9,0	15,7	11,4	9,7	9,2	8,1
8	5,1	10,3	8,8	6,6	5,9	6,9
9	3,0	9,0	7,1	6,3	7,7	7,8
10	7,4	11,2	12,9	15,3	12,0	9,1
11	9,8	12,7	11,4	10,4	9,0	7,8
12	4,9	15,6	12,2	11,5	9,0	7,2
média	8,3	12,5	11,8	11,2	9,7	8,4
DP	3,5	2,5	2,6	3,6	2,3	1,9
EP	1,1	0,8	0,8	1,1	0,7	0,6

TABELA 29: Valores individuais e níveis médios de cortisol após a administração de ghrelina em pacientes com doença de Cushing antes do tratamento com cetoconazol

	cortisol (μg/dL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	23,9	31,7	42,1	46,1	49,1	39,0
7	37,0	44,1	62,4	58,0	68,8	69,2
8	20,0	25,0	30,4	28,8	25,0	21,0
9	29,2	30,8	36,0	35,8	37,4	30,1
11	20,5	29,4	33,2	35,6	35,9	29,8
12	25,8	38,8	50,0	54,4	56,3	41,5
13	21,9	29,9	32,2	26,7	23,6	18,3
14	11,4	10,7	23,6	28,7	32,3	25,1
média	23,7	30,1	38,7	39,3	41,1	34,3
DP	7,5	9,8	12,4	12,1	15,8	16,2
EP	2,6	3,5	4,4	4,3	5,6	5,7

TABELA 30: Valores individuais e níveis médios de cortisol após a administração de ghrelina em pacientes com doença de Cushing após 3 meses do tratamento com cetoconazol

	cortisol (μg/dL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	5,9	7,4	11,3	13,4	15,5	17,3
7	21,4	26,2	28,4	29,0	33,9	33,5
8	15,2	16,3	20,2	19,3	17,4	16,3
9	15,0	16,8	17,1	19,2	19,1	18,5
11	17,1	18,9	17,8	20,0	19,9	18,8
12	21,3	24,5	34,3	34,0	34,6	33,7
13	7,0	11,0	14,1	12,8	15,9	16,7
14	12,8	17,1	23,1	20,8	21,3	18,3
média	14,5	17,3	20,8	21,1	22,2	21,6
DP	5,8	6,2	7,6	7,2	7,7	7,4
EP	2,0	2,2	2,7	2,6	2,7	2,6

TABELA 31: Valores individuais e níveis médios de cortisol após a administração de ghrelina em pacientes com doença de Cushing após 6 meses do tratamento com cetoconazol

	cortisol (µg/dL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	25,3	25,4	28,3	30,0	34,0	34,9
7	13,6	14,0	14,9	18,1	20,0	21,8
8	20,3	24,3	27,0	26,3	25,8	23,8
9	14,8	16,8	17,7	18,8	21,8	19,4
11	13,2	15,3	16,4	16,3	17,6	17,0
12	16,0	20,8	25,4	26,2	29,4	31,5
13	7,1	8,5	8,8	11,9	13,3	13,3
14	17,4	21,7	26,0	29,6	31,9	30,2
média	16,0	18,4	20,6	22,2	24,2	24,0
DP	5,4	5,7	7,1	6,7	7,3	7,6
EP	1,9	2,0	2,5	2,4	2,6	2,7

TABELA 32: Valores individuais e níveis médios de cortisol após a administração de GHRP-6 em pacientes com doença de Cushing antes do tratamento com cetoconazol

	cortisol (µg/dL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	22,6	31,0	38,2	43,2	47,9	46,7
7	26,6	38,4	46,8	52,0	54,7	58,7
8	16,7	20,9	22,6	19,6	18,1	21,3
9	34,1	36,6	38,9	32,7	30,4	30,1
11	21,0	26,2	28,7	28,5	27,5	21,3
12	16,6	34,0	51,8	44,8	51,9	40,6
13	17,3	23,9	30,9	28,2	25,4	19,3
14	10,1	19,9	22,4	21,6	23,4	21,6
média	20,6	28,9	35,0	33,8	34,9	32,5
DP	7,3	7,1	10,8	11,7	14,3	14,7
EP	2,6	2,5	3,8	4,1	5,1	5,2

TABELA 33: Valores individuais e níveis médios de cortisol após a administração de GHRP-6 em pacientes com doença de Cushing após 3 meses do tratamento com cetoconazol

	cortisol (µg/dL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	28,5	22,3	31,0	32,7	38,3	43,1
7	22,6	23,5	24,8	30,1	32,9	35,8
8	19,3	16,7	19,1	18,1	18,0	15,8
9	16,5	16,9	18,5	21,0	18,8	19,4
11	15,9	17,8	21,0	18,7	19,3	18,2
12	24,3	23,9	26,9	34,6	29,6	33,3
13	9,9	10,8	14,5	15,5	17,8	17,1
14	15,3	18,7	20,9	22,0	25,2	29,5
média	19,0	18,8	22,1	24,1	25,0	26,5
DP	5,9	4,4	5,2	7,3	7,9	10,3
EP	2,1	1,5	1,9	2,6	2,8	3,6

TABELA 34: Valores individuais e níveis médios de cortisol após a administração de GHRP-6 em pacientes com doença de Cushing após 6 meses do tratamento com cetoconazol

	cortisol (µg/dL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	12,1	14,6	19,3	25,8	29,3	30,7
7	17,2	16,4	17,8	18,3	19,5	17,9
8	20,6	20,0	21,2	19,5	20,8	21,3
9	16,8	20,5	24,3	23,6	23,3	18,9
11	11,4	11,4	13,6	14,1	14,3	13,2
12	13,5	15,7	18,7	21,9	22,0	27,8
13	4,3	4,6	6,5	7,6	8,0	7,2
14	10,1	12,0	14,6	15,8	18,4	19,9
média	13,3	14,4	17,0	18,3	19,5	19,6
DP	5,0	5,1	5,4	5,8	6,3	7,5
EP	1,8	1,8	1,9	2,1	2,2	2,6

TABELA 35: Resposta do cortisol expressa em pico e AUC (área sob a curva) após a administração de ghrelina e GHRP-6 em controles

Indiv.	PICO (μg/dL)		AUC (μg/dL.90 min)	
	ghrelina	GHRP-6	ghrelina	GHRP-6
1	23,8	16,3	1799	1214
2	15,2	16,8	1258	1330
3	18,1	12,9	1319	946
4	18,5	10,4	1365	757
5	20,1	12,6	1565	914
6	15,6	15,7	1142	948
8	12,5	10,3	911	660
9	10,7	9,0	760	649
10	21,5	15,3	1524	1053
11	14,9	12,7	1040	911
12	11,2	15,6	851	937
média	16,6	13,4	1230	938
DP	4,2	2,7	325	209
EP	1,3	0,8	98	63

TABELA 36: Resposta do cortisol expressa em Δ AUC após a administração de ghrelina e GHRP-6 em controles

Indiv.	Δ AUC ghrelina (μg/dL.90 min)	
	ghrelina	GHRP-6
1	1043	233
2	394	43
3	662	-215
4	834	199
5	737	176
6	611	138
8	47	201
9	193	379
10	849	387
11	419	29
12	374	496
média	560	188
DP	303	196
EP	91	59

TABELA 37: Resposta do cortisol expressa em pico e AUC (área sob a curva) após a administração de ghrelina em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	PICO (µg/dL)			AUC (µg/dL.90 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	49,1	17,3	34,9	3668	1134	2734
7	69,2	33,9	21,8	5331	2680	1584
8	30,4	20,2	27,0	2291	1587	2254
9	37,4	19,2	21,8	3051	1616	1692
11	35,9	20,0	17,6	2882	1709	1470
12	56,3	34,6	31,5	4231	2836	2340
13	32,2	16,7	13,3	2302	1229	990
14	32,3	23,1	31,9	2134	1765	2461
média	42,9	23,1	25,0	3236	1820	1941
DP	14,0	7,1	7,6	1115	621	594
EP	5,0	2,5	2,7	394	220	210

TABELA 38: Resposta do cortisol expressa em pico e AUC (área sob a curva) após a administração de GHRP-6 em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	PICO (µg/dL)			AUC (µg/dL.90 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	47,9	43,1	30,7	3634	3012	2106
7	58,7	35,8	19,5	4369	2623	1624
8	22,6	19,3	21,3	1799	1595	1853
9	38,9	21,0	24,3	3014	1684	1960
11	28,7	21,0	14,3	2347	1689	1192
12	51,9	34,6	27,8	3860	2629	1858
13	30,9	17,8	8,0	2234	1343	601
14	23,4	29,5	19,9	1885	2048	1424
média	37,9	27,8	20,7	2893	2078	1577
DP	13,7	9,4	7,3	973	604	494
EP	4,8	3,3	2,6	344	214	175

TABELA 39: Resposta do cortisol expressa em ∆ AUC após a administração de ghrelina e GHRP-6 em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	∆ AUC ghrelina (µg/dL.90 min)			∆ AUC GHRP-6 (µg/dL.90 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	1517	603	457	1600	447	1017
7	2001	754	360	1975	589	76
8	491	219	427	296	-142	-1
9	423	266	360	-55	199	448
11	1037	170	282	457	258	166
12	1909	919	900	2366	442	643
13	331	599	351	677	452	214
14	1108	613	895	976	671	515
média	1102	518	504	1037	365	385
DP	662	271	248	860	256	340
EP	234	95,7	87,8	304	90,6	120

TABELA 40: Valores individuais e níveis médios de ACTH após a administração de ghrelina em controles

	ACTH (pg/mL) Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
1	7,6	66,1	42,4	30,2	25,0	12,4
2	33,4	67,1	58,9	47,4	38,1	25,4
3	5,0	21,4	32,4	25,1	16,5	5,0
4	23,7	144,0	129,0	77,7	50,0	31,5
5	9,5	60,0	57,5	33,4	26,3	13,5
6	11,5	50,9	44,0	31,4	22,2	13,4
8	15,7	21,9	19,6	16,5	12,8	11,9
9	10,0	19,2	21,4	16,2	14,0	11,0
10	11,1	30,3	28,3	21,5	16,5	9,6
11	23,6	82,6	68,6	51,6	34,7	20,5
12	15,0	112,0	74,2	48,5	30,5	14,2
média	15,1	61,4	52,4	36,3	26,1	15,3
DP	8,5	39,9	31,4	18,5	11,5	7,6
EP	2,6	12,0	9,5	5,6	3,5	2,3

TABELA 41: Valores individuais e níveis médios de ACTH após a administração de GHRP-6 em controles

	ACTH (pg/mL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
1	6,9	49,4	29,4	19,9	15,3	9,6
2	21,8	27,3	22,0	18,9	17,1	14,9
3	10,4	10,8	8,5	7,0	7,8	7,6
4	19,0	33,0	27,0	20,0	16,0	21,0
5	6,7	13,6	12,8	10,4	7,7	6,5
6	14,6	26,9	20,5	17,3	12,8	12,9
8	13,1	21,0	16,5	14,0	13,2	19,0
9	7,0	20,0	15,0	14,5	16,4	19,0
10	8,1	24,0	18,3	15,0	13,0	11,0
11	20,8	28,9	22,6	19,0	16,8	14,6
12	18,1	87,1	56,1	34,1	21,7	12,5
média	13,3	31,1	22,6	17,3	14,3	13,5
DP	5,9	21,2	12,7	6,9	4,1	4,8
EP	1,8	6,4	3,8	2,1	1,2	1,4

TABELA 42: Valores individuais e níveis médios de ACTH após a administração de ghrelina em pacientes com doença de Cushing antes do tratamento com cetoconazol

	ACTH (pg/mL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	75,6	300,0	372,0	328,0	235,0	116,0
7	67,7	169,0	192,0	281,0	277,0	187,0
8	72,7	140,0	132,0	114,0	101,0	72,1
9	54,6	126,0	116,0	98,8	76,8	57,7
11	31,4	140,0	160,0	142,0	94,3	41,2
12	36,3	269,0	241,0	182,0	120,0	54,6
13	37,4	124,0	82,6	44,6	30,6	14,0
14	35,0	606,0	706,0	413,0	277,0	82,9
média	51,3	234,0	250,0	200,0	152,0	78,2
DP	18,5	165,0	205,0	128,0	96,7	53,2
EP	6,6	58,2	72,5	45,1	34,2	18,8

TABELA 43: Valores individuais e níveis médios de ACTH após a administração de ghrelina em pacientes com doença de Cushing após 3 meses de tratamento com cetoconazol

	ACTH (pg/mL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	47,0	284,0	357,0	361,0	285,0	181,0
7	110,0	578,0	762,0	702,0	652,0	391,0
8	98,0	187,0	203,0	147,0	130,0	84,0
9	104,0	277,0	278,0	234,0	178,0	102,0
11	57,0	270,0	281,0	241,0	177,0	52,0
12	46,7	250,0	291,0	211,0	155,0	73,0
13	37,8	606,0	617,0	373,0	291,0	101,0
14	54,4	321,0	314,0	257,0	193,0	114,0
média	69,4	347,0	388,0	316,0	258,0	137,0
DP	29,4	156,0	195,0	173,0	170,0	109,0
EP	10,4	55,2	68,9	61,2	59,9	38,6

TABELA 44: Valores individuais e níveis médios de ACTH após a administração de ghrelina em pacientes com doença de Cushing após 6 meses de tratamento com cetoconazol

	ACTH (pg/mL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	109,0	232,0	358,0	324,0	294,0	176,0
7	48,0	202,0	430,0	519,0	539,0	403,0
8	220,0	392,0	337,0	297,0	237,0	117,0
9	79,0	578,0	459,0	327,0	254,0	166,0
11	93,0	264,0	327,0	267,0	203,0	97,0
12	63,5	541,0	561,0	455,0	315,0	150,0
13	69,9	701,0	759,0	559,0	368,0	178,0
14	67,1	452,0	558,0	475,0	306,0	110,0
média	93,7	420,0	474,0	403,0	315,0	175,0
DP	54,3	180,0	147,0	112,0	104,0	97,4
EP	19,2	63,7	52,1	39,5	36,8	34,4

TABELA 45: Valores individuais e níveis médios de ACTH após a administração de GHRP-6 em pacientes com doença de Cushing antes do tratamento com cetoconazol

	ACTH (pg/mL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	86,0	438,0	606,0	567,0	470,0	136,0
7	74,0	245,0	304,0	331,0	278,0	208,0
8	58,9	77,2	78,4	78,6	68,0	58,8
9	54,6	121,0	106,0	83,4	69,0	55,5
11	24,6	75,7	68,1	56,0	44,1	24,5
12	24,0	163,0	172,0	121,0	84,5	47,2
13	31,6	206,0	166,0	99,0	56,3	22,5
14	46,9	613,0	628,0	430,0	348,0	182,0
média	50,1	242,0	266,0	221,0	177,0	91,8
DP	22,8	190,0	229,0	195,0	165,0	73,0
EP	8,1	67,2	80,9	69,0	58,2	25,8

TABELA 46: Valores individuais e níveis médios de ACTH após a administração de GHRP-6 em pacientes com doença de Cushing após 3 meses de tratamento com cetoconazol

	ACTH (pg/mL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	50,0	209,0	297,0	307,0	445,0	714,0
7	71,0	187,0	269,0	263,0	237,0	137,0
8	85,0	126,0	145,0	113,0	119,0	95,0
9	61,0	132,0	126,0	104,0	84,0	70,0
11	60,0	222,0	218,0	173,0	132,0	77,0
12	83,5	430,0	374,0	308,0	239,0	153,0
13	56,5	549,0	531,0	457,0	336,0	138,0
14	46,9	433,0	517,0	475,0	424,0	272,0
média	64,2	286,0	310,0	275,0	252,0	207,0
DP	14,3	161,0	155,0	142,0	139,0	215,0
EP	5,1	56,8	54,6	50,2	49,0	75,8

TABELA 47: Valores individuais e níveis médios de ACTH após a administração de GHRP-6 em pacientes com doença de Cushing após 6 meses de tratamento com cetoconazol

	ACTH (pg/mL)					
	Tempo (minutos)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
6	48,0	325,0	448,0	418,0	364,0	307,0
7	112,0	224,0	263,0	178,0	173,0	116,0
8	137,0	185,0	175,0	150,0	120,0	88,0
9	66,0	229,0	176,0	130,0	96,0	70,0
11	83,0	248,0	213,0	201,0	162,0	112,0
12	80,1	614,0	590,0	580,0	485,0	267,0
13	79,1	522,0	488,0	365,0	346,0	342,0
14	59,3	287,0	342,0	344,0	282,0	158,0
média	83,1	329,0	337,0	296,0	254,0	183,0
DP	28,9	155,0	157,0	158,0	138,0	107,0
EP	10,2	54,9	55,4	55,8	48,7	37,7

TABELA 48: Resposta do ACTH expressa em pico e AUC (área sob a curva) após administração de ghrelina e GHRP-6 em controles

Indiv.	**PICO (pg/mL)**		**AUC (pg/mL.90 min)**	
	ghrelina	**GHRP-6**	**ghrelina**	**GHRP-6**
1	66,1	49,4	2886	2021
2	67,1	27,3	4090	1795
3	32,4	10,8	1667	762
4	144,0	33,0	7035	2018
5	60,0	13,6	3128	873
6	50,9	26,9	2681	1562
8	21,9	21,0	1454	1453
9	21,4	20,0	1407	1449
10	30,3	24,0	1800	1378
11	82,6	28,9	2457	2054
12	112,0	87,1	4007	3820
média	62,6	31,1	3181	1690
DP	38,7	21,2	1716	719
EP	11,7	6,4	517	217

TABELA 49: Resposta do ACTH expressa em ∆ AUC após a administração de ghrelina e GHRP-6 em controles

Indiv.	∆ AUC (pg/mL.90 min - basal)	
	ghrelina	GHRP-6
1	2202	1400
2	1084	-167
3	1217	-174
4	4902	308
5	2273	270
6	1646	248
8	41	274
9	507	819
10	801	649
11	2183	-61,5
12	3182	1842
média	1822	492
DP	1368	644
EP	413,0	194

TABELA 50: Resposta do ACTH expressa em pico e AUC (área sob a curva) após a administração de ghrelina em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	PICO (pg/mL)			AUC (pg/mL.90 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	372,0	361,0	358,0	22595	24510	23783
7	281,0	762,0	539,0	19175	51990	35798
8	140,0	203,0	392,0	9689	12975	24128
9	126,0	278,0	578,0	8115	18150	29258
11	160,0	281,0	327,0	9605	17070	19590
12	269,0	250,0	561,0	14171	16213	33169
13	124,0	617,0	759,0	4947	32286	41759
14	706,0	321,0	558,0	33614	19841	31313
média	272,0	384,0	509,0	15239	24129	29850
DP	197,0	198,0	143,0	9469	12728	7226
EP	69,6	70,0	50,5	3348	4500	2555

TABELA 51: Resposta do ACTH expressa em pico e AUC (área sob a curva) após a administração de GHRP-6 em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	PICO (pg/mL)			AUC (pg/mL.90 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	606,0	714,0	448,0	37425	33293	31020
7	331,0	269,0	263,0	23130	18705	16448
8	79,0	145,0	185,0	6367	10500	12698
9	121,0	132,0	229,0	7451	8828	11730
11	75,7	222,0	248,0	4541	13770	15878
12	172,0	430,0	614,0	9629	24979	42278
13	206,0	549,0	522,0	8906	33109	34133
14	613,0	517,0	344,0	35977	35347	23755
média	276,0	372,0	357,0	16678	22316	23493
DP	222,0	212,0	155,0	13593	10833	11246
EP	78,4	75,0	54,8	4806	3830	3976

TABELA 52: Resposta do ACTH expressa em ∆ AUC após a administração de ghrelina e GHRP-6 em pacientes com doença de Cushing antes e após 3 e 6 meses de tratamento com cetoconazol

Indiv.	∆ AUC ghrelina (pg/mL.90 min)			∆ AUC GHRP-6 (pg/mL.90 min)		
	pré	3 meses	6 meses	pré	3 meses	6 meses
6	15791	20280	13973	29685	28793	26700
7	13082	42090	31478	16470	12315	6368
8	3146	4155	4328	1066	2850	368
9	3201	8790	22148	2537	3338	5790
11	6779	11940	11220	2327	8370	8408
12	10904	12010	27454	7469	17464	35069
13	1581	28884	35468	6062	28024	27014
14	30464	14945	25274	31756	31126	18418
média	10619	17887	21418	12172	16535	16017
DP	9517	12319	10703	12428	11600	12560
EP	3365	4355	3784	4394	4101	4441

TABELA 53: Valores individuais e níveis médios de glicose após a administração de ghrelina em controles

	glicose (mg/dL)				
	Tempo (minutos)				
Indiv.	**0**	**30**	**60**	**90**	**120**
1	85	99	89	90	92
2	81	86	83	75	85
3	84	91	94	90	93
4	81	87	81	83	83
5	82	86	85	89	92
6	102	105	103	102	100
8	73	77	75	75	75
9	84	90	89	86	86
10	81	90	85	90	87
11	92	97	96	101	95
12	79	90	79	81	92
média	84	91	87	87	89
DP	8	8	8	9	7
EP	2	2	2	3	2

TABELA 54: Valores individuais e níveis médios de glicose após a administração de ghrelina em pacientes com doença de Cushing antes do tratamento com cetoconazol

	glicose (mg/dL)				
	Tempo (minutos)				
Indiv.	**0**	**30**	**60**	**90**	**120**
6	87	97	97	102	86
7	177	177	175	174	172
8	88	99	100	92	93
9	118	117	124	132	135
11	133	131	143	119	148
12	148	153	168	172	175
13	90	95	97	100	99
14	86	89	93	112	106
média	116	120	125	125	127
DP	34	32	34	32	36
EP	12	11	12	11	13

TABELA 55: Valores individuais e níveis médios de glicose após a administração de ghrelina em pacientes com doença de Cushing 3 meses após o tratamento com cetoconazol

	glicose (mg/dL)				
	Tempo (minutos)				
Indiv.	**0**	**30**	**60**	**90**	**120**
6	85	82	83	82	86
7	114	125	123	120	122
8	99	111	108	98	99
9	118	111	115	113	114
11	97	100	101	101	89
12	131	139	137	141	146
13	93	97	98	95	100
14	72	83	77	74	79
média	101	106	105	103	104
DP	19	20	20	21	22
EP	7	7	7	8	8

TABELA 56: Valores individuais e níveis médios de glicose após a administração de ghrelina em pacientes com doença de Cushing 6 meses após o tratamento com cetoconazol

	glicose (mg/dL)				
	Tempo (minutos)				
Indiv.	**0**	**30**	**60**	**90**	**120**
6	77	86	91	78	87
7	99	101	99	107	100
8	93	124	120	107	107
9	108	106	106	111	111
11	90	93	96	95	97
12	127	137	139	138	142
13	73	73	73	67	75
14	87	97	92	94	92
média	94	102	102	100	101
DP	17	20	20	22	20
EP	6	7	7	8	7

TABELA 57: Níveis médios de glicose durante os testes de ghrelina, GHRP-6 e GHRH em pacientes com doença de Cushing antes do tratamento com cetoconazol

Indiv.	glicose (mg/dL)		
	ghrelina	GHRP-6	GHRH
6	94	87	82
7	175	85	185
8	94	90	92
9	125	171	123
11	135	137	137
12	163	158	130
13	96	92	93
14	97	82	93
média	122	113	117
DP	33	37	34
EP	12	13	12

TABELA 58: Níveis médios de glicose durante os testes de ghrelina, GHRP-6 e GHRH em pacientes com doença de Cushing após 3 meses de tratamento com cetoconazol

Indiv.	glicose (mg/dL)		
	ghrelina	GHRP-6	GHRH
6	84	102	74
7	121	122	114
8	103	93	96
9	114	100	102
11	98	93	91
12	139	129	131
13	97	84	87
14	77	85	71
média	104	101	96
DP	20	17	20
EP	7	6	7

TABELA 59: Níveis médios de glicose durante os testes de ghrelina, GHRP-6 e GHRH em pacientes com doença de Cushing após 6 meses de tratamento com cetoconazol

Indiv.	glicose (mg/dL)		
	ghrelina	GHRP-6	GHRH
6	84	81	83
7	101	97	96
8	110	99	96
9	108	105	112
11	94	95	90
12	137	127	124
13	72	77	68
14	92	82	83
média	100	95	94
DP	20	16	18
EP	7	6	6

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