SERGIO DE OLIVA NASCIF

EFEITOS DA GHRELINA, GHRP-6 E GHRH SOBRE A SECREÇÃO DE GH, ACTH E CORTISOL NO HIPERTIREOIDISMO

Tese apresentada à Universidade Federal de São Paulo – Escola Paulista de Medicina para a obtenção do título de Doutor em Ciências

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Orientadora:

Prof. Dra. Ana Maria Judith Lengyel

Coordenador:

Prof. Dr. Sergio Atala Dib

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ÍNDICE

INTRODUÇÃO

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

Na década de 70, antes da descoberta do GHRH, Bowers e col. identificaram pequenos peptídeos sintéticos a partir da molécula de met-encefalina que eram capazes de liberar GH. Estudos posteriores baseados em cálculos de energia conformacional, modificações químicas e testes de atividade biológica resultaram no desenvolvimento de peptídeos mais potentes, incluindo um hexapeptídeo denominado *growth hormone-releasing peptide-6* (GHRP-6) (Bowers *et al.*, 1984). Este peptídeo promove a liberação de GH, tanto *in vitro* como *in vivo*, em todas as espécies de animais testadas e este efeito é obtido por diferentes vias de administração, incluindo endovenosa e mesmo por via oral (Ghigo *et al.*, 1997; Isidro & Cordido, 2006). 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), uma vez que exercem sua atividade por mecanismo diferente daquele utilizado pelo GHRH (Korbonits & Grossman, 1995). A presença de receptores específicos para os GHS, tanto no hipotálamo quanto na hipófise, sugeria a possível existência de

um peptídeo endógeno semelhante ainda não 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). Finalmente, em 1999, Kojima e col. clonaram o ligante endógeno dos GHS, que foi isolado a partir de extrato de 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 (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). Este peptídeo é o mais potente estímulo para a secreção de GH em humanos, promovendo uma maior liberação de GH quando comparado à hexarelina (um GHS) e ao 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 receptores de GHS (GHS-R) em culturas 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 destes

peptídeos são reduzidos ou abolidos 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 & Bowers, 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 & Bowers, 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 ocorre através da ativação do sistema da proteína quinase C, com elevação de diacilglicerol e inositol trifosfato, acarretando aumento do cálcio intracelular (Howard *et al.*, 1996; Chen *et al.*, 1996), ao passo que o GHRH estimula o sistema da proteína quinase A após ativação do AMPc intracelular. (Goth *et al.*, 1992). Mais recentemente, foi demonstrado em somatotrofos de suínos (Malagón *et al.*, 2003) e de babuínos (Kineman & Luque, 2007), que a ghrelina também é capaz de ativar o AMPc e estimular os sistemas de influxo de cálcio intracelular, ação esta mais ampla que a dos GHS sintéticos, o que poderia explicar sua maior potência. A descoberta da ghrelina comprovou a existência de uma terceira via de regulação de secreção de GH. Entretanto, o papel desta via na fisiologia e fisiopatologia da secreção de GH ainda não é conhecido (Lengyel, 2006).

Os hormônios tireoidianos participam da síntese e secreção de GH. Apesar do local preciso e mecanismo de ação ainda serem desconhecidos, há evidências que estes hormônios atuam tanto no hipotálamo quanto na hipófise

(Dieguez *et al.*, 1985; Jones *et al.*, 1990; Valcavi *et al.*, 1992; Giustina & Wehrenberg, 1995).

Distúrbios da função tireoidiana cursam com alterações na secreção de GH (Valcavi *et al.*, 1992). No hipertireoidismo, a responsividade do GH à diversos estímulos farmacológicos, incluindo o GHRH, está diminuída (Burgess *et al.*, 1966; Giustina *et al.*, 1991; Valcavi *et al.*, 1993; Ramos-Dias *et al.*, 1995). Os mecanismos responsáveis por estas alterações não estão claros. Um aumento no tônus hipotalâmico de somatostatina é improvável pois foi demonstrado que compostos que inibem a liberação de somatostatina são incapazes de normalizar a resposta do GH aos estímulos farmacológicos (Yeung, 1973; Valcavi *et al.*, 1991; Ramos-Dias *et al.*, 1995). Embora controverso (Iranmanesh *et al.*, 1991), a diminuição da secreção de GHRH hipotalâmico poderia estar envolvida (Jones *et al.*, 1990; Kamegai *et al.*, 2004), ou poderia haver um efeito direto do excesso de hormônios tireoidianos sobre os somatotrofos (Dieguez *et al.*, 1985; Jones *et al.*, 1990; Valcavi *et al.*, 1992; Giustina & Wehrenberg, 1995).

A ghrelina e os GHS também são capazes de estimular o eixo hipotálamo-hipófise-adrenal em indivíduos normais, sendo que a ghrelina tem um efeito mais potente (Takaya *et al.*, 2000; Arvat *et al.*, 2001). A ação destes peptídeos sobre a liberação de ACTH e cortisol é exclusivamente hipotalâmica, já que não promovem a secreção de ACTH em fragmentos de hipófise *in vitro* (Elias *et al.*, 1995; Kojima *et al.*, 1999), e os GHS-R não foram encontrados em corticotrofos normais (Smith *et al.*, 1997). Além disso, na desconexão hipotálamo-hipofisária o efeito da ghrelina e dos GHS sobre o ACTH e cortisol é abolido (Popovic *et al.*, 1995; Popovic *et al.*, 2003). Foi demonstrado que o

GHRP-6 estimula a liberação de arginina-vasopressina (AVP) em fragmentos hipotalâmicos *in vitro* (Korbonits *et al.*, 1999b), ao passo que a ghrelina tem uma ação mais ampla, promovendo 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).

Estudos prévios sugerem que os hormônios tireoidianos podem participar da regulação do eixo hipotálamo-hipófise adrenal por mecanismos que ainda não estão completamente elucidados. Classicamente, sabe-se que na tireotoxicose há aumento compensatório da taxa de produção de cortisol secundário a uma maior degradação (Levin & Daughaday, 1955; Peterson, 1958; Gordon & Southren, 1977). Dessa forma, no hipertireoidismo prolongado, as adrenais estão estimuladas cronicamente e, dependendo da severidade clínica e da duração da doença, a resposta de cortisol após diferentes estímulos como ACTH, CRH e teste de tolerância à insulina pode estar diminuída (Jackson *et al.*, 1966; Goswami & Kochupillai, 2001; Tsatsoulis *et al.*, 2000; Yamakita *et al.*, 2001). Entretanto, resposta normal de cortisol após indução de hipoglicemia também tem sido observada (Jackson *et al.*, 1966; Giustina *et al.*, 1971; Brauman *et al.*, 1973; Moghetti *et al.*, 1994). O comprometimento da reserva adrenocortical durante o hipertireoidismo, e sua normalização após obtenção do eutireoidismo, pode estar relacionada à diminuição dos níveis da globulina ligadora de corticoesteróides (CBG) nos indivíduos não-tratados, uma vez que o tratamento do hipertireoidismo cursa com aumento nos níveis de CBG (Dumoulin *et al.*, 1995; Mishra *et al.*, 2007).

Entretanto, a elevação da concentração de corticosterona no líquor de ratos tireotóxicos e também da razão cortisol/CBG no hipertireoidismo (Kamilaris *et al.*, 1991; Johnson *et al.*, 2005; Mishra *et al.*, 2007) sugere que um aumento de cortisol circulante está realmente presente por excesso de secreção de cortisol na vigência de elevação dos hormônios tireoidianos. Essa hipótese é reforçada pelos poucos estudos que mostram aumento dos níveis de ACTH basal (Moghetti *et al.*, 1994; Yamakita *et al.*, 2001; Mishra *et al.*, 2007) e após estímulo com CRH e hipoglicemia em indivíduos portadores de hipertireoidismo (Moghetti *et al.*, 1994; Lizcano & Salvador, 2008).

A administração de ghrelina também acarreta aumento dos níveis de glicose plasmática em indivíduos normais (Broglio *et al.*, 2001), provavelmente por estimular a liberação de glicose hepática (Gauna *et al.*, 2005). Esta ação é independente do GH, uma vez que está presente mesmo em pacientes com deficiência de GH (Gauna *et al.*, 2004). Sabe-se que o hipertireoidismo pode cursar com resistência à insulina e hiperinsulinemia (O'Meara *et al.*, 1993; Dimitriadis *et al.*, 2008) mas não há relatos sobre os efeitos da ghrelina/GHS nos níveis circulantes de glicose em pacientes com excesso de hormônios tireoidianos.

OBJETIVOS

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(1) Avaliar os efeitos da ghrelina e do GHRP-6 sobre a liberação GH e glicose em pacientes com hipertireoidismo. Os níveis de GH após a administração de GHRH também foram estudados.

(2) Avaliar os efeitos da ghrelina e do GHRP-6 sobre a liberação de ACTH e cortisol em pacientes tireotóxicos.

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DECREASED GHRELIN-INDUCED GH RELEASE IN THYROTOXICOSIS: COMPARISON WITH GH-RELEASING PEPTIDE-6 (GHRP-6) AND GHRH

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Decreased ghrelin-induced GH release in thyrotoxicosis: comparison with GH-releasing peptide-6 (GHRP-6) and GHRH

Sergio Oliva Nascif · Silvia Regina Correa-Silva ·
Marcos Roberto Silva · Ana-Maria Judith Lengyel

Abstract In thyrotoxicosis GH response to several stimuli is impaired, but there is no data on ghrelin-induced GH release in these patients. Ghrelin is a potent GH secretagogue and it also increases glucose levels in men. The aim of this study was to evaluate the effects of ghrelin (1 µg/kg), GHRP-6 (1 µg/kg) and GHRH (100 µg), i.v., on GH levels in 10 hyperthyroid patients and in 8 controls. Glucose levels were also measured during ghrelin and GHRP-6 administration. In control subjects and hyperthyroid patients peak GH (µg/l; mean ± SE) values after ghrelin injection (controls: 66.7 ± 13.6; hyper: 19.3 ± 2.4) were significantly higher than those obtained after GHRP-6 (controls: 26.7 ± 5.1; hyper: 12.6 ± 1.3) and GHRH (controls: 13.5 ± 4.3; hyper: 5.3 ± 1.3). There was a significant decrease in GH responsiveness to ghrelin, GHRP-6 and GHRH in the hyperthyroid group compared to controls. In control subjects and hyperthyroid patients basal glucose (mmol/l) values were 4.5 ± 0.1 and 4.7 ± 0.2, respectively. There was a significant increase in glucose levels 30 min after ghrelin injection (controls: 4.9 ± 0.1; hyper: 5.2 ± 0.2), which remained elevated up to 120 min. When the two groups were compared no differences in glucose values were observed. GHRP-6 administration was not able to increase glucose levels in both groups. Our data shows that GH release after ghrelin, GHRP-6 and GHRH administration is decreased in thyrotoxicosis. This suggests that thyroid hormone excess interferes with GH-releasing pathways activated by these peptides. Our results also suggest that ghrelin's ability to increase glucose levels is not altered in thyrotoxicosis.

Keywords Ghrelin · Thyrotoxicosis · GH · GHRP-6 · GHRH

Introduction

Pulsatile GH secretion is modulated by an interplay between hypothalamic GHRH and somatostatin. Several studies have proposed that GH secretagogues (GHS) might also have a role in this process, acting at both pituitary and hypothalamic receptors [1–3]. Ghrelin, the endogenous ligand of GHS-receptor (GHS-R), has been recently discovered in the stomach, but is also present in the hypothalamic arcuate nucleus [4–6]. Interestingly, the chemical structure of this acylated peptide is different from GHS [4]. The post-translational fatty acid chain modification (n-octanoyl residue) is essential for its biological effects on GH release and also on the increase in circulating glucose levels [4, 6]. Non-acylated ghrelin, which is the main circulating form, might have non-endocrine actions [4]. Ghrelin can cross the blood–brain barrier [7] and is able to release GH in a potent manner after i.v. injection both in animals and in men [8–10]. The main site of action of this peptide is at hypothalamic level as its GH-releasing property is greatly decreased in hypothalamic–pituitary disconnection, similarly to what is observed with GHS [11, 12]. However, ghrelin and GHS also act at pituitary level, through different receptors and intracellular mechanisms than those of

S. O. Nascif (✉) · S. R. Correa-Silva ·
M. R. Silva · A.-M. J. Lengyel
Division of Endocrinology, Universidade Federal de São Paulo, UNIFESP/EPM, Rua Pedro de Toledo, 910, 04039-002 São Paulo, SP, Brazil
e-mail: snascif@uol.com.br

GHRH [4, 13, 14]. It has been proposed that GHS activate hypothalamic GHRH release, amplify GHRH effects at the somatotroph and also act as functional somatostatin antagonists [15, 16]. Ghrelin is also able to increase circulating glucose levels in humans, by a direct hepatic action [17, 18]. It has been recently shown that non-acylated ghrelin counteracts the increase in glucose levels induced by the acylated peptide [19].

Thyroid hormones participate in GH synthesis and secretion. Their site and mechanism of action are still unknown, but theses hormones could act at both pituitary and hypothalamic level [20–23]. Impaired GH release has been found in patients with thyroid dysfunctions [22]. In hyperthyroidism a blunted GH response to several pharmacological stimuli, such as GHRH, has been demonstrated [24–27]. We have previously shown that GH response to GHRP-6 is normal in thyrotoxicosis while GH responsiveness to GHRH is decreased [28]. The mechanisms involved in these alterations are unclear. An increase in hypothalamic somatostatin is unlikely, as compounds that inhibit somatostatin release are unable to normalise the blunted GH response to pharmacological stimuli in thyrotoxicosis [27, 29, 30]. Although controversial [31], a decrease in hypothalamic GHRH secretion could be involved [21, 32] and/or a direct effect of thyroid hormones at the somatotroph [20–23]. The effect of thyroid hormone excess on hypothalamic ghrelin has not been studied, but a decrease in gastric [33] and pituitary ghrelin mRNA levels has been reported in thyrotoxic rats [32]. It has also been shown that circulating ghrelin levels are decreased in hyperthyroidism, both in rats and in men [33–35]. There is no data in the literature about the effects of ghrelin on GH release in hyperthyroidism both in men and in animals. As ghrelin and GHS have different structures and several receptors for these peptides are likely to exist [4, 5, 36], the aim of this work was to evaluate the GH and glucose-releasing effects of ghrelin in thyrotoxicosis, in comparison with GHRP-6. GHRH-induced GH release was also studied, as this peptide releases GH by different pathways than those of GHS.

Subjects and methods

Subjects

We studied 10 patients (eight women and two men) with hyperthyroidism due to Graves' disease. Their mean age was 33 years (range, 26–44), with a mean body mass index (BMI) of 22.8 kg/m^2 (range, 20.3–26.5). All patients had diffuse goiter and/or thyroid ophthalmopathy and had clinical symptoms and signs of thyrotoxicosis. Three subjects had been previously treated with antithyroid drugs alone or associated with beta-adrenoreceptor blockers and one of them had been previously submitted to radioiodine therapy. The diagnosis of hyperthyroidism was confirmed by high levels of free T4 (95.2 ± 15.4 pmol/l; normal range 10.3–21.9), total T3 (9.0 ± 1.2 nmol/l; normal range 0.9–2.8), and by suppressed levels of TSH (0.02 ± 0.01 mU/l; normal range 0.5–5.5). TSH receptor antibody (TRAb) was positive in seven patients, varying from 11 to 300 U/l (normal range <11). None of the patients had other associated diseases and was taking any medication for at least 2 months before the study.

Eight subjects (three women and five men) with no history of thyroid disease were also studied as a control group. Their mean age was 30 years (range, 20–36) and their mean BMI was 23.2 kg/m^2 (19.5–25.7). All subjects had normal thyroid function and were free of any medication at the time of the study. 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 were studied after giving informed consent. The tests were performed after an overnight fast and the subjects remained recumbent throughout it. Each subject underwent three tests, randomly, with an interval of at least 48 h between them. Forty-five minutes before starting the test, an indwelling catheter was inserted into an antecubital vein and kept patent by slow 0.9% saline infusion. After the first blood sample each subject received acylated ghrelin (Neosystem, Strasbourg, France) at a dose of 1 µg/kg, GHRP-6 (Bachem, San Carlos, USA) at a dose of 1 µg/kg, or GHRH (Clinalfa, Läufelfingen, Switzerland) at a dose of 100 µg, i.v., in bolus. Blood samples were collected every 15 min until 120 min for subsequent GH determination. Glucose levels were measured every 30 min. Baseline blood samples were also obtained for free T4, total T3 and TSH. Control subjects were also submitted to the tests using the same procedure as above.

Assays

Serum GH was measured in duplicate by immunofluorometric assay (Wallac Oy, Turku, Finland). The sensitivity of the method is 0.01 µg/l, with mean

intra- and interassay coefficients of variation of 6.7 and 7%, respectively. Free T4, total T3 and TSH were determined by immunochemiluminometric assays (Advia Centaur, Bayer, USA). TRAb was measured by a radioimmunoassay (RSR Limited, Cardiff, United Kingdom). Plasma glucose levels were determined by the hexokinase method (Advia 1650, Bayer, USA).

Statistical analysis

Friedman's analysis of variance was performed to compare GH and glucose levels after the injection of each peptide and to compare GH responses in the same group. The Mann–Whitney rank sum test was performed for comparisons between thyrotoxic patients and control subjects. The responses were also analysed by the area under the curve (AUC), which was calculated by trapezoidal integration. Undetectable GH levels were considered to be equal to 0.01 µg/l for statistical purposes. Results are shown as mean ± SE. $P < 0.05$ was considered statistically significant.

Results

In controls subjects peak GH (µg/l) and AUC (µg/l 120 min) values after ghrelin administration (66.7 ± 13.6; 3,773 ± 878) were significantly higher than those obtained after GHRP-6 (26.7 ± 5.1; 1,454 ± 333) and GHRH (13.5 ± 4.3; 1,038 ± 346) (Figs. 1 and 2). No significant differences were seen in GH responses to GHRP-6 and GHRH, despite the higher magnitude of GH levels after GHRP-6.

In thyrotoxicosis peak GH values after ghrelin injection (19.3 ± 2.4) were significantly higher than after GHRP-6 (12.6 ± 1.3) and GHRH (5.3 ± 1.3). In terms of AUC values, ghrelin (873 ± 98) and GHRP-6-induced (652 ± 78) GH release did not reach statistical significance, while both were higher than that of GHRH (467 ± 84).

When thyrotoxic patients were compared to controls, there was a significant decrease in GH responsiveness to ghrelin, GHRP-6 and GHRH in the hyperthyroid group, both in terms of peak GH and AUC values, except for GHRH AUC, which did not reach statistical significance despite lower levels, due to the variability of responses.

In control subjects and hyperthyroid patients basal glucose (mmol/l) values were 4.5 ± 0.1 and 4.7 ± 0.2, respectively (Fig. 3). There was a significant increase in glucose levels 30 min after ghrelin injection (controls: 4.9 ± 0.1; hyper: 5.2 ± 0.2), which remained elevated up to 120 min. The absolute increment was 0.4 mmol/l in controls and 0.5 mmol/l in thyrotoxic patients, while the AUC (mmol/l 120 min) was 10,298 ± 223 and 10,727 ± 322, respectively. When the two groups were compared, no differences in glucose values before or after ghrelin administration were observed. GHRP-6 administration was not able to increase glucose levels in both groups.

No significant differences in age and BMI were observed in the two study groups.

Side effects

Hunger sensation was reported in five thyrotoxic patients and in five controls after ghrelin administration. Nausea was seen after GHRP-6 in four patients and in one control. Most subjects experienced transient facial flushing after GHRH injection.

Fig. 1 Mean GH values after ghrelin (1 µg/kg), GHRP-6 (1 µg/kg) and GHRH (100 µg) administration in 8 control subjects and in 10 hyperthyroid patients (mean ± SE)
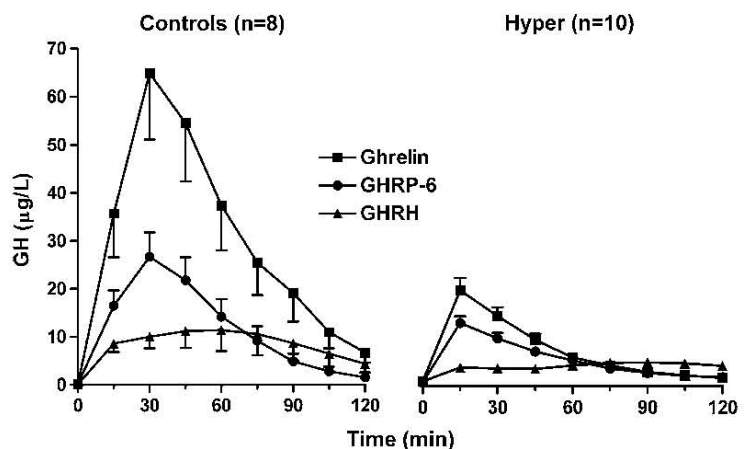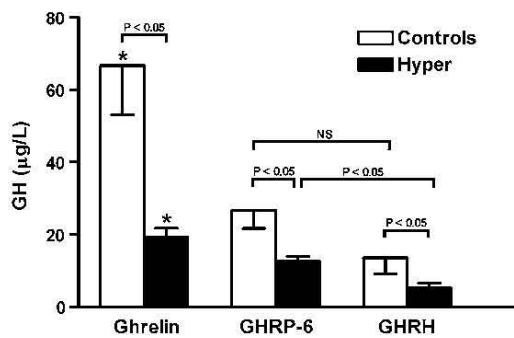
Fig. 2 Mean peak GH levels after ghrelin (1 μg/kg), GHRP-6 (1 μg/kg) and GHRH (100 μg) administration in 8 control subjects and in 10 hyperthyroid patients (mean ± SE; *$P < 0.05$ compared to GHRP-6 and GHRH in each group; NS, not significant)
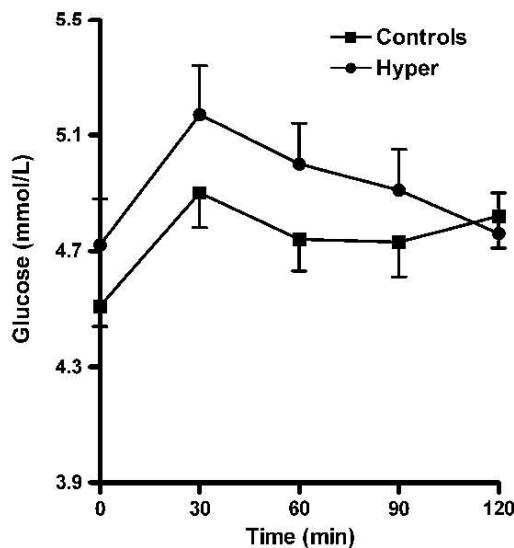
Fig. 3 Mean glucose values after ghrelin (1 μg/kg) administration in 8 control subjects and in 10 hyperthyroid patients (mean ± SE)

Discussion

In our study GH responsiveness to ghrelin in normal subjects was higher than seen with GHRP-6 and GHRH, confirming a previous report with another GHS, hexarelin [10].

In patients with hyperthyroidism peak GH values after ghrelin were also higher than with GHRP-6 and GHRH, which could be due to the greater potency of ghrelin in relation to the other peptides [10]. It is also possible that thyroid hormone excess has less impact on ghrelin-modulated pathways of GH release.

When the two groups were compared, a significant decrease in GH response to ghrelin was observed in

thyrotoxic patients. Moreover, in hyperthyroidism peak GH values after GHRH were also blunted, confirming earlier studies [25–27]. However, surprisingly, we also found a decrease in GH response to GHRP-6, which is in contrast to our previous report showing normal GH response to GHRP-6 associated with blunted GH responsiveness to GHRH in thyrotoxicosis [28]. The reasons for these divergent findings are not clear. When we compared these two studies, despite a trend to higher T3 and free T4 levels in our actual work, no significant differences in the degree of thyrotoxicosis were observed. However, in the present report hyperthyroid patients were somewhat older and with slightly higher BMI, which eventually could have contributed to the observed decrease in GH responsiveness to GHRP-6 [37, 38]. It has been previously suggested that alterations in hypothalamic–pituitary adrenal function in thyrotoxicosis become more pronounced as the duration of thyroid dysfunction increases [39]. Whether the GH axis is also progressively inhibited during long-term exposure to thyroid hormone excess is still unknown. In our data there were no apparent differences in the duration of thyrotoxicosis in the two studies (data not shown). Another possibility would be related to the different GH assays employed in these reports. In the present work GH was determined by a specific 22 kDa isoform assay, while in our former study an assay which measures both 22 kDa and 20 kDa isoforms was used. Although no changes in GH molecular forms have been reported in baseline blood samples from hyperthyroid patients [40] it is not known whether the proportion of each isoform is maintained after simulation with GHRP-6. Interestingly, Marino et al. [41] have previously shown that the use of assays which are non-specific for the 22 kDa isoform could overestimate serum GH levels in some circumstances.

Our data show that thyroid hormone excess interferes with ghrelin-activated pathways of GH release, which is a novel finding. However, the mechanisms involved in the decreased GH response to ghrelin in thyrotoxicosis are still unknown. A decrease in circulating ghrelin levels has been described in hyperthyroid patients and could eventually be implicated [34, 35]. However, patients with gastrectomy, with low ghrelin levels, have an enhanced sensitivity to ghrelin-induced GH release [42]. Ghrelin and GHS modulate GH secretion acting at both hypothalamic and pituitary level [2, 4, 11, 12]. In patients with hypothalamic–pituitary disconnection there is a major decrease of GH release after the administration of these compounds, indicating that their main site of action is the hypothalamus [11, 12]. GHS and ghrelin activate

GHS-R, which are present in GHRH neurons in the arcuate nucleus [16]. Moreover, for their full effect to occur an intact GHRH system is necessary [16, 43]. Thyroid hormones could interfere with hypothalamic mechanisms and/or with the different receptors and intracellular pathways activated by GHRH and GHS/ghrelin at the somatotroph. GHRH and GHS/ghrelin bind to specific pituitary receptors, but there is evidence of cross-talk between them [44]. GHRH stimulates cyclic AMP and protein-kinase A pathways [13], while ghrelin/GHS activate protein-kinase C transduction systems, via inositol triphosphate [2, 14]. In porcine somatotrophs ghrelin stimulates several interdependent pathways of GH release [45]. Interestingly, it has been demonstrated that glucocorticoids downregulate human GHS-R [46], which could eventually interfere with both ghrelin/GHS stimulated pathways at the somatotroph and also with GHS-R located in hypothalamic GHRH neurons [16]. This could be a hypothetical mechanism to explain the decreased GH response to GHS and ghrelin in patients with Cushing's disease [47, 48]. However, differently than glucocorticoids, thyroid hormones either increase [46] or do not alter human GHS-R [49]. Therefore, it is unlikely that this mechanism is involved in the blunted GH responsiveness to ghrelin/GHS in thyrotoxicosis. We have previously shown that a decrease of circulating T3 levels, with iopanoic acid administration for 15 days, is able to increase, although not normalise, GH responsiveness to GHRH in patients with thyrotoxicosis, indicating that T3 might interfere with GH-releasing pathways [50]. Furthermore, it has been previously suggested that there is an impairment of hypothalamic GHRH production in thyroid hormone excess [21]. In hyperthyroid rats a decrease in hypothalamic GHRH mRNA levels has been demonstrated [21, 32]. If also true for humans, this could eventually explain our findings as chronic GHRH deficiency decreases GH release after the acute administration of ghrelin/GHS and also of GHRH [11, 12, 51]. However, further studies are necessary to substantiate this hypothesis.

It has been recently demonstrated that the increase in glucose values after acylated ghrelin administration is independent of GH, as it is preserved in GH-deficient patients [52]. Moreover, acylated ghrelin enhances glucose release from porcine hepatocytes in culture, while the unacylated peptide has the opposite effect [18]. In our study ghrelin injection induced a significant rise in circulating glucose values in hyperthyroid patients, which was similar to that observed in control subjects. GHRP-6 administration did not alter glucose levels in both groups, confirming earlier studies [17, 53].

Therefore, our data suggest that thyroid hormone excess does not impair ghrelin's ability to increase glucose levels from hepatocytes.

In summary, in patients with thyrotoxicosis there is a decrease in ghrelin-induced GH release, which is a novel finding. Peak GH levels after ghrelin are higher than those observed with GHRP-6 and GHRH, which could be due to the greater potency of this new peptide. Our results suggest that thyroid hormone excess interferes with hypothalamic and pituitary mechanisms of GH release activated by both ghrelin/GHS and GHRH. Further studies are necessary, however, to elucidate the mechanisms involved in these alterations. Our data also suggest that the ability of ghrelin to increase glucose levels is not impaired in thyrotoxicosis.

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GHRELIN AND GHRP-6-INDUCED ACTH AND CORTISOL RELEASE IN THYROTOXICOSIS

Estudo 2

Ghrelin and GHRP-6-induced ACTH and cortisol release in thyrotoxicosis

Sergio O. Nascif, Patrícia Molica, Silvia R. Correa-Silva, Marcos R. Silva, Ana-Maria J. Lengyel

Division of Endocrinology, Universidade Federal de São Paulo, UNIFESP/EPM, São Paulo, Brazil

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Corresponding author:

Sergio de Oliva Nascif

Rua Pedro de Toledo, 910

04039-002 – São Paulo/SP – Brazil

Phone/fax: 55-11-55748432

E-mail: snascif@uol.com.br

Abstract

Thyroid hormones have a role in the regulation of hypothalamic-pituitary-adrenal (HPA) axis, although the mechanisms are still not clear. Ghrelin is a GH-secretagogue and it also increases ACTH and cortisol levels, similarly to GHRP-6, after intravenous administration in humans. The aim of this study was to evaluate the effects of ghrelin and GHRP-6 on ACTH and cortisol levels in 20 hyperthyroid patients due to Graves' disease (mean age: 31 yr; BMI: 23.0 kg/m^2) and in 9 controls (mean age: 29 yr; BMI: 24.1 kg/m^2). Mean basal cortisol (μg/dL) levels were significantly higher in hyperthyroid patients compared to normal subjects (10.7$\pm$0.7 *vs.* 8.1$\pm$0.7). In controls mean AUC cortisol (μg/dL.90min) values after ghrelin injection (1225$\pm$120) were higher than after GHRP-6 (957$\pm$75) and there was a trend to higher peak values after ghrelin administration (16.4$\pm$1.6 *vs.* 13.5$\pm$0.9; *P*=0.055). In patients with hyperthyroidism ghrelin induced a higher cortisol response (peak: 19.1$\pm$0.9; AUC: 1415$\pm$78) than seen after GHRP-6 (15.8$\pm$0.9; 1114$\pm$69). There were no significant differences of cortisol responses to both peptides in terms of peak and AUC between thyrotoxic patients and controls. Similar Δ AUC cortisol values were observed in thyrotoxic patients (ghrelin: 484$\pm$80; GHRP-6: 115$\pm$63) and controls (524$\pm$107; 192$\pm$73). Mean basal ACTH (pg/mL) levels were increased in thyrotoxic patients compared to normal subjects (21.5$\pm$2.9 *vs.*13.5$\pm$1.8). In controls mean peak ACTH and AUC (pg/mL.90min) values after ghrelin administration were 54.9$\pm$10.3 and 2808$\pm$426, respectively. This response was significantly higher than that obtained after GHRP-6 (31.3$\pm$7.9;

1668±265), both in terms of peak ACTH and AUC levels. In thyrotoxicosis mean peak ACTH and AUC values after ghrelin injection (149.7±39.8; 6209±1556) were also significantly higher than after GHRP-6 injection (53.9±11.2; 2767±487). Ghrelin-induced peak ACTH release was increased in hyperthyroid patients when compared to controls and there was a trend in terms of AUC levels (P=0.063). Peak ACTH and AUC values after GHRP-6 in thyrotoxic patients did not reach statistical significance compared to normal subjects. When the Δ AUC values were analyzed, there was a significant increase in ACTH levels after ghrelin in the thyrotoxic group (patients: 4189±1202; controls: 1499±338). After GHRP-6 administration Δ ACTH values in thyrotoxic patients did not reach statistical significance compared to control subjects (patients: 927±330; controls: 539±237). In summary, our results show that cortisol responsiveness to ghrelin and GHRP-6 is normal in thyrotoxicosis. ACTH release after ghrelin is increased, but it does not reach statistical significance with GHRP-6. Our results suggest that the pathways of ACTH release mediated by ghrelin might be activated by thyroid hormone excess, but adrenocortical reserve is maintained.

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is regulated mainly by corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which stimulate the release of ACTH and cortisol. However, several neurotransmitters and peptides could have an additional role in the modulation of CRH, AVP and ACTH secretion (Melmed & Kleinberg, 2008).

Thyroid hormones may have a role in the regulation of HPA axis by mechanisms that are not fully elucidated. There is an increase in the production rate of cortisol in thyrotoxicosis because its degradation is accelerated (Levin & Daughaday, 1955; Peterson, 1958; Gordon & Southren, 1977). Thus, in prolonged hyperthyroidism the adrenals secrete at their maximal rate and, depending on the severity/duration of the disease, cortisol responsiveness to different stimuli such as hypoglycemia, ACTH and CRH might be decreased (Jackson *et al.*, 1966; Tsatsoulis *et al.*, 2000; Goswami & Kochupillai, 2001; Yamakita *et al.*, 2001). However, normal cortisol responses to hypoglycemia have also been observed (Jackson *et al.*, 1966; Giustina *et al.*, 1971; Brauman *et al.*, 1973; Moghetti *et al.*, 1994). The impairment of adrenocortical reserve in hyperthyroidism and its normalization after treatment could be due to a decrease in cortisol-binding globulin (CBG) levels, as this globulin is reduced in thyrotoxicosis and returns to normal in the euthyroid state (Dumoulin *et al.*, 1995; Mishra *et al.*, 2007). However, corticosterone in the cerebrospinal fluid and free cortisol index (cortisol/CBG), which reflect free cortisol, are increased in hyperthyroidism (Kamilaris *et al.*, 1991; Johnson *et al.*, 2005; Mishra *et al.*,

2007), suggesting that high circulating cortisol levels are really present and are due to enhanced secretion in this setting.

There are few data in the literature about basal and stimulated ACTH levels in hyperthyroidism. Experimental studies have suggested that thyrotoxicosis is associated with hyperactivity of the HPA axis (Kamilaris *et al.*, 1991; Johnson *et al.*, 2005) and higher basal ACTH levels have been observed in hyperthyroid subjects (Moghetti *et al.*, 1994; Yamakita *et al.*, 2001; Mishra *et al.*, 2007). It has also been shown that these patients have normal or higher ACTH responsiveness to hypoglycemia or CRH stimulation test (Moghetti *et al.*, 1994; Yamakita *et al.*, 2001; Lizcano & Salvador, 2008).

Ghrelin, the endogenous ligand of growth hormone secretagogue (GHS) receptor (GHS-R), was discovered in the stomach, but is also present in the hypothalamus, mainly in the arcuate nucleus (Kojima *et al.*, 1999; Gnanapavan *et al.*, 2002; van der Lely *et al.*, 2004). The active form of this peptide is acylated and its chemical structure is different from GHS (Kojima *et al.*, 1999). Ghrelin and GH-releasing peptide-6 (GHRP-6), a GHS, induce GH, ACTH and cortisol release (Arvat *et al.*, 2001; Correa-Silva *et al.*, 2006). Their main site of action is the hypothalamus, as in hypothalamic-pituitary disconnection these effects are reduced or abolished (Popovic *et al.*, 1995; Popovic *et al.*, 2003). A direct adrenal action of these peptides on cortisol release has been suggested as GHS-R mRNA expression was found in this tissue (Gnanapavan *et al.*, 2002), although in much lower concentrations than in the hypothalamic nuclei (Bennett *et al.*, 1997; Guan *et al.*, 1997; Mozid *et al.*, 2003; Korbonits *et al.*, 2004). However, this hypothesis seems unlikely as the effects of ghrelin/GHS on cortisol release are abolished in hypothalamic-pituitary disconnection.

There are no data in the literature about the effect of thyroid hormone excess on ACTH and cortisol release after the administration of ghrelin and GHRP-6. Therefore, the aim of our study was to evaluate ACTH and cortisol responses to ghrelin and GHRP-6 in thyrotoxic patients due to Graves´ disease.

Subjects and methods

Subjects

We studied 20 patients (sixteen women and four men) with hyperthyroidism due to Graves' disease. Their mean age was 31 yr (range: 17 – 44), with a mean body mass index (BMI) of 23.0 kg/m^2 (20.3 – 26.5). All patients had diffuse goiter and/or thyroid ophthalmopathy and had clinical symptoms and signs of thyrotoxicosis. Seven subjects had been previously treated with antithyroid drugs alone or associated with beta-adrenoreceptor blockers and one of them had been previously submitted to radioiodine therapy. The diagnosis of hyperthyroidism was confirmed by high levels of free T4 (7.7$\pm$0.8 ng/mL; mean$\pm$SE; normal range 0.89–1.76), total T3 (644$\pm$50 ng/dL; normal range 60–180), and by suppressed levels of TSH (0.01$\pm$0.01 mU/L; normal range 0.5–5.5). TSH receptor antibody (TRAb) was positive in sixteen patients, varying from 11 to 567 U/L (normal range < 11). None of the patients had other associated diseases and was taking any medication for at least 2 months before the study.

Nine subjects (three women and six men) with no history of thyroid disease were also studied as a control group. Their mean age was 29 yr (range: 20 – 35) and their mean BMI was 24.1 kg/m^2 (20.9 – 25.9). All subjects had normal thyroid function and were free of any medication at the time of the study. 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 were studied after giving informed consent. The tests were performed after an overnight fast and the subjects remained recumbent throughout it. Each subject underwent two tests, randomly, with an interval of at least 48 h between them. Forty-five minutes before starting the test, an indwelling catheter was inserted into an antecubital vein and kept patent by slow 0.9% saline infusion. After the first blood sample each subject received acylated ghrelin (Neosystem, Strasbourg, France) at a dose of 1 μg/kg or GHRP-6 (Bachem, San Carlos, USA) at a dose of 1 μg/kg, i.v., in bolus. Blood samples were collected every 15 minutes until 90 minutes for subsequent ACTH and cortisol determination. Baseline blood samples were also obtained for free T4, total T3 and TSH. Control subjects were also submitted to the tests using the same procedure as above.

Assays

Serum ACTH was measured by an immunochemiluminometric assay (DPC, Los Angeles, USA). The sensitivity of the method is 5 pg/mL, with mean inter- and intra-assay coefficients of variation of 3.6% and 2.8%, respectively. Serum cortisol levels were measured in duplicate by a fluoroimmunoassay (Wallac Oy, Turku, Finland), with sensitivity of 0.2 μg/dL, and mean inter- and intra-assay coefficients of variation of 8.2% and 6.2%, respectively. Free T4, total T3 and TSH were determined by immunochemiluminometric assays (Advia Centaur, Bayer, USA). TRAb was measured by a radioimmunoassay (RSR Limited, Cardiff, United Kingdom).

Statistical analysis

Friedman's analysis of variance was performed to compare ACTH and cortisol levels after the injection of each peptide. Wilcoxon signed rank test was used for comparisons of ACTH and cortisol values within the same group. Mann-Whitney rank sum test was performed for comparisons between thyrotoxic patients and control subjects. Mean basal levels were calculated using all individual values obtained before the injection of each peptide. The responses were also analyzed by the area under the curve (AUC), which was calculated by trapezoidal integration. Delta (Δ) AUC values subtracting baseline were also calculated when appropriate. The Spearman correlation coefficient was calculated when appropriate. Results are shown as mean $\pm$ SE. $P < 0.05$ was considered statistically significant.

Results

No significant differences in age and BMI were observed in the two study groups.

Mean basal cortisol (μg/dL) levels were significantly higher in hyperthyroid patients (10.7$\pm$0.7) when compared to normal subjects (8.1$\pm$0.7). In control subjects mean AUC cortisol (μg/dL.90min) values after ghrelin injection (1225$\pm$120) were higher than after GHRP-6 (957$\pm$75). In terms of peak levels (Fig. 1), there was a trend to higher values after ghrelin administration (16.4$\pm$1.6 *vs.* 13.5$\pm$0.9; *P*=0.055). In patients with hyperthyroidism ghrelin induced a higher cortisol response (peak: 19.1$\pm$0.9; AUC: 1415$\pm$78) than seen after GHRP-6 (15.8$\pm$0.9; 1114$\pm$69).

When patients with hyperthyroidism were compared to controls, there were no significant differences of cortisol responses to both peptides in terms of peak and AUC. Also, similar Δ AUC cortisol values were observed in thyrotoxic patients (ghrelin: 484$\pm$80; GHRP-6: 115$\pm$63) and controls (524$\pm$107; 192$\pm$73).

Mean basal ACTH (pg/mL) levels were increased in thyrotoxic patients compared to normal subjects (21.5$\pm$2.9 *vs.*13.5$\pm$1.8). In controls mean peak ACTH and AUC (pg/mL.90min) values after ghrelin administration were 54.9$\pm$10.3 and 2808$\pm$426, respectively (Figs. 2 and 3). This response was significantly higher than that obtained after GHRP-6 (31.3$\pm$7.9; 1668$\pm$265), both in terms of peak ACTH and AUC levels. In thyrotoxicosis mean peak ACTH and AUC values after ghrelin injection (149.7$\pm$39.8; 6209$\pm$1556) were also significantly higher than after GHRP-6 injection (53.9$\pm$11.2; 2767$\pm$487).

Ghrelin-induced peak ACTH release was increased in hyperthyroid patients when compared to controls and there was a trend in terms of AUC levels (P=0.063). Peak ACTH and AUC values after GHRP-6 in thyrotoxic patients did not reach statistical significance compared to normal subjects. When the Δ AUC values were analyzed, there was a significant increase in ACTH levels after ghrelin in the thyrotoxic group (patients: 4189$\pm$1202; controls: 1499$\pm$338). After GHRP-6 administration Δ ACTH values in thyrotoxic patients did not reach statistical significance compared to control subjects (patients: 927$\pm$330; controls: 539$\pm$237).

No significant correlations were found between free T4 and other parameters and basal and stimulated cortisol and ACTH values.

Side effects

Hunger sensation, nausea, sleepiness and facial flushing were reported occasionally after ghrelin administration. Facial flushing was the most prominent symptom after GHRP-6 injection.

Fig. 1: Mean peak cortisol levels after ghrelin and GHRP-6 administration in hyperthyroid patients and in control subjects (mean±SE; a, P = 0.055 vs. GHRP-6; b, P < 0.05 vs. GHRP-6).
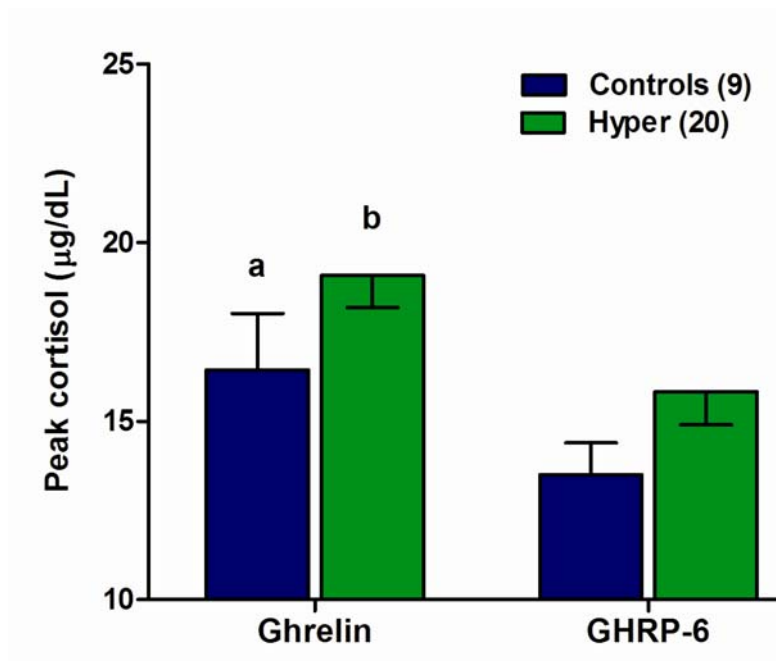
Fig. 2: Mean ACTH values after ghrelin and GHRP-6 administration in hyperthyroid patients and in control subjects (mean±SE).
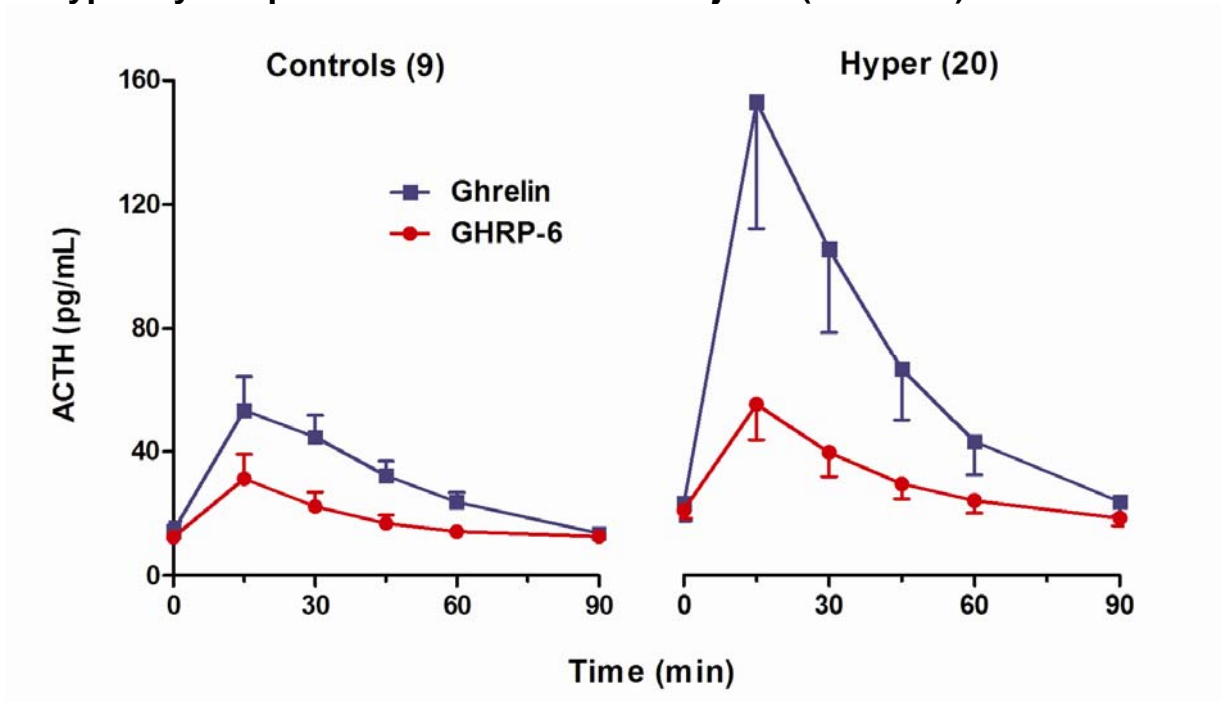
Fig. 3: Mean peak ACTH levels after ghrelin and GHRP-6 administration in hyperthyroid patients and in control subjects (mean±SE; a, P < 0.05 vs. GHRP-6).
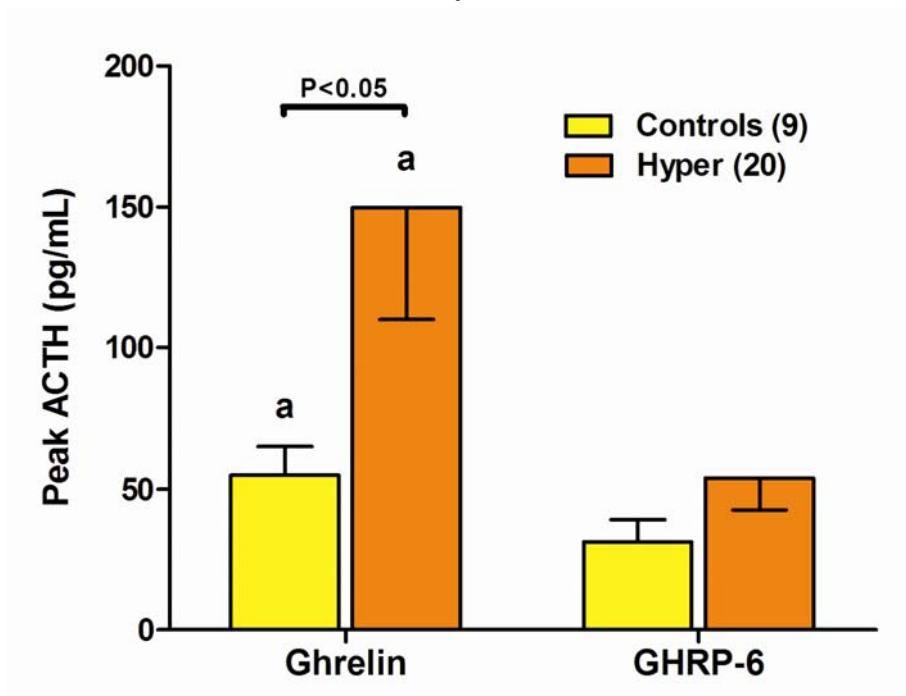
Discussion

In our study cortisol responsiveness to ghrelin in normal controls and in thyrotoxic patients was higher than seen with GHRP-6. In normal subjects this has been previously described (Correa-Silva *et al.*, 2006) and was also observed with another GHS, hexarelin (Arvat *et al.*, 2001). This could be due to the greater potency of ghrelin in relation to the synthetic analogues (Arvat *et al.*, 2001). It is also possible that ghrelin activates additional pathways to stimulate the HPA axis (Wren *et al.*, 2002; Mozid *et al.*, 2003).

When thyrotoxic patients and controls were compared, no significant differences in terms of peak and AUC cortisol levels were observed between the two study groups, although mean basal cortisol levels were slightly higher in hyperthyroid patients, confirming our previous findings (Molica *et al.*, 2007). Classically, thyroid hormones increase the conversion of cortisol to cortisone. Therefore, the disposal of cortisol is accelerated, but as its rate of secretion is also increased, plasma cortisol concentrations remain normal (Gordon & Southren, 1977; Davies & Larsen, 2008). However, conflicting results about fasting cortisol values have been reported in hyperthyroid patients, with slightly higher (Jackson *et al.*, 1966), normal (Moghetti *et al.*, 1994; Yamakita *et al.*, 2001; Mishra *et al.*, 2007; Lizcano & Salvador, 2008) or even reduced values compared to normal subjects (Goswami & Kochupillai, 2001). Our finding of slightly higher basal cortisol levels in hyperthyroidism is in agreement with previous reports in humans and also in thyrotoxic rats, which have an increase in circulating corticosterone values, the main glucocorticoid in rodents (Jackson *et al.*, 1966; Johnson *et al.*, 2005). In addition, it has been suggested, in

experimentally-induced hyperthyroidism, that alterations in hypothalamic-pituitary-adrenal function become more pronounced as the duration and severity of thyroid dysfunction increases (Levin & Daughaday, 1955; Johnson *et al.*, 2005), but this information is lacking in thyrotoxic patients. Although there are few data in the literature to allow this analysis, normal plasma cortisol concentrations were found in patients who had lower T4 values (Mishra *et al.*, 2007) compared to our study group. Therefore, although no correlations were found between cortisol and T4 values in our patients, it is possible that differences in duration and/or clinical severity might explain the discrepancies in mean basal cortisol levels in the literature.

The excessive catabolism of cortisol and continuing hyperactivity of the HPA axis in thyrotoxicosis may result in exhaustion of adrenocortical reserve (Gordon & Southren, 1977; Goswami & Kochupillai, 2001). Although controversial, it has been suggested that severely thyrotoxic patients might have an impaired cortisol response to insulin-induced hypoglycemia (Jackson *et al.*, 1966) and to both high and low-dose ACTH stimulation, associated or not with dexamethasone (Tsatsoulis *et al.*, 2000; Goswami & Kochupillai, 2001; Mishra *et al.*, 2007). After normalization of thyroid function this impairment disappears, which suggests that adrenal autoimmune disease is an unlikely cause for the reduced adrenocortical reserve (Goswami & Kochupillai, 2001; Mishra *et al.*, 2007). Moreover, it has been previously shown that in hyperthyroidism there is a significant reduction in CBG (Dumoulin *et al.*, 1995; Mishra *et al.*, 2007), which could decrease serum total cortisol measurements and contribute to these findings. However, our results show that cortisol responsiveness to ghrelin and GHRP-6 is similar in hyperthyroid patients

compared to normal subjects, suggesting that adrenocortical reserve is preserved. This is in agreement with our previous observations (Molica *et al.*, 2007) and also with reports of normal cortisol responsiveness to hypoglycemia and CRH in hyperthyroidism (Giustina *et al.*, 1971; Brauman *et al.*, 1973; Moghetti *et al.*, 1994; Lizcano & Salvador, 2008). It is possible that these different findings are related to the variable clinical features of thyrotoxic patients.

In our study, ghrelin and GHRP-6 were able to release ACTH in normal subjects and in hyperthyroid patients, as previously reported (Frieboes *et al.*, 1995; Arvat *et al.*, 2001; Correa-Silva *et al.*, 2006, Molica *et al.*, 2007). The mean peak and AUC ACTH levels after ghrelin administration were significantly higher than seen after GHRP-6 injection in the two study groups.

Interestingly, we observed an increase in basal ACTH values and also in the ACTH response to ghrelin in thyrotoxicosis compared to normal subjects, which did not reach statistical significance with GHRP-6. It has been previously demonstrated that thyroid hormone excess stimulates the conversion of cortisol to cortisone, which is biologically inactive and unable to inhibit pituitary function (Gordon & Southren, 1977; Davies & Larsen, 2008). Therefore, there is an enhancement in ACTH release, which could explain the increase in basal ACTH values observed by us and by others (Moghetti *et al.*, 1994; Yamakita *et al.*, 2001; Mishra *et al.*, 2007). Also, ACTH values decrease after treatment in hyperthyroid patients (Yamakita *et al.*, 2001; Mishra *et al.*, 2007). There are few studies in the literature about ACTH responsiveness to stimulation in hyperthyroidism. Normal and higher ACTH responses to hypoglycemia and CRH have been observed both in short- and long-term thyrotoxicosis in humans

and in rats (Moghetti *et al.*, 1994; Yamakita, 2001 *et al.*; Johnson *et al.*, 2005; Lizcano & Salvador, 2008). Some of these studies suggest hyperactivity of HPA axis in hyperthyroidism, which support our findings of enhanced ACTH responsiveness to ghrelin in hyperthyroid patients and also of a decrease in ACTH values after ghrelin, GHRP-6 and CRH with normalization of thyroid function (Molica *et al.*, 2007; Lizcano & Salvador, 2008).

The central mechanisms modulating the increased ACTH release in thyrotoxicosis are still unknown. It is possible that hypothalamic pathways, which are activated by hypoglycemia, and/or pituitary sensitivity to CRH are enhanced in this condition.

It has been shown that the main action of ghrelin/GHS on ACTH/cortisol secretion is exerted at hypothalamic level as these effects are abolished after hypothalamic-pituitary disconnection (Popovic *et al.*, 1995; Popovic *et al.*, 2003). Moreover, GHS are not able to release ACTH in pituitary cells *in vitro* (Elias *et al.*, 1995; Kojima *et al.*, 1999) and normal corticotrophs lack GHS-R (Smith *et al.*, 1997). It has been suggested that HPA activation induced by these peptides probably occurs via stimulation of AVP and/or CRH release. (Thomas *et al.*, 1997; Korbonits *et al.*, 1999a). Reinforcing this hypothesis, it has been shown that GHS-R are located in the paraventricular and arcuate nucleus of the hypothalamus, where AVP, CRH and NPY neurons are found (Bennett *et al.*, 1997; Guan *et al.*, 1997; Korbonits *et al.*, 2004). Experimental studies have shown that GHRP-6 increases mainly AVP release from hypothalamic fragments *in vitro* (Korbonits *et al.*, 1999b), while ghrelin has apparently a broader effect, enhancing AVP, CRH and NPY secretion, with a predominant action on AVP (Wren *et al.*, 2002; Mozid *et al.*, 2003). Although

controversial (Kaji *et al.,* 2001), thyroid hormones enhance the activity of the human GHS-R promoter (Petersenn *et al.*, 2001). Therefore, it is possible that these pathways are activated in thyrotoxicosis, which might explain our findings.

Furthermore, it has been previously suggested that a centrally mediated stimulation of ACTH release is present in hyperthyroidism, which is reinforced by the finding of bilateral increase in the adrenal glands found both in thyrotoxic patients and rats (Goswami & Kochupillai, 2001; Johnson *et al.*, 2005), together with high ACTH and cortisol/corticosterone levels (Moghetti *et al.*, 1994; Yamakita *et al.*, 2001; Johnson *et al.*, 2005; Mishra *et al.*, 2007).

In summary, our results show that cortisol responsiveness to ghrelin and GHRP-6 is normal in thyrotoxicosis. ACTH release after ghrelin is increased, although not reaching statistical significance with GHRP-6. Our results suggest that the pathways of ACTH release mediated by ghrelin might be activated by thyroid hormone excess, but adrenocortical reserve is maintained.

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SUMÁRIO E CONCLUSÕES

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Estudo 1

A ghrelina demonstrou ser um estímulo mais potente do que o GHRP-6 e o GHRH para a secreção de GH em controles e em pacientes com hipertireoidismo. Porém, a resposta do GH após todos os estímulos foi menor na tireotoxicose quando comparada aos indivíduos normais. Portanto, o excesso de hormônios tireoidianos interfere com as vias hipotalâmicas e hipofisárias de liberação de GH ativadas por ghrelina, GHRP-6 e GHRH.

Os valores basais de glicemia dos pacientes com hipertireoidismo não foram significativamente diferentes dos observados em controles. A ghrelina promoveu um aumento similar nas concentrações plasmáticas de glicose em ambos os grupos, enquanto que o GHRP-6 não alterou os níveis circulantes de glicose. Portanto, o excesso de hormônios tireoidianos não interfere nos mecanismos de liberação de glicose estimulados pela ghrelina.

Estudo 2

Foi observado um aumento nos valores basais de ACTH e de cortisol nos pacientes com hipertireoidismo comparado com controles, sugerindo que o excesso de hormônios tireoidianos interfere com o eixo hipotálamo-hipófise adrenal.

A administração da ghrelina promoveu uma liberação maior de ACTH e de cortisol em ambos os grupos, confirmando que a ghrelina é um estímulo potente para ativação do eixo hipotálamo-hipófise adrenal.

A resposta do cortisol à ghrelina e ao GHRP-6 em pacientes com hipertireoidismo foi semelhante a dos controles. A liberação de ACTH após ghrelina na tireotoxicose foi maior que em indivíduos normais, enquanto que com GHRP-6 os valores não alcançaram significância estatística.

Nossos dados sugerem que as vias de liberação de ACTH estimuladas pela ghrelina são ativadas pelo excesso de hormônios tireoidianos, porém sem repercussões significativas na resposta adrenocortical.

ANEXOS – Estudo 1

Tabela 1 – Dados clínicos dos indivíduos normais.

Indivíduo	Sexo	Idade (anos)	IMC (kg/m^2)*
1	M	28	25,7
2	M	36	19,5
3	M	28	23,3
4	M	30	22,4
5	M	30	25,5
6	F	34	20,9
7	F	35	23,7
8	F	20	24,3
Média		30,1	23,2
DP		5,1	2,2
EP		1,8	0,8

* IMC = índice de massa corpórea

Tabela 2 – Dados laboratoriais dos indivíduos normais.

Indivíduo	T3 total (ng/dL)[a]	T4 livre (ng/mL)[b]	TSH (mU/L)[c]
1	103	1,1	2,0
2	123	1,3	1,1
3	115	1,1	1,5
4	121	1,3	1,3
5	111	1,1	0,6
6	118	1,1	1,3
7	156	1,1	2,4
8	126	1,1	3,0
Média	121,6	1,2	1,7
DP	15,7	0,1	0,8
EP	5,5	0,03	0,3

a. Valor normal: 60 – 180

b. Valor normal: 0,89 – 1,76

c. Valor normal: 0,5 – 5,5

Tabela 3 – Dados clínicos dos pacientes com hipertireoidismo.

Indivíduo	Sexo	Idade (anos)	IMC* (kg/m²)	Tratamento prévio
1	F	34	23,1	Sim
2	F	26	22,1	Não
3	F	44	20,3	Não
4	F	27	24,4	Sim
5	F	36	26,5	Não
6	F	28	24,7	Sim
7	F	36	23,3	Não
8	M	31	20,5	Não
9	F	42	22,4	Não
10	M	28	20,8	Não
Média		33,2	22,8	
DP		6,3	2,0	
EP		2,0	0,6	

* IMC = índice de massa corpórea

Tabela 4 – Valores individuais e níveis médios de T3 total, T4 livre, TSH e TRAb nos pacientes com hipertireoidismo.

Indivíduo	T3 total (ng/dL)[a]	T4 livre (ng/mL)[b]	TSH (mU/L)[c]	TRAb (U/L)[d]
1	316	4,2	< 0,01	94,0
2	> 800	9,9	< 0,01	300,0
3	> 800	> 12,0	< 0,01	< 11,0
4	785	11,4	< 0,01	< 11,0
5	247	2,7	< 0,01	< 11,0
6	285	2,7	0,1	15,0
7	> 800	> 12,0	< 0,01	45,0
8	332	3,5	< 0,01	11,0
9	> 800	9,0	< 0,01	58,0
10	680	6,6	< 0,01	233,7
Média	584,5	7,4	0,02	
DP	252,7	3,9	0,03	
EP	79,9	1,2	0,01	

a. Valor normal: 60 – 180

b. Valor normal: 0,89 – 1,76

c. Valor normal: 0,5 – 5,5

d. Valor normal: < 11,0

Tabela 5 – Valores individuais e níveis médios de GH após a administração de ghrelina (1 μg/kg), ev, em 8 indivíduos normais.

	GH (μg/L)								
	Tempo (min)								
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	0,2	7,3	50,8	65,8	45,8	25,4	13,5	6,9	3,7
3	0,02	37,5	95,8	90,4	57,3	33,1	41,1	23,9	15,3
4	0,2	35,2	43,5	29,0	19,3	14,4	8,6	4,8	3,0
5	0,02	17,5	25,1	18,5	10,4	8,5	6,0	4,2	2,7
6	0,08	16,8	41,9	32,4	21,2	13,0	6,4	5,2	2,3
7	0,07	23,0	27,9	17,0	7,0	3,7	2,5	1,0	0,6
8	0,06	78,1	123,5	102,3	80,8	53,1	34,1	16,9	11,6
Média	0,09	35,7	64,9	54,5	37,4	25,5	19,1	11,0	6,7
DP	0,07	25,8	38,9	34,3	26,7	19,3	16,6	9,6	6,0
EP	0,03	9,1	13,8	12,1	9,4	6,8	5,9	3,4	2,1

Tabela 6 – Valores individuais e níveis médios de GH após a administração de GHRP-6 (1 μg/kg), ev, em 8 indivíduos normais.

	GH (μg/L)								
	Tempo (min)								
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	0,13	11,6	14,2	8,4	4,2	2,4	1,0	0,5	0,3
3	0,04	12,1	29,1	26,3	15,8	8,9	4,7	2,6	1,4
4	0,07	19,5	24,7	17,9	9,2	4,8	2,4	1,5	1,2
5	0,01	13,3	17,7	15,9	11,8	6,7	2,4	1,2	0,8
6	0,7	11,0	25,2	19,3	12,7	6,9	3,7	2,1	1,1
7	0,2	6,2	10,6	9,6	4,3	3,0	2,3	2,0	1,4
8	0,16	34,6	56,5	51,5	37,3	28,6	15,4	8,8	4,3
Média	0,21	16,5	26,7	21,8	14,2	9,2	4,9	2,8	1,6
DP	0,23	9,1	14,5	13,6	10,6	8,4	4,6	2,6	1,2
EP	0,08	3,2	5,1	4,8	3,7	3,0	1,6	0,9	0,4

Tabela 7 – Valores individuais e níveis médios de GH após a administração de GHRH (100 μg), ev, em 8 indivíduos normais.

	GH (μg/L)								
	Tempo (min)								
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	0,04	3,4	3,6	2,2	2,3	1,8	1,2	0,8	0,8
3	0,03	8,3	9,2	6,8	7,5	7,3	5,7	3,8	2,4
4	0,03	6,2	5,0	4,0	3,5	2,7	2,2	1,4	1,1
5	0,01	3,9	4,8	5,4	4,4	3,2	1,9	1,2	0,6
6	0,06	7,5	8,1	6,6	4,8	2,4	1,4	0,8	0,4
7	0,13	16,0	21,5	24,8	22,6	19,8	13,7	18,2	13,8
8	0,6	8,0	8,1	11,0	8,2	9,4	12,5	7,1	5,3
Média	0,12	8,6	10,0	11,2	11,4	10,6	8,7	6,5	4,4
DP	0,2	4,8	6,8	10,0	12,4	12,7	10,2	7,7	5,2
EP	0,07	1,7	2,4	3,5	4,4	4,5	3,6	2,7	1,8

Tabela 8 – Valores individuais e níveis médios de GH após a administração de ghrelina (1 μg/kg), ev, em 10 pacientes com hipertireoidismo.

	GH (μg/L)								
	Tempo (min)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
1	0,2	12,9	13,3	8,3	4,1	1,9	1,1	0,7	0,4
2	1,2	31,5	18,2	12,7	8,0	4,3	2,6	1,5	1,3
3	1,4	18,3	11,0	6,4	4,8	3,0	2,5	2,0	1,5
4	1,2	24,2	14,4	7,0	5,0	3,0	2,1	1,3	0,9
5	0,1	28,4	25,1	15,9	9,6	5,4	2,8	1,8	1,1
6	0,1	7,8	9,6	5,6	3,0	1,4	0,7	0,4	0,2
7	0,5	13,7	13,0	10,8	6,0	4,7	2,9	1,8	1,2
8	2,2	25,3	17,7	13,2	9,2	8,1	6,5	4,4	3,6
9	0,3	17,0	10,2	6,8	2,9	4,5	2,2	1,6	1,3
10	1,5	11,9	7,0	4,8	4,5	4,7	5,0	5,5	4,9
Média	0,9	19,1	14,0	9,2	5,7	4,1	2,8	2,1	1,6
DP	0,7	7,9	5,2	3,8	2,4	1,9	1,7	1,6	1,5
EP	0,2	2,5	1,7	1,2	0,8	0,6	0,5	0,5	0,5

Tabela 9 – Valores individuais e níveis médios de GH após a administração de GHRP-6 (1 μg/kg), ev, em 10 pacientes com hipertireoidismo.

	GH (μg/L)								
	Tempo (min)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
1	0,6	19,7	16,4	11,8	8,7	5,6	3,7	2,3	1,3
2	1,9	15,3	11,3	9,0	8,4	5,8	3,5	2,7	2,3
3	1,1	9,9	9,0	6,1	4,1	2,5	1,9	1,3	1,4
4	0,7	13,3	8,9	5,7	3,5	2,2	1,4	1,0	0,8
5	0,2	15,7	9,9	6,1	3,0	0,7	0,6	0,8	0,4
6	0,1	6,8	3,8	2,5	1,7	1,3	0,7	0,3	0,2
7	0,7	8,3	6,3	3,7	2,4	1,7	0,8	0,5	0,5
8	0,6	17,0	11,1	8,7	7,2	5,9	5,4	4,4	4,1
9	0,1	10,6	10,6	7,4	5,8	3,8	3,4	3,3	2,8
10	0,5	9,7	8,1	7,7	6,4	5,3	4,5	3,6	3,3
Média	0,7	12,6	9,5	6,9	5,1	3,5	2,6	2,0	1,7
DP	0,5	4,2	3,3	2,7	2,5	2,0	1,7	1,4	1,3
EP	0,2	1,3	1,1	0,8	0,8	0,6	0,5	0,5	0,4

Tabela 10 – Valores individuais e níveis médios de GH após a administração de GHRH (100 μg), ev, em 10 pacientes com hipertireoidismo.

	GH (μg/L)								
	Tempo (min)								
Indiv.	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
1	0,2	1,0	2,1	2,5	3,6	4,3	4,1	4,2	3,5
2	1,3	4,0	3,8	3,7	4,8	5,7	6,3	5,7	4,3
3	2,3	3,9	3,3	3,3	2,8	2,9	3,1	2,8	3,3
4	1,1	2,6	2,4	2,6	3,2	3,7	3,3	3,3	2,9
5	0,1	1,5	1,4	2,7	4,0	4,4	3,1	1,6	1,0
6	1,2	10,7	9,4	9,6	11,5	10,6	10,7	9,9	8,8
7	0,6	2,9	2,5	2,1	1,9	2,3	2,1	2,5	2,0
8	0,3	3,6	4,0	3,9	3,9	5,8	6,7	6,3	5,7
9	1,0	2,9	3,0	2,5	2,0	2,0	2,7	3,2	2,7
10	0,7	3,5	2,7	2,5	3,5	4,5	5,1	5,9	5,7
Média	0,9	3,7	3,5	3,4	4,1	4,6	4,7	4,5	4,0
DP	0,7	2,7	2,2	2,2	2,7	2,4	2,6	2,5	2,3
EP	0,2	0,8	0,7	0,7	0,9	0,8	0,8	0,8	0,7

Tabela 11 – Valores individuais e níveis médios do pico de GH e da área sob a curva após a administração de ghrelina (1 μg/kg), GHRP-6 (1 μg/kg) ou GHRH (100 μg), ev, em 8 indivíduos normais.

Indiv.	Pico GH (μg/L)			ASC (μg/L.120min)		
	Ghrelina	GHRP-6	GHRH	Ghrelina	GHRP-6	GHRH
1	110,4	35,5	38,5	6674	1907	2925
2	65,8	14,2	3,6	3262	638	236
3	95,8	29,1	9,2	5802	1503	747
4	43,5	24,7	6,2	2346	1210	383
5	25,1	17,7	5,4	1373	1041	377
6	41,9	25,2	8,1	2071	1227	477
7	27,9	10,6	24,8	1237	582	2153
8	123,5	56,5	12,5	7419	3524	1009
Média	66,7	26,7	13,5	3773	1454	1038
DP	38,5	14,5	12,1	2485	941	979
EP	13,6	5,1	4,3	878	333	346

Tabela 12 – Valores individuais e níveis médios do pico de GH e da área sob a curva após a administração de ghrelina (1 μg/kg), GHRP-6 (1 μg/kg) ou GHRH (100 μg), ev, em 10 pacientes com hipertireoidismo.

Indiv.	Pico GH (μg/L)			ASC (μg/L.120min)		
	Ghrelina	GHRP-6	GHRH	Ghrelina	GHRP-6	GHRH
1	13,3	19,7	4,3	639	1037	355
2	31,5	15,3	6,3	1201	872	552
3	18,3	9,9	3,9	742	541	374
4	24,2	13,3	3,7	871	551	346
5	28,4	15,7	4,4	1344	557	289
6	9,6	6,8	11,5	430	259	1161
7	13,7	8,3	2,9	806	365	264
8	25,3	17,0	6,7	1310	931	559
9	17,0	10,6	3,2	690	695	302
10	11,9	9,7	5,9	695	708	464
Média	19,3	12,6	5,3	873	652	467
DP	7,6	4,2	2,5	309	247	265
EP	2,4	1,3	1,3	98	78	84

Tabela 13 – Valores individuais e níveis médios de glicemia (mg/dL) após a administração de ghrelina (1 μg/kg), ev, em 8 indivíduos normais.

	Glicemia (mg/dL)				
	Tempo (min)				
Indiv.	**0**	**30**	**60**	**90**	**120**
1	85	99	89	90	92
2	80	88	84	87	86
3	81	86	83	75	85
4	84	91	94	90	93
5	82	86	85	89	92
6	73	77	75	75	75
7	84	90	89	86	86
8	81	90	85	90	87
Média	81,3	88,4	85,5	85,3	87,0
DP	3,8	6,2	5,6	6,5	5,8
EP	1,3	2,2	2,0	2,3	2,1

Tabela 14 – Valores individuais e níveis médios de glicemia (mg/dL) após a administração de GHRP-6 (1 μg/kg), ev, em 8 indivíduos normais.

	Glicemia (mg/dL)				
	Tempo (min)				
Indiv.	0	30	60	90	120
1	86	91	90	86	100
2	93	93	93	92	95
3	88	86	82	85	84
4	88	93	93	97	93
5	85	89	88	91	93
6	75	73	75	77	78
7	82	82	83	83	84
8	86	88	85	85	84
Média	85,4	86,9	86,1	87,0	88,9
DP	5,2	6,7	6,2	6,2	7,4
EP	1,9	2,4	2,2	2,2	2,6

Tabela 15 – Valores individuais e níveis médios de glicemia (mg/dL) após a administração de ghrelina (1 μg/kg), ev, em 10 pacientes com hipertireoidismo.

	Glicemia (mg/dL)				
	Tempo (min)				
Indiv.	**0**	**30**	**60**	**90**	**120**
1	84	89	91	90	86
2	82	91	89	87	85
3	64	71	71	70	71
4	92	109	100	98	92
5	95	96	95	94	97
6	87	95	94	91	89
7	88	98	93	93	83
8	95	98	96	97	95
9	81	85	83	81	76
10	83	101	90	85	84
Média	85,1	93,3	90,2	88,6	85,8
DP	9,0	10,3	8,1	8,4	8,1
EP	2,9	3,2	2,6	2,7	2,6

Tabela 16 – Valores individuais e níveis médios de glicemia (mg/dL) após a administração de GHRP-6 (1 μg/kg), ev, em 10 pacientes com hipertireoidismo.

	Glicemia (mg/dL)				
	Tempo (min)				
Indiv.	**0**	**30**	**60**	**90**	**120**
1	82	84	83	84	83
2	-	-	-	-	-
3	88	85	79	75	73
4	95	100	99	97	94
5	91	92	91	92	92
6	96	96	93	90	90
7	101	102	102	104	110
8	85	93	89	91	90
9	87	87	85	85	81
10	94	100	97	97	96
Média	91,0	93,2	90,9	90,6	89,9
DP	6,0	6,8	7,7	8,5	10,5
EP	2,0	2,3	2,6	2,8	3,5

Tabela 17 – Valores individuais e níveis médios do pico de glicemia e da área sob a curva após a administração de ghrelina (1 μg/kg) ou GHRP-6 (1 μg/kg), ev, em 8 indivíduos normais.

Indiv.	Pico glicemia (mg/dL)		ASC (mg/dL.120min)	
	Ghrelina	GHRP-6	Ghrelina	GHRP-6
1	99	100	10995	10800
2	88	95	10260	11160
3	86	86	9810	10170
4	94	97	10905	11205
5	92	93	10410	10710
6	77	78	9030	9045
7	90	84	10500	9930
8	90	88	10470	10290
Média	89,5	90,1	10298	10414
DP	10,7	7,4	631	717
EP	3,8	2,6	223	254

Tabela 18 – Valores individuais e níveis médios do pico de glicemia e da área sob a curva após a administração de ghrelina (1 μg/kg) ou GHRP-6 (1 μg/kg), ev, em 10 pacientes com hipertireoidismo.

	Pico glicemia (mg/dL)		ASC (mg/dL.120min)	
Indiv.	Ghrelina	GHRP-6	Ghrelina	GHRP-6
1	91	84	10650	10005
2	91	-	10515	-
3	71	85	8385	9585
4	109	100	11970	11715
5	97	92	11430	10995
6	95	96	11040	11160
7	98	110	11085	12405
8	98	93	11580	11115
9	85	87	9825	10230
10	101	100	10785	11670
Média	93,6	94,1	10727	10987
DP	10,2	8,4	1019	905
EP	3,2	2,8	322	302

ANEXOS – Estudo 2

Tabela 1 – Dados clínicos do grupo controle.

Indivíduo	Sexo	Idade (anos)	IMC (kg/m^2)*
1	M	28	25,7
2	M	28	23,3
3	M	30	22,4
4	M	30	25,5
5	F	34	20,9
6	F	35	23,7
7	F	20	24,3
8	M	26	24,9
9	M	28	25,9
Média		28,8	24,1
DP		4,4	1,7
EP		1,5	0,6

* IMC = índice de massa corpórea

Tabela 2 – Valores individuais e níveis médios das dosagens basais do grupo controle.

Indivíduo	TSH (mU/L)[a]	ACTH (pg/mL)[b]	Cortisol (µg/dL)[c]
1	2,0	7,3	9,7
2	1,5	27,6	12
3	1,3	7,7	10,1
4	0,6	8,1	8,7
5	1,3	14,4	7,4
6	2,4	8,5	4,7
7	3,0	9,6	7,5
8	1,3	22,2	8,4
9	1,2	16,6	5,1
Média	1,6	13,6	8,2
DP	0,7	7,3	2,3
EP	0,2	2,4	0,8

a. Valor normal: 0,5 – 5,5

b. Valor normal: até 46,0

c. Valor normal: 7,0 – 25,0

Tabela 3 – Dados clínicos dos pacientes com hipertireoidismo.

Indivíduo	Sexo	Idade (anos)	IMC* (kg/m²)	Tratamento prévio
1	F	34	23,1	Sim
2	F	26	22,1	Não
3	F	44	20,3	Não
4	F	27	24,4	Sim
5	F	36	26,5	Não
6	F	28	24,7	Sim
7	F	36	23,3	Não
8	M	31	20,5	Não
9	F	42	22,4	Não
10	M	28	20,8	Não
11	F	38	25,5	Sim
12	F	26	24,8	Sim
13	F	17	24,0	Não
14	F	31	23,2	Não
15	M	32	24,7	Não
16	F	22	21,5	Não
17	M	25	22,3	Não
18	F	42	21,3	Sim
19	F	31	22,2	Não
20	F	22	21,5	Sim
Média		30,9	23,0	
DP		7,2	1,8	
EP		1,6	0,4	

* IMC = índice de massa corpórea

Tabela 4 – Valores individuais e níveis médios das dosagens basais nos pacientes com hipertireoidismo.

Indivíduo	T3 (ng/dL)[a]	T4 livre (ng/mL)[b]	TSH (mU/L)[c]	TRAb (U/L)[d]
1	316,0	4,2	< 0,01	94,0
2	> 800,0	9,9	< 0,01	300,0
3	> 800,0	> 12,0	< 0,01	< 11,0
4	784,6	11,4	< 0,01	< 11,0
5	246,8	2,7	< 0,01	< 11,0
6	284,5	2,7	0,1	15,0
7	> 800,0	> 12,0	< 0,01	45,0
8	332,2	3,5	< 0,01	11,0
9	> 800,0	9,0	< 0,01	58,0
10	680,0	6,6	< 0,01	233,7
11	494,2	5,9	< 0,01	51,6
12	> 800,0	> 12,0	< 0,01	567,0
13	> 800,0	10,0	< 0,01	> 405,0
14	> 800,0	8,8	< 0,01	233,6
15	679,3	7,8	< 0,01	33,5
16	> 800,0	9,0	< 0,01	< 11,0
17	> 800,0	4,7	< 0,01	14,4
18	265,6	2,8	< 0,01	59,7
19	> 800,0	7,7	< 0,01	268,8
20	> 800,0	> 12,0	< 0,01	> 405,0
Média	644,2	7,7	0,01	
DP	223,3	3,4	0,02	
EP	49,9	0,8	0,01	

a. Valor normal: 60 – 180

b. Valor normal: 0,89 – 1,76

c. Valor normal: 0,5 – 5,5

d. Valor normal: até 11,0

Tabela 5 – Valores individuais e níveis médios de cortisol após administração de ghrelina em controles.

	Cortisol (µg/dL)					
	Tempo (min)					
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	9,2	15,2	17,7	20,1	19,6	17,3
5	9,6	10,6	12,5	10,2	9,4	8,5
6	6,3	8,6	10,7	9,3	7,6	7,5
7	7,5	21,5	18,8	17,7	16,7	14,8
8	6,9	11,3	14,9	14,2	11,4	8,4
9	5,3	9,1	11,0	9,4	11,2	7,8
Média	7,8	12,8	15,7	15,2	14,5	12,2
DP	1,5	4,2	4,2	5,0	4,9	4,4
EP	0,5	1,4	1,4	1,7	1,7	1,5

Tabela 6 – Valores individuais e níveis médios de cortisol após administração de GHRP-6 em controles.

	Cortisol (μg/dL)					
	Tempo (min)					
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	8,2	12,6	11,7	10,3	9,5	8,0
5	5,1	10,3	8,8	6,6	5,9	6,9
6	3,0	9,0	7,1	6,3	7,7	7,8
7	7,4	11,2	12,9	15,3	12,0	9,1
8	9,8	12,7	11,4	10,4	9,0	7,8
9	4,9	15,6	12,2	11,5	9,0	7,2
Média	8,5	12,5	12,0	11,6	9,9	8,7
DP	3,8	2,2	2,9	3,9	2,5	2,0
EP	1,3	0,7	1,0	1,3	0,8	0,7

Tabela 7 – Valores individuais e níveis médios de ACTH após a administração de ghrelina em controles.

	ACTH (pg/mL)					
	Tempo (min)					
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	9,5	60,0	57,5	33,4	26,3	13,5
5	15,7	21,9	19,6	16,5	12,8	11,9
6	10,0	19,2	21,4	16,2	14,0	11,0
7	11,1	30,3	28,3	21,5	16,5	9,6
8	23,6	82,6	68,6	51,6	34,7	20,5
9	15,0	112,0	74,2	48,5	30,5	14,2
Média	14,5	53,4	44,8	32,4	23,8	13,7
DP	8,9	32,4	20,6	13,9	9,4	6,0
EP	3,0	10,8	6,9	4,6	3,1	2,0

Tabela 8 – Valores individuais e níveis médios de ACTH após a administração de GHRP-6 em controles.

	ACTH (pg/mL)					
	Tempo (min)					
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	6,7	13,6	12,8	10,4	7,7	6,5
5	13,1	21,0	16,5	14,0	13,2	19,0
6	7,0	20,0	15,0	14,5	16,4	19,0
7	8,1	24,0	18,3	15,0	13,0	11,0
8	20,8	28,9	22,6	19,0	16,8	14,6
9	18,1	87,1	56,1	34,1	21,7	12,5
Média	12,5	31,3	22,4	17,0	14,3	12,7
DP	6,2	23,7	14,0	7,7	4,5	4,5
EP	2,1	7,9	4,7	2,6	1,5	1,5

Tabela 9 – Valores individuais e níveis médios de cortisol após a administração de ghrelina em pacientes com hipertireoidismo.

	Cortisol (μg/dL)					
	Tempo (min)					
Indiv.	0	15	30	45	60	90
1	9,9	19,8	20,5	22,1	21,6	17,4
2	2,5	11,3	15,4	18,4	19,0	16,5
3	12,5	21,6	22,7	26,1	28,0	27,9
4	8,4	14,5	14,1	12,1	9,4	4,8
5	8,6	16,1	18,8	20,1	16,8	12,8
6	7,0	12,1	16,1	17,3	14,8	11,7
7	10,1	16,5	19,4	10,3	18,3	12,7
8	7,5	16,5	22,3	21,5	18,6	13,1
9	14,9	12,4	13,9	12,6	10,4	10,8
10	16,7	20,7	21,3	22,8	23,4	21,5
11	7,6	13,9	16,4	17,9	16,6	11,5
12	8,8	13,8	14,9	15,9	13,5	9,1
13	7,4	12,4	13,3	13,5	9,4	5,1
14	13,5	17,1	18,3	15,9	17,3	8,0
15	18,6	20,4	20,9	22,4	21,9	15,9
16	6,4	10,8	14,2	14,9	18,2	9,7
17	6,8	11,5	14,5	14,6	10,9	6,7
18	20,7	22,6	24,9	21,3	17,9	11,0
19	11,9	16,0	19,4	20,1	21,6	15,1
20	7,1	9,9	14,3	14,7	13,2	11,2
Média	10,3	15,5	17,8	17,7	17,0	12,6
DP	4,6	3,9	3,5	4,2	5,0	5,5
EP	1,0	0,9	0,8	1,2	1,1	1,2

Tabela 10 – Valores individuais e níveis médios de cortisol após a administração de GHRP-6 em pacientes com hipertireoidismo.

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

Tabela 11 – Valores individuais e níveis médios de ACTH após administração de ghrelina em pacientes com hipertireoidismo.

	ACTH (pg/mL)					
	Tempo (min)					
Indiv.	0	15	30	45	60	90
1	19,4	98,6	74,6	57,4	48,7	27,5
2	6,7	181,0	146,0	80,2	45,4	28,7
3	20,0	336,0	205,0	120,0	87,2	61,4
4	13,6	74,7	57,1	25,0	17,7	10,1
5	10,8	44,0	47,1	24,7	18,3	12,3
6	7,0	23,0	28,5	18,9	12,1	5,8
7	13,1	140,0	100,0	64,0	33,2	19,0
8	12,0	130,0	78,3	54,0	32,0	18,0
9	5,2	67,5	52,0	41,0	23,0	16,4
10	94,2	817,0	559,0	349,0	225,0	90,8
11	12,2	103,0	60,1	36,3	23,4	12,0
12	20,4	160,0	105,0	67,8	42,9	26,1
13	9,6	28,4	18,2	15,4	11,0	8,3
14	23,0	62,5	45,0	33,7	24,4	16,5
15	71,3	287,0	152,0	91,7	61,3	28,1
16	11,1	50,6	53,9	40,0	25,2	16,4
17	12,9	52,9	45,8	32,1	22,2	13,9
18	61,8	202,0	118,0	66,7	30,0	15,5
19	12,7	66,4	61,7	39,9	27,1	16,2
20	12,0	58,3	47,9	37,6	29,4	18,9
Média	22,5	149,1	102,8	64,8	42,0	23,1
DP	24,1	178,3	117,1	71,9	46,7	19,8
EP	5,4	39,9	26,2	16,1	10,4	4,4

Tabela 12 – Valores individuais e níveis médios de ACTH após administração de GHRP-6 em pacientes com hipertireoidismo.

	ACTH (pg/mL)					
	Tempo (min)					
Indiv.	**0**	**15**	**30**	**45**	**60**	**90**
1	13,3	16,6	12,3	11,3	7,4	8,1
2	12,3	121,0	92,4	64,8	46,3	25,9
3	13,5	41,7	29,3	22,7	17,1	24,9
4	16,8	44,9	27,7	23,2	22,2	12,7
5	14,0	19,5	16,1	12,4	10,2	8,5
6	12,5	12,6	10,3	9,1	10,1	9,0
7	9,0	14,3	14,5	16,0	15,6	16,0
8	18,5	81,5	45,0	35,0	26,0	15,0
9	23,0	55,3	45,0	33,0	29,0	22,0
10	60,2	222,0	155,0	96,0	84,7	57,8
11	23,7	49,6	32,5	25,8	20,1	18,7
12	20,8	60,7	49,5	41,9	39,4	27,8
13	13,1	23,8	25,2	21,5	14,8	11,2
14	23,6	31,6	25,6	23,1	17,4	14,0
15	26,5	32,3	27,5	22,9	20,4	22,2
16	15,7	30,4	19,9	14,1	18,3	14,4
17	29,7	112,0	61,9	40,2	24,8	14,8
18	29,5	59,7	51,8	38,3	29,0	18,6
19	25,8	34,8	22,1	14,4	11,3	10,5
20	7,5	10,4	8,3	7,9	6,2	6,9
Média	20,5	53,7	38,6	28,7	23,5	18,0
DP	11,5	50,1	34,2	21,1	17,6	11,2
EP	2,6	11,2	7,6	4,7	3,9	2,5

Tabela 13 – Resposta do cortisol expressa em pico e ASC após administração de ghrelina e GHRP-6 em controles.

Indiv.	Pico (μg/dL)		ASC (μg/dL.90min)	
	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	20,1	12,6	1565	914
5	12,5	10,3	911	660
6	10,7	9,0	760	649
7	21,5	15,3	1524	1053
8	14,9	12,7	1040	911
9	11,2	15,6	851	937
Média	16,4	13,5	1225	957
DP	4,7	2,7	359	224
EP	1,6	0,9	120	75

Tabela 14 – Resposta do ACTH expressa em pcio e ASC após administração de ghrelina e GHRP-6 em controles.

Indiv.	Pico (pg/mL)		ASC (pg/mL.90min)	
	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	60,0	13,6	3128	873
5	21,9	21,0	1454	1453
6	21,4	20,0	1407	1449
7	30,3	24,0	1800	1378
8	82,6	28,9	4307	1811
9	112,0	87,1	4532	3471
Média	54,9	31,3	2808	1668
DP	30,9	23,7	1279	794
EP	10,3	7,9	426	265

Tabela 15 – Resposta do cortisol expressa em pico e ASC após administração de grhelina e GHRP-6 em pacientes com hipertireoidismo.

Indiv.	Pico (µg/dL)		ASC (µg/dL.90min)	
	Ghrelina	GHRP-6	Ghrelina	GHRP-6
1	22,1	18,5	1757	1139
2	19,0	16,4	1370	1222
3	28,0	17,1	2198	1159
4	14,5	13,5	957	967
5	20,1	15,4	1460	929
6	17,3	10,9	1244	752
7	19,4	10,7	1371	872
8	22,3	22,0	1576	1486
9	13,9	12,1	1091	986
10	23,4	20,7	1946	1646
11	17,9	19,2	1326	1369
12	15,9	16,3	1175	1291
13	13,5	13,9	932	947
14	18,3	15,6	1380	1098
15	22,4	14,7	1826	1084
16	18,2	13,7	1202	902
17	14,6	21,5	1006	1485
18	24,9	17,7	1755	1205
19	21,6	21,1	1634	1462
20	14,7	5,7	1102	287
Média	19,1	15,8	1415	1114
DP	4,0	4,2	350	309
EP	0,9	0,9	78	69

Tabela 16 – Resposta do ACTH expressa em pico e ASC após administração de ghrelina e GHRP-6 em pacientes com hipertireoidismo.

Indiv.	Pico (pg/mL)		ASC (pg/mL.90min)	
	Ghrelina	GHRP-6	Ghrelina	GHRP-6
1	98,6	16,6	5113	991
2	181,0	121,0	7610	5696
3	336,0	41,7	12948	2265
4	74,7	44,9	3004	2253
5	47,1	19,5	2414	1182
6	28,5	12,6	1468	936
7	140,0	16,0	5690	1331
8	130,0	81,5	5015	3371
9	67,5	55,3	3210	3155
10	817,0	222,0	33006	10319
11	103,0	49,6	3789	2529
12	160,0	60,7	6502	3741
13	28,4	25,2	1374	1657
14	62,5	31,6	3087	1983
15	287,0	32,3	10296	2231
16	53,9	30,4	3064	1712
17	52,9	112,0	2767	4214
18	202,0	59,7	7172	3400
19	66,4	34,8	3468	1675
20	58,3	10,4	3192	698
Média	149,7	53,9	6209	2767
DP	178,0	50,0	6957	2176
EP	39,8	11,2	1556	487

Tabela 17 – Resposta do cortisol expressa em Δ ASC após administração de ghrelina e GHRP-6 em controles.

Indiv.	Δ ASC (μg/dL.90min)	
	Ghrelina	**GHRP-6**
1	1043	233
2	394	43
3	662	-215
4	737	176
5	47	201
6	193	379
7	849	387
8	419	29
9	374	496
Média	524	192
DP	322	218
EP	107	73

Tabela 18 – Resposta do ACTH expressa em Δ ASC após administração de ghrelina e GHRP-6 em controles.

Indiv.	Δ ASC (pg/mL.90min)	
	Ghrelina	GHRP-6
1	2202	1400
2	1084	-167
3	1217	-174
4	2273	270
5	41	274
6	507	819
7	801	649
8	2183	-61
9	3182	1842
Média	1499	539
DP	1015	711
EP	338	237

Tabela 19 – Resposta do cortisol expressa em Δ ASC após administração de ghrelina e GHRP-6 em pacientes com hipertireoidismo.

Indiv.	Δ ASC (μg/dL.90min)	
	Ghrelina	**GHRP-6**
1	866	-274
2	1145	691
3	1073	169
4	201	130
5	686	56
6	614	-256
7	462	305
8	901	658
9	-250	14
10	443	242
11	642	262
12	383	337
13	266	389
14	165	-117
15	152	-59
16	626	-142
17	394	117
18	-108	152
19	563	-356
20	463	-10
Média	484	115
DP	360	283
EP	80	63

Tabela 20 – Resposta do ACTH expressa em Δ ASC após administração de ghrelina e GHRP-6 em pacientes com hipertireoidismo.

Indiv.	Δ ASC (pg/mL.90min)	
	Ghrelina	GHRP-6
1	3367	-206
2	7007	4589
3	11148	1050
4	1780	741
5	1442	-78
6	838	-189
7	4511	521
8	3935	1706
9	2742	1085
10	24528	4901
11	2691	396
12	4666	1869
13	510	478
14	1017	-141
15	3879	-154
16	2065	299
17	1606	1541
18	1610	745
19	2325	-647
20	2112	23
Média	4189	927
DP	5377	1476
EP	1202	330

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