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Integrated Assessment of Endocrine Axes in Chronic Chagas Disease Using a Combined Stimulation Test

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ABSTRACT

Background: Chronic Chagas disease (CD) is characterized by cardiac and digestive manifestations, but its potential effects on neuroendocrine function remain poorly understood. This study aimed to evaluate the integrity of the hypothalamic-pituitary-target gland axes in patients with chronic CD using a combined hypothalamic stimulation test.

Methods: In this prospective study, 15 patients with chronic CD (five with the indeterminate form and ten with the cardiac form) underwent simultaneous stimulation with corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), and luteinizing hormone-releasing hormone (LHRH/GnRH). Dynamic responses of the hypothalamic-pituitary-adrenal (HPA), hypothalamic-pituitary-thyroid (HPT), hypothalamic-pituitary-gonadal (HPG), and prolactin axes were evaluated by serial measurements of ACTH, cortisol, TSH, prolactin, FSH, LH, thyroid hormones, testosterone, estradiol, and reverse T3.

Results: Pituitary function was preserved in all patients, as demonstrated by normal ACTH and TSH responses to CRH and TRH stimulation, respectively. Two patients exhibited reduced cortisol responses despite normal basal cortisol concentrations, suggesting decreased adrenocortical reserve. Reduced free thyroxine (FT4) levels were observed in eight patients, while low T3 syndrome was identified in three patients. Reverse T3 concentrations remained within the normal range in all cases. Prolactin responses were preserved in all but one patient, and basal hyperprolactinemia was observed in two men. Baseline testosterone concentrations and LH responses were normal in male patients, whereas isolated abnormalities in FSH responses and estradiol levels were identified without evidence of clinically significant gonadal dysfunction.

Conclusions: Chronic CD is not associated with generalized dysfunction of the hypothalamic-pituitary endocrine axes. Preserved pituitary responses indicate intact central neuroendocrine regulation, whereas reduced adrenocortical reserve and alterations in thyroid hormone metabolism suggest selective peripheral endocrine adaptations. These findings expand current knowledge of the endocrine manifestations

of chronic CD and support further investigation of their clinical significance in larger patient populations.

Keywords: Chagas disease, hypothalamic-pituitary axis, adrenal reserve, thyroid hormones, endocrine function, combined stimulation test

RESUMO

Introdução: A doença de Chagas (DC) crônica apresenta manifestações predominantemente cardíacas e digestivas, porém seus possíveis efeitos sobre a função neuroendócrina permanecem pouco esclarecidos. Este estudo avaliou a integridade dos eixos hipotálamo-hipófise-glândulas-alvo em pacientes com DC crônica por meio de um teste combinado de estimulação hipotalâmica.
Métodos: Estudo prospectivo envolvendo 15 pacientes com DC crônica, sendo cinco com a forma indeterminada e dez com a forma cardíaca. Os participantes foram submetidos à estimulação simultânea com hormônio liberador de corticotrofina (CRH), hormônio liberador de tireotrofina (TRH) e hormônio liberador de gonadotrofinas (GnRH/LHRH). As respostas dos eixos hipotálamo-hipófise-adrenal, hipotálamo-hipófise-tireoide, hipotálamo-hipófise-gonadal e do eixo da prolactina foram avaliadas por dosagens seriadas de ACTH, cortisol, TSH, prolactina, FSH, LH, hormônios tireoidianos, testosterona, estradiol e T3 reverso.
Resultados: A função hipofisária mostrou-se preservada em todos os pacientes, evidenciada pelas respostas normais de ACTH e TSH aos estímulos com CRH e TRH. Dois pacientes apresentaram resposta reduzida do cortisol, apesar de concentrações basais normais, sugerindo diminuição da reserva adrenocortical. Oito pacientes apresentaram redução dos níveis de T4 livre e três preencheram critérios para síndrome do T3 baixo, enquanto o T3 reverso permaneceu normal em todos os casos. As respostas da prolactina foram preservadas, exceto em um paciente, e hiperprolactinemia basal foi observada em dois homens. Nos pacientes do sexo masculino, as concentrações basais de testosterona e as respostas de LH foram normais, sendo observadas apenas alterações isoladas de FSH e estradiol, sem repercussão clínica.
Conclusões: A DC crônica não se associa à disfunção generalizada dos eixos hipotálamo-hipofisários. A preservação das respostas hipofisárias indica integridade da regulação neuroendócrina central, enquanto a redução da reserva adrenocortical e as alterações do metabolismo dos hormônios tireoidianos sugerem adaptações endócrinas periféricas seletivas, que merecem investigação em estudos com maior número de pacientes.

Palavras-chave: Doença de Chagas, eixo hipotálamo-hipófise, reserva adrenocortical, hormônios tireoidianos, função endócrina, teste combinado de estimulação

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1. INTRODUCTION

Chagas disease (CD) is a parasitic disease caused by the protozoan *Trypanosoma cruzi*, transmitted primarily through contact with triatomine insects, as well as by oral, transfusional, and congenital routes. During the chronic phase, which may remain asymptomatic for decades, approximately 30-40% of infected individuals develop potentially severe clinical manifestations, most notably Chagas cardiomyopathy, characterized by cardiac arrhythmias, heart failure, and an increased risk of sudden cardiac death, in addition to digestive forms such as megaesophagus and megacolon. These manifestations reflect the progressive involvement of the cardiac muscle and the autonomic nervous system, resulting in significant impairment of patients' quality of life and functional capacity[1].

From a public health perspective, CD remains a major health concern in Latin America and is classified as a neglected tropical disease, closely associated with socioeconomic vulnerability and limited access to diagnosis and treatment[2]. Despite substantial advances in vector control and the prevention of transfusion-transmitted infection, significant challenges remain in the early detection of the disease, clinical management, and the expansion of etiological treatment[3]. Furthermore, increasing international migration has contributed to the global spread of CD, with cases now reported in non-endemic regions such as North America, Europe, and Asia. This epidemiological shift underscores the need for adaptation of healthcare systems, implementation of screening programs in blood banks, and greater awareness among healthcare professionals worldwide[4].

Although the cardiac and gastrointestinal manifestations of CD have been extensively characterized, systematic investigations of endocrine alterations during the chronic phase remain scarce. The limited available evidence has largely been derived from experimental animal studies focusing on the acute phase of *Trypanosoma cruzi* infection[5]. Given the systemic nature of *Trypanosoma cruzi* infection and its potential to affect the autonomic nervous system, it has been hypothesized that neuroendocrine axes may also be impaired, particularly in patients with more advanced clinical forms[6]. However, the available literature remains limited and fragmented and is, in many cases, based on small sample sizes or isolated hormonal assessments, making it difficult to

achieve a comprehensive understanding of the functional status of the hypothalamic-pituitary-target gland axes in this clinical setting[7].

In this context, the combined hypothalamic-pituitary stimulation test represents a well-established dynamic approach for the simultaneous assessment of multiple anterior pituitary axes through the administration of hypothalamic releasing hormones, including corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), and gonadotropin-releasing hormone (GnRH, formerly LHRH). This test provides an integrated evaluation of pituitary secretory reserve and peripheral endocrine gland responsiveness, encompassing the hypothalamic-pituitary-adrenal, hypothalamic-pituitary-thyroid, hypothalamic-pituitary-gonadal, and prolactin axes. By reproducing physiological hypothalamic stimulation, dynamic testing offers a more comprehensive assessment of neuroendocrine function than isolated basal hormone measurements and may reveal subtle or subclinical abnormalities that would otherwise remain undetected. Given the limited understanding of endocrine involvement in chronic Chagas disease, this integrated functional approach may provide valuable insights into neuroendocrine adaptations associated with chronic *Trypanosoma cruzi* infection[8].

The aim of this study was to evaluate the hypothalamic-pituitary endocrine axes, including the adrenal, thyroid, prolactin, and gonadal axes, in patients with the chronic form of CD.

2. METHODS

This prospective study included patients diagnosed with chronic CD who were referred to the outpatient center of the Evandro Chagas National Institute of Infectious Diseases (INI) of the Oswaldo Cruz Foundation (Fiocruz) between January 1994 and December 1996. After the serological diagnosis of chronic CD was confirmed using two distinct reactive serological techniques, all patients underwent an initial evaluation protocol, which included sociodemographic information, epidemiological history of CD, clinical anamnesis, and physical examination focused on chronic CD-related cardiovascular signs and symptoms, including a 12-lead electrocardiogram (ECG) and a two-dimensional Doppler echocardiogram. Based on the presence of symptoms related to the digestive form of CD, upper gastrointestinal endoscopy, esophagography, colonoscopy, and contrast barium enemas were performed. The clinical forms of chronic

CD were retrospectively classified according to the 2nd Brazilian Consensus on Chagas Disease (2015), in which the cardiac form is stratified into stages A, B1, B2, C, or D, and the digestive form into megaesophagus, megacolon, or both.

A questionnaire was administered prior to the procedure to assess clinical complaints related to adrenal, thyroid, prolactin, testicular, and ovarian functions.

The endocrinological evaluation was carried out as follows procedure. On Day 1, patients were admitted on the evening prior to the examination and fasted from 10:00 p.m. onward. Preparation of materials was also initiated on the previous day, when eight 10-mL plastic syringes intended for blood collection were heparinized and refrigerated. On Day 2, at 7:00 a.m., venipuncture of a large-caliber vein in the antecubital fossa was performed and maintained with a slow infusion of glucose solution. At 8:00 a.m., a baseline blood sample (8 mL) was obtained. Subsequently, 100 µg of CRHh (BACHEM Biosciences, Philadelphia, USA), 200 µg of TRH (Fudap EPM), and 100 µg of LHRH (Relisorm Serono) were administered intravenously over a slow infusion. Following hormone administration, blood samples were collected at 15, 30, 45, 60, 90, 120, and 150 minutes. All chilled blood samples were centrifuged, and plasma and serum aliquots were transferred into Eppendorf tubes and stored at −20°C until hormonal assays were performed.

The following hormones were measured by radioimmunoassay (Diagnostic Products Corporation): cortisol, total T3, total T4, free T4 (FT4), thyroid-stimulating hormone (TSH), prolactin, total testosterone, free testosterone, estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Adrenocorticotropic hormone (ACTH) was measured by radioimmunoassay (CIS Biointernational). Reverse T3 (rT3) was measured by radioimmunoassay (Serono).

The normal response pattern to the combined test was defined as follows: prolactin and TSH responses to TRH were considered normal when there was at least a twofold increase over baseline at 30 minutes. For FSH and LH responses to LHRH, an increase of at least 1.5 times the baseline value was considered normal. Regarding cortisol and ACTH responses after CRH administration, a delta increase of ≥7 µg/dL and ≥5 pg/mL, respectively, was considered normal.

In female patients, the combined test was performed between the 5th and 8th days of the menstrual cycle.

Data analysis

The results were expressed as median and mean ± standard deviation. Statistical analysis was performed using the Wilcoxon test. The significance level was set at 5%.

Ethical approval

In Brazil, the requirement that all research involving human subjects be submitted for ethical review was formally established in 1996 with the publication of CNS Resolution No. 196/1996 by the National Health Council. Thus, at the time this study was conducted, submission to a research ethics committee was not mandatory. However, all patients were enrolled in the study after reading and signing the informed consent form, which was approved by the Research Ethics Committee of Fiocruz.

3. RESULTS

A total of fifteen patients were evaluated, including five with the indeterminate form and ten with the cardiac form of the disease, of whom five were classified as stage A and five as stage B. Nine patients were male, with a median age of 47 years (range: 25–62), and six were female, with a median age of 35.5 years (range: 28–45). There were no reports of any clinical complaints before or after the administration of the combined test. The results are presented in Table 1.

Table 1. Characteristics of study population stratified according demographic aspects and clinical presentation (n= 15).

Patient	Sex	Age	Clinical form		Patient	Sex	Age	Clinical form
1	M	44	I		***9***	F	31	B
2	M	25	I		***10***	F	42	I
3	M	52	A		***11***	M	39	B
4	M	62	A		***12***	M	49	B
5	M	47	I		***13***	F	33	I
6	M	45	A		***14***	F	38	B
7	F	45	A		***15***	F	28	A
8	M	56	B					

M = male, F = female. Age (years). According to the 2nd Brazilian Consensus on Chagas Disease: I = indeterminate form, A = cardiac form, stage A, B = cardiac form, stage B

Baseline cortisol levels were outside the normal range in four patients. In one patient, plasma cortisol measurements could not be performed due to technical and operational reasons. Regarding the response to CRHh, two patients exhibited a reduced cortisol response, although they had normal baseline cortisol levels. The four patients with low baseline cortisol showed an adequate response to CRHh. The results of baseline serum cortisol levels and peak cortisol after CRH are shown in Table 2.

Table 2. Baseline serum cortisol levels and peak levels after CRHh administration.

Patient	Baseline cortisol / post-CRHh cortisol (µg/dL)
1	-
2	**4.8** / 16.5
3	8.5 / **14.5**
4	10.0 / 19.0
5	9.5 / **14.5**
6	**2.0** / 17.5
7	11.5 / 50.0
8	13.3 / 31.5
9	8.0 / 16.0
10	5.0 / 30.0
11	24.0 / 54.0
12	18.0 / 50.0
13	**4.2** / 14.0
14	**3.6** / 17.0
15	8.0 / 28.0

Normal baseline cortisol = 5–25 µg/dL
Normal post-CRHh cortisol response: a delta increase of ≥7 µg/dL

All patients had normal baseline serum ACTH levels, as well as normal responses to CRHh. The results are presented in Table 3.

Table 3. Baseline serum ACTH levels and peak levels after CRHh administration.

Patient	Baseline ACTH / post-CRHh ACTH (pg/mL)
1	4.1 / 10.3
2	4.1 / 9.10
3	4.1 / 16.0
4	5.9 / 19.0
5	4.0 / 13.0

6	5.9 / 14.6
7	7.8 / 13.2
8	2.0 / 8.7
9	3.6 / 11.4
10	3.1 / 15.8
11	3.5 / 15.5
12	5.9 / 16.4
13	6.8 / 22.7
14	7.3 / 19.9
15	3.1 / 15.9

Normal baseline ACTH < 60 pg/dL
Normal post-CRHh ACTH response: a delta increase of ≥5 pg/dL

Total T3 levels ranged from 50 to 170 ng/dL, with three patients presenting decreased values. Total T4 levels varied between 4.8 and 12 µg/dL and were within the normal range in all patients. Free T4 levels ranged from 0.5 to 1 ng/dL; reduced levels were observed in eight patients, while no elevations were detected. Reverse T3 levels ranged from 0.13 to 0.33 and remained within normal limits in all cases. The results are presented in Table 4.

Table 4: Baseline serum levels of total T3 and T4, free T4, and reverse T3

Patient	T3 (ng/dL)	T4 (µg/dL)	Free T4 (ng/mL)	T3 reverse (ng/mL)
1	**68**	12.0	1.00	0.33
2	165	8.0	0.95	0.18
3	155	6.6	**0.52**	0.18
4	165	6.1	**0.75**	0.15
5	108	7.7	**0.75**	0.15
6	90	7.5	**0.75**	0.18
7	109	8.0	0.95	0.20
8	100	6.1	**0.50**	0.16
9	145	8.0	0.90	0.18
10	145	7.7	0.85	0.17
11	**50**	4.8	0.90	0.15
12	100	5.4	**0.70**	0.15
13	170	7.5	**0.75**	0.13
14	95	6.1	0.80	0.18
15	**75**	6.1	**0.66**	0.13

Normal baseline: T3 total = 86-187 ng/dL, T4 total = 4.5-12.5µg/dL, free T4 = 0.8-2.0 ng/dL and T3 reverse = 0.09-0.35 /mL T3

Serum TSH levels ranged from 0.4 to 4.0 µIU/mL, and all patients had values within the normal range. Regarding the TSH response to TRH, all patients showed a normal response, with a significant increase at 30 minutes compared to 60 minutes. The results are presented in Table 5.

Table 5. Baseline serum TSH levels and peak levels after TRH administration.

Patient	TSH (µIU/mL) - 0’	TSH (µIU/mL) - 30’	TSH (µIU/mL) - 60’
1	0.4	4.0	3.0
2	1.5	15.0	9.5
3	4.0	26.0	18.0
4	0.8	8.4	4.6
5	1.3	19.0	14.0
6	0.7	6.2	5.2
7	1.2	15.0	11.4
8	3.0	29.0	21.0
9	1.3	17.6	11.0
10	1.0	12.3	8.0
11	1.1	8.4	5.2
12	1.1	17.0	11.0
13	3.3	23.8	20.6
14	0.7	8.5	4.0
15	1.2	12.4	7.7

Normal baseline: TSH = 0.4-4.5 µIU/mL

Serum prolactin levels ranged from 0 to 34 ng/mL in men and from 9.5 to 27 ng/mL in women. Hyperprolactinemia was present in two men. The prolactin response to TRH was normal in eight men and six women and reduced in one man. The results are presented in Table 6.

Table 6. Baseline serum prolactin levels and peak levels after TRH administration.

Patient	Sex	Prolactin (ng/mL) – 0’	Prolactin (ng/mL) – 30’	Prolactin (ng/mL) – 60’	Prolactin (ng/mL) – 90’
1	M	6.0	4.0	11	17
2	M	**18**	24	14	20
3	M	6.0	33	8.0	10
4	M	7.6	26	7.5	22
5	M	4.0	88	18	31
6	M	0	21	16.5	6.9
7	F	20	83	60	66
8	M	**34**	75	29	42
9	F	18	49	28	18
10	F	27	120	75	42
11	M	13	78	32	22
12	M	7.5	23	17	9.5
13	F	16.5	24	31	9.5
14	F	11	38	22	21
15	F	9.5	60	18	22

Normal baseline prolactin in men: up to 15 ng/mL.

Normal baseline prolactin in women: up to 20 ng/mL

Total testosterone levels in male patients ranged from 530 to 870 ng/dL, with no cases of decreased values. Free testosterone levels ranged from 7.3 to 23 pg/mL, with only one patient exhibiting a reduced level. The results are presented in Table 7.

Table 7. Baseline serum levels of total and free testosterone in men.

Patient	Total testosterone (ng/dL)	Free testosterone (ng/dL)
1	600	**7.3**
2	640	19.0
3	800	11.0
4	640	13.0
5	790	19.0
6	870	16.0
8	640	12.0
11	530	14.0
12	600	23.0

Normal baseline total testosterone in men: 245 – 1836 ng/dL
Normal baseline free testosterone in men: 10.8 - 40

Baseline plasma levels in women ranged from 20.0 to 64.0 pg/mL. According to normal values for women of reproductive age during the follicular phase, five patients had reduced levels. The results are presented in Table 8.

Table 8. Baseline serum levels estradiol in women.

Patient	Estradiol (pg/mL)
7	**20.0**
9	64.0
10	**28.0**
13	**20.0**
14	**42.0**
15	**20.0**

Normal baseline estradiol in women: 60-200 pg/mL

Plasma FSH levels in men ranged from 1.5 to 5.2 mIU/mL and were within normal limits in all cases. In women, levels ranged from 2.5 to 36.0 mIU/mL, with two patients presenting elevated values and two presenting reduced values. Following LHRH stimulation, two men exhibited a diminished response and two showed no response. Among women, two demonstrated a reduced response and one showed no response. No significant increase in FSH levels was observed at either 30 or 60 minutes, and no

significant differences were found between sexes.

Serum LH levels in men ranged from 1.5 to 3.0 mIU/mL and were within normal limits in all cases. In women, levels ranged from 1.5 to 38.0 mIU/mL, with two patients presenting elevated values. The LH response to LHRH was normal in all participants. No significant differences in LH levels were observed between sexes. The results are presented in Table 9.

Table 9. Baseline serum FSH and LH levels and peak levels after TRH administration.

Patient	Sex	FSH (mIU/mL) 0'	FSH (mIU/mL) 30'	FSH (mIU/mL) 60'	LH (mIU/mL) 0'	LH (mIU/mL) 30'	LH (mIU/mL) 60'
1	M	3.0	7.0	8.0	1.5	31.5	42.0
2	M	2.5	1.9	2.5	3.0	9.0	9.0
3	M	1.9	3.8	1.9	1.5	3.0	3.0
4	M	4.4	7.5	10.0	1.5	13.0	9.0
5	M	3.2	3.8	4.4	1.5	17.0	17.0
6	M	5.2	7.0	8.0	1.5	19.0	15.0
7	F	**36.0**	44.0	58.0	**15.0**	42.0	42.0
8	M	3.8	3.0	3.0	1.5	17.0	13.0
9	F	6.6	7.5	8.5	1.5	3.0	1.5
10	F	**3.0**	5.0	3.8	1.5	6.0	3.0
11	M	5.0	6.6	6.6	1.5	13.0	7.5
12	M	1.5	5.6	1.9	1.7	9.0	6.0
13	F	**30.0**	34.0	30.0	**38.0**	60.0	7.5
14	F	**2.5**	3.2	5.6	1.5	3.0	3.0
15	F	5.0	6.85	7.55	1.5	5.0	3.0

Normal baseline FSH in men: 1.1-13.5 mIU/mL
Normal baseline FSH in women: 3.3-8.8 mIU/mL
Normal baseline LH in men: 0.4-5.7 mIU/mL
Normal baseline LH in women: 0.6-6.2 mIU/mL

4. DISCUSSION

To our knowledge, this is one of the few studies to provide an integrated functional assessment of multiple hypothalamic-pituitary endocrine axes in patients with chronic CD using a combined stimulation test. Although chronic *Trypanosoma cruzi* infection is well recognized for its cardiac, digestive, and autonomic nervous system involvement, its effects on neuroendocrine regulation have received comparatively little attention. Our findings demonstrate that the overall integrity of hypothalamic-pituitary

function is preserved in most patients with chronic CD, while subtle alterations are observed in adrenal reserve and thyroid hormone metabolism.

The hypothalamic-pituitary-adrenal (HPA) axis showed preserved pituitary responsiveness, as evidenced by normal ACTH secretion following CRH stimulation in all patients. However, two individuals exhibited an impaired cortisol response despite normal basal cortisol concentrations, suggesting reduced adrenocortical reserve rather than central dysfunction. Conversely, patients with low basal cortisol concentrations demonstrated adequate responses to CRH, indicating preserved adrenal responsiveness under stimulated conditions. These findings suggest that basal hormone measurements alone may underestimate mild adrenal dysfunction and reinforce the value of dynamic endocrine testing in chronic systemic diseases. Although overt adrenal insufficiency does not appear to be a characteristic feature of chronic CD, a reduction in adrenal functional reserve could become clinically relevant during periods of acute physiological stress, severe infection, or surgery[9].

The mechanisms responsible for this reduced adrenal reserve remain speculative. Chronic immune activation, persistent low-grade inflammation, autonomic nervous system dysfunction, and possible microvascular alterations of the adrenal cortex have all been implicated in the pathophysiology of chronic CD and could contribute to subtle impairment of adrenal steroidogenesis[10]. Nevertheless, the preserved ACTH responses observed in the present study argue against hypothalamic or pituitary dysfunction as the primary mechanism.

Evaluation of the hypothalamic-pituitary-thyroid (HPT) axis also demonstrated preserved pituitary function, since all patients exhibited appropriate TSH responses following TRH administration. Interestingly, reduced free T4 concentrations were observed in more than half of the patients, whereas low T3 syndrome was identified in a smaller proportion. Despite these findings, reverse T3 concentrations remained within the normal range. This hormonal profile differs somewhat from the classical pattern of non-thyroidal illness syndrome (NTIS), in which elevated reverse T3 levels are generally expected[11]. One possible explanation is that patients with stable chronic CD exhibit adaptive metabolic changes distinct from those observed during acute critical illness[12]. Alternatively, preserved 5'-monodeiodinase activity may have prevented reverse T3 accumulation despite reductions in circulating thyroid hormones[13]. These observations

suggest that alterations in thyroid hormone metabolism in chronic CD are likely adaptive rather than indicative of primary thyroid disease.

The prolactin axis remained essentially intact. Basal hyperprolactinemia was detected in only two male patients, while nearly all participants demonstrated normal prolactin responses following TRH stimulation. Since prolactin secretion is highly sensitive to hypothalamic dopaminergic regulation, these findings further support preservation of hypothalamic and pituitary regulatory mechanisms despite the well-described autonomic nervous system abnormalities associated with chronic CD[14].

Similarly, the hypothalamic-pituitary-gonadal axis was largely preserved. Basal testosterone concentrations remained within the normal range in male patients, and LH responses to GnRH stimulation were normal in all individuals. Although a small number of patients exhibited reduced FSH responses, these abnormalities were isolated and were not accompanied by consistent alterations in gonadal steroid concentrations. In women, reduced estradiol levels should be interpreted cautiously, given the physiological variability of ovarian steroid secretion during the menstrual cycle and the relatively small sample size. Overall, these findings do not support clinically significant gonadotroph dysfunction in chronic CD.

Collectively, our results indicate that chronic CD is not associated with generalized hypothalamic-pituitary failure. Instead, the endocrine abnormalities identified appear to reflect selective functional adaptations involving peripheral endocrine organs rather than impairment of central neuroendocrine regulation. This distinction is important because chronic inflammatory diseases frequently induce metabolic and hormonal adaptations that differ substantially from those observed in primary endocrine disorders [10].

The present study has several strengths. The simultaneous evaluation of four endocrine axes using a standardized combined stimulation test provides a comprehensive assessment of neuroendocrine function that cannot be obtained through isolated basal hormone measurements. Furthermore, the use of dynamic testing allows detection of subtle abnormalities that might otherwise remain clinically silent.

Some limitations should also be acknowledged. The relatively small sample size limits statistical power and precludes subgroup analyses according to the different

clinical forms of CD. In addition, the absence of a healthy control group prevents direct comparison with non-infected individuals. Because the study was originally conducted during the 1990s, hormonal assays were performed using radioimmunoassay techniques, whereas current practice relies predominantly on highly sensitive immunometric assays and liquid chromatography-mass spectrometry for selected hormones. Nevertheless, the physiological principles underlying the stimulation tests remain valid, and the present findings provide valuable information that has not been extensively investigated in more recent studies.

Future investigations including larger cohorts, healthy controls, patients with advanced cardiac and digestive disease, and contemporary endocrine methodologies will be important to confirm these observations and determine whether the subtle endocrine alterations identified influence long-term clinical outcomes, disease progression, exercise tolerance, or prognosis in chronic Chagas disease.

5. CONCLUSION

The present study demonstrates that, in patients with Chagas disease, the function of most hypothalamic–pituitary axes remains preserved, although specific functional alterations can be identified. Evaluation of the hypothalamic-pituitary-adrenal (HPA) axis revealed preserved hypothalamic–pituitary function associated with reduced adrenocortical reserve, suggesting impairment of the adrenal gland's functional capacity despite preserved basal hormone secretion. This finding may reflect subclinical changes associated with disease progression and warrants attention because of its potential implications for the physiological stress response.

The hypothalamic-pituitary-thyroid (HPT) axis was found to be globally preserved. However, the high frequency of reduced serum thyroxine (T4) levels, together with the occurrence of low T3 syndrome in a subset of patients, indicates alterations in peripheral thyroid hormone metabolism consistent with non-thyroidal illness syndrome (NTIS). The maintenance of reverse T3 concentrations within the normal range suggests preserved 5'-monodeiodinase activity, indicating that the observed hormonal changes are more likely to represent adaptive mechanisms related to the patients' clinical condition rather than primary dysfunction of the thyroid axis.

Pituitary lactotroph function was also preserved, as were both the male and

female hypothalamic-pituitary-gonadal (HPG) axes, with hormonal profiles consistent with normal physiological function. These findings further support the absence of significant impairment of anterior pituitary and gonadal endocrine function in the study population.

Taken together, these findings indicate that, despite its systemic nature, Chagas disease is not associated with generalized dysfunction of the hypothalamic-pituitary axes. The endocrine alterations identified were mainly confined to reduced adrenocortical functional reserve and changes in thyroid hormone metabolism, suggesting endocrine–metabolic adaptations rather than overt hormonal insufficiency. These results contribute to a broader understanding of the endocrine manifestations of Chagas disease and provide a basis for future investigations into the underlying pathophysiological mechanisms and their clinical significance, particularly in patients with chronic forms of the disease.

Conflict of interest

The authors have no conflict of interest to declare.

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