Physiology & Behavior 142 (2015) 90–96

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Endocrine and metabolic function in male Carioca High-conditioned Freezing rats

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HIGHLIGHTS

- Endocrine and metabolic functions are affected in a rat model of anxiety disorder.
- Anxiety may contribute to the development of metabolic diseases.
- A rat model of anxiety disorder induces an increase in corticosterone serum levels.

ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 21 January 2015
Accepted 22 January 2015
Available online 23 January 2015

Keywords:
Anxiety
Corticosterone
Thyroid function
Metabolism
Fat depots

ABSTRACT

The aim of this study was to characterize Carioca High-conditioned Freezing rats (CHF) regarding their endocrine and metabolic backgrounds. We found an increase in serum corticosterone (CTRL: 96.7 ± 21.65 *vs* CHF: 292.0 ± 40.71 ng/ml) and leptin (CTRL: 9.5 ± 1.51 *vs* CHF: 19.2 ± 4.32 ng/ml). Serum testosterone (CTRL: 3.3 ± 0.29 *vs* CHF: 2.0 ± 0.28 ng/ml) and T3 (CTRL: 52.4 ± 2.74 *vs* CHF: 42.7 ± 2.94 ng/dl) were decreased in the CHF group, but serum TSH and T4 were unaffected. Body weight and food intake were unchanged, nevertheless retroperitoneal fat (CTRL: 2.2 ± 0.24 *vs* CHF: 4.8 ± 0.64 g) and epididymal fat (CTRL: 2.6 ± 0.20 *vs* CHF: 4.8 ± 0.37 g) depot weights were around 2-fold higher in CHF animals. BAT weight was similar in both groups. Serum triglycerides (CTRL: 41.4 ± 6.03 *vs* CHF: 83.2 ± 17.09 mg/dl) and total cholesterol (CTRL: 181.6 ± 5.61 *vs* CHF: 226.4 ± 13.04 mg/dl) were higher in the CHF group. Fasting glycemia (CTRL: 68.7 ± 3.04 *vs* CHF: 82.3 ± 2.99 mg/dl) was also higher in the CHF group, however glucose tolerance test response and serum insulin levels were similar between the groups. Oxygen consumption (CTRL: 10.5 ± 0.40 *vs* CHF: 7.9 ± 0.58 VO_2 ml/min/kg$^{0.75}$) and BAT D2 activity (CTRL: 0.7 ± 0.17 *vs* CHF: 0.3 ± 0.04 fmol T4/min/mg ptn) were lower in the CHF group. Our data show that anxiety could impair endocrine and metabolic functions and may contribute to the development of metabolic diseases.

1. Introduction

Anxiety can be defined as a response to a potentially dangerous situation and is accompanied by a characteristic set of behavioral and physiological responses, including avoidance, vigilance, arousal, and activation of the hypothalamic–pituitary–adrenal (HPA) and sympathoadrenal axes. This set of responses has adaptive value and is evoked to protect the individual from danger [39,40]. However, for some individuals, anxiety responses can become persistent, uncontrolled, excessive, and inappropriate, without any adaptive meaning; thus, it can become a disorder that requires clinical intervention [35]. Anxiety disorders represent one of the most prevalent mental disorders worldwide [2,22].

A common characteristic of anxiety disorders is deregulation of the HPA axis, resulting in increased serum glucocorticoid levels [30,44]. Glucocorticoids (cortisol in humans and corticosterone in rodents) are metabolically active hormones that play an important role in the stress response and act on different biological systems. However, sustained elevations for long periods of time may have deleterious effects on different systems and thus lead to metabolic dysfunction, such as an increase in fat depots, insulin resistance, and dyslipidemia [42]. Some evidence indicates that anxiety disorders in rats are related to metabolic disturbances [6]. Human data also show a correlation between anxiety disorders and obesity [1,25].

Anxiety has been studied from neuroanatomical, neurochemical, and behavioral perspectives, but metabolic studies are scarce. Our

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http://dx.doi.org/10.1016/j.physbeh.2015.01.028
0031-9384/

group [19] produced two rat lines, named Carioca High- and Low-conditioned Freezing (CHF and CLF), that were selectively bred for high and low levels of defensive freezing behavior in response to contextual cues previously associated with footshock. This defensive freezing response has been shown to be an important animal model of anxiety disorders [7]. The CHF line was validated behaviorally by tests used in other animal model of anxiety, including the elevated plus maze, the social interaction test, and defensive responses induced by electrical stimulation of the dorsal periaqueductal gray [11,16,20,21]. In the present study we evaluated the relationship between sustained elevated corticosterone serum levels and development of metabolic dysfunctions in the CHF line. In order to test whether CHF animals would be more vulnerable to the development of metabolic diseases we evaluated the endocrine and metabolic profiles in CHF animals as compared to control animals (CTRL) that were not selectively bred.

2. Materials and methods

2.1. Animals

The animals used in the present study were born in the colony room of the PUC-Rio Psychology Department under a controlled room temperature (24 ± 1 °C) and a 12 h/12 h light/dark cycle (lights on 7:00 AM–7:00 PM). To assign a control number for each animal, one toe from each foot was amputated, and a small incision was made on one of the ears 6 to 8 days after birth. Upon weaning at 21 days of age, each animal was separated by sex and housed in groups of five to seven in polycarbonate cages (18 × 31 × 38 cm) according to their respective lines with food and water available *ad libitum*. The CHF animals were generated according to procedures described in previous work [19]. Briefly, albino Wistar rats were selectively bred for differences in defensive freezing behavior in a contextual fear-conditioning paradigm. Male rats from both the CHF and CTRL groups were 2 to 3 months old at the beginning of the experiment. Bodyweight varied (254–379 g for CHF and 250–394 g for CTRL). The experimental procedures were approved by the Institutional Committee for Use of Animals in Research, and the procedures used were compliant with the International Guiding Principles for Biomedical Research Involving Animals, Council for International Organizations of Medical Sciences (Geneva, Switzerland), and the guiding principles for care and use of the American Physiological Society. Animal handling and the methods of sacrifice were reviewed and approved by the Committee for Animal Care and Use of PUC-Rio (protocol no. 20/2009). Euthanasia by decapitation was performed in the morning after 1–2 weeks of adaptation to an inverted dark–light cycle (light off 7:00 h AM to 7:00 h PM), at the Laboratório de Fisiologia Endócrina Doris Rosenthal, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.

2.2. Radioimmunoassays

For serum hormone quantification, blood was collected and centrifuged at 1200 ×*g* at 4 °C for 15 min, and serum was stored at −20 °C for further measurements of corticosterone, testosterone, T3, T4, thyroid stimulating hormone (TSH), leptin, and insulin. Serum corticosterone (Coat-A-Count, Rat Corticosterone, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) was determined using specifically coated tubes in a radioimmunoassay (RIA) kit. The intra- and inter-assay coefficients of variation were 4.0–12.2% and 4.8–14.9%, respectively, and the sensitivity was 5.7 ng/ml. Serum testosterone (DSL 4100, Diagnostic Systems Laboratories, Texas, USA) was also determined using specifically coated tubes in a RIA kit. The intra- and inter-assay coefficients of variation were 6.7–8.1% and 8.1–10.5%, respectively, and the sensitivity was 0.05 ng/ml. Serum insulin (Linco Research, St. Charles, Missouri, USA) was measured using a specific RIA kit. The intra- and inter-assay coefficients of variation were 2.2–4.6% and 8.9–9.4%, respectively, and the sensitivity was 0.078 ng/ml. Serum leptin (Linco Research, St. Charles, Missouri, USA) was also measured using a specific RIA kit. The intra- and inter-assay coefficients of variation were 2.0–4.6% and 3.0–5.7%, respectively, and the sensitivity was 0.5 ng/ml. Serum T3 and T4 were determined using specifically coated tubes in RIA kits (Diagnostic Systems Laboratories, Texas, USA). The intra- and inter-assay coefficients of variation for T3 were 5.0–6.5% and 4.2–6.0%, respectively, and the sensitivity was 4.3 ng/dl. The intra- and inter-assay coefficients of variation for T4 were 2.9–5.1% and 7.1–7.4%, respectively, and the sensitivity was 0.4 ng/dl. All of the procedures were performed according to the manufacturers' recommendations, and the kits presented no significant cross-reactivity with other iodothyronines. Serum TSH RIA measurements were performed using a kit supplied by the National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD, USA) and are expressed in terms of the preparation (RP-3) provided.

2.3. Food intake, body weight, and fat depot evaluation

Food Intake was measured for 7 days before sacrifice and is expressed as daily food intake per animal in each cage. Body weight was measured before euthanasia. After euthanasia by decapitation, fat depots were excised, dissected, and weighed using a precision balance (Digimed, KN, São Paulo, Brazil).

2.4. Serum total cholesterol and triglycerides

Serum triglycerides and total cholesterol levels were measured using an enzymatic colorimetric method (BioClin Systems, São Paulo, Brazil), and the procedures were performed according to the manufacturer's recommendations.

2.5. Fasting glycemia and glucose tolerance test

The animals were fasted for 12 h until the beginning of the test. At time 0, glucose was measured in tail blood using a glucometer (Accu Check Advantage II) to determine the fasting glucose level. Glucose values were also measured 30, 60, 120, and 180 min after orogastric administration of a dextrose solution (1.83×10^{-3} mol/100 g body weight).

2.6. Oxygen consumption

The animals were allocated to metabolic cages (Panlab, Physiocage, Harvard Instruments, Barcelona, Spain) to measure oxygen consumption (Panlab, LE 405 Gas Analyzer, Harvard Instruments, Barcelona, Spain) for 24 consecutive hours (12 h during the light cycle and 12 h during the dark cycle). The data were obtained using specific software (Panlab, Metabolism, Harvard Instruments, Barcelona, Spain).

2.7. Brown adipose tissue type 2 iodothyronine deiodinase activity

Type 2 iodothyronine deiodinase (D2) activity was determined using methods previously published [31]. Briefly, brown adipose tissue (BAT) was excised and dissected on ice to avoid muscular and white fat contamination. Previously frozen BAT (15 mg) was homogenized on 1 ml sucrose–dithiothreitol (DTT; 0.25 M sucrose and 10 mM DTT) buffer and stored at −70 °C until the day of the assay. Additionally, 20 μl of each sample was separately stored at −20 °C, and the protein content was determined using the method of Bradford [9].

D2 activity was measured using the homogenate (20 μg of protein) in PE buffer (100 mM sodium phosphate and 1 mM EDTA, pH 6.9), 20 mM DTT, 100 nM propylthiouracil, and 1 nM T4. Total reaction volume was 300 μl, to which were added 100 μl (100,000 cpm) of the freshly purified tracer (Sephadex LH20) T4-^{125}I (Perkin-Elmer Life

Sciences, Boston, MA, USA), and the reaction was incubated at 37 °C for 3 h. After incubation, the reaction was stopped on ice, and 200 μl bovine serum and 100 μl of 50% trichloroacetic acid were added. The tubes were then centrifuged at 8000 ×*g* for 3 min. The supernatant (360 μl) was transferred to counting tubes to measure radiation on a gamma counter (CompuGama, LKB, 1470 Wallak, Turkey, Finland).

3. Statistical analyses

The data are expressed as mean ± SEM and were analyzed using an unpaired *t*-test and Prism 4 software (GraphPad, San Diego, CA, USA). Values of $p < 0.05$ were considered statistically significant.

4. Results

4.1. Endocrine function

As shown in Table 1, CHF animals exhibited an increase in serum levels of corticosterone ($t_{28} = 4.23$, $p < 0.001$) and leptin ($t_{16} = 2.14$, $p < 0.05$), whereas the serum levels of testosterone ($t_{12} = 3.09$, $p < 0.01$) and T3 ($t_{29} = 2.40$, $p < 0.05$) decreased as compared to CTRL animals. No differences were found between CHF and CTRL animals in serum T4, TSH, and insulin levels (all $p > 0.05$).

4.2. Body weight and fat depots

Table 2 presents several body composition parameters. CHF animals had greater retroperitoneal ($t_{30} = 3.84$, $p < 0.001$) and epididymal ($t_{30} = 5.10$, $p < 0.001$) fat depots than the controls. No differences in body weight were found between CHF and CTRL animals.

4.3. Metabolism

Fig. 1 shows the mean ± SEM serum levels of total cholesterol (Fig. 1A) and triglycerides (Fig. 1B). CHF animals exhibited higher levels of both parameters compared with CTRL animals (total cholesterol: $t_{17} = 3.28$, $p < 0.05$; triglycerides: $t_{17} = 2.41$, $p < 0.05$). Serum total cholesterol levels were 181.6 ± 5.61 mg/dl in the CTRL group and 226.4 ± 13.04 mg/dl in the CHF group (Fig. 1A). Serum triglyceride levels were 41.4 ± 6.03 mg/dl in the CTRL group and 83.2 ± 17.09 mg/dl in the CHF group (Fig. 1B).

Carbohydrate metabolism was also impaired. Fasting glycemia was higher in CHF animals (68.7 ± 3.04 mg/dl for CTRL *vs.* 82.3 ± 2.99 mg/dl for CHF; Fig. 2A), although the glucose tolerance test was unaffected (18,070 ± 658.7 area-under-the-curve [AUC] for CTRL *vs.* 19,680 ± 624.7 AUC for CHF; Fig. 2C).

Table 1
Serum total corticosterone, testosterone, T3, T4, TSH, leptin, and insulin levels in control (CTRL) and Carioca High-conditioned Freezing (CHF) rats (corticosterone, $n = 15$; testosterone, $n \geq 6$; T3, $n \geq 15$; T4, $n \geq 10$; TSH, $n \geq 17$; leptin, $n = 9$; insulin, $n \geq 9$). The results are expressed as mean ± SEM. $^*p < 0.05$, *vs.* CTRL group.

	CTRL	CHF
Corticosterone (ng/ml)	96.7 ± 21.65	292.0 ± 40.71*
Testosterone (ng/ml)	3.3 ± 0.29	2.0 ± 0.28*
T3 (ng/dl)	52.4 ± 2.74	42.7 ± 2.94*
T4 (μg/dl)	3.4 ± 0.38	4.2 ± 0.45
TSH (ng/ml)	1.0 ± 0.12	1.3 ± 0.2
Leptin (ng/ml)	9.5 ± 1.51	19.2 ± 4.32*
Insulin (ng/ml)	2.0 ± 0.30	2.1 ± 0.27

Table 2
Body weight, daily food intake, retroperitoneal fat depot weight, epididymal fat depot weight, and brown adipose tissue weight in control (CTRL) and Carioca High-conditioned Freezing (CHF) rats ($n \geq 16$ per group). The results are expressed as mean ± SEM. $^*p < 0.05$, *vs.* CTRL group.

	CTRL	CHF
Body weight (g)	327.3 ± 8.16	322.1 ± 10.16
Food intake (g/day)	20.2 ± 1.37	23.1 ± 1.25
Retroperitoneal fat depot (g)	2.2 ± 0.24	4.8 ± 0.64*
Epididymal fat depot (g)	2.6 ± 0.20	4.8 ± 0.37*
Brown adipose tissue fat depot (g)	0.24 ± 0.01	0.29 ± 0.02

4.4. Oxygen consumption

The animals in the CHF group developed an endocrine profile that affected metabolism and some white adipose tissue depots, without substantial differences in food intake or body weight. Therefore, we decided to evaluate energy expenditure. The CHF group had lower energy consumption over 24 h (10.5 ± 0.40 VO_2 ml/min/$kg^{0.75}$ for CTRL *vs.* 7.9 ± 0.58 VO_2 ml/min/$kg^{0.75}$ for CHF) in both the dark and light phases (Fig. 3). The group difference with regard to energy expenditure was not attributable to motor effects because locomotor activity was similar between CTRL and CHF animals (Fig. 3C).

4.5. Brown adipose tissue type 2 iodothyronine deiodinase activity

Brown adipose tissue is clearly involved in the control of energy expenditure, and this effect appears to depend on D2 induction in BAT, which enables an increase in T3 generated in this tissue. We evaluated D2 activity in BAT in both CTRL and CHF animals. We detected lower BAT D2 activity in CHF animals compared with CTRL animals (0.7 ± 0.17 fmol T4/min/mg for CTRL *vs.* 0.3 ± 0.04 fmol T4/min/mg protein for CHF; Fig. 4).

5. Discussion

Anxiety has been an important trait during the evolution of mammals. It has adaptive value in the environment, protects species from danger, and contributes to the perpetuation of the species [5]. However, anxiety can negatively interfere in individual's life. Most basic research studies have focused on neuroanatomy, neurochemistry, and behavior associated with anxiety. Investigations of metabolic disorders have been scarce compared with other levels of investigation. We used CHF animals and found that anxiety disorders may impair metabolism and possibly contribute to the development of chronic diseases.

We found a three-fold increase in circulating serum corticosterone levels in CHF animals, but the mechanism involved in this increase is not completely understood. Studies that used rat strains selected for unconditioned fear reported a hyperreactive HPA axis in rats with high anxiety-like behavior [26]. Furthermore, animals obtained and bred using conditioned fear paradigms exhibit elevations in basal serum corticosterone levels and high emotionality [6,18]. In humans, individuals with anxiety disorders have high serum glucocorticoid levels [30,44]. Although the mechanism is not completely understood, this increase suggests possible deregulation of the HPA axis.

Different brain structures are known to be involved in the control of the HPA axis. Lesions of the hippocampus increase glucocorticoid secretion [15], suggesting that the hippocampus plays an important inhibitory role in the regulation of the HPA axis. In fact, the hippocampus has high densities of glucocorticoid receptors (GRs) among limbic structures in the brain [34] and the pharmacological blockade of hippocampal

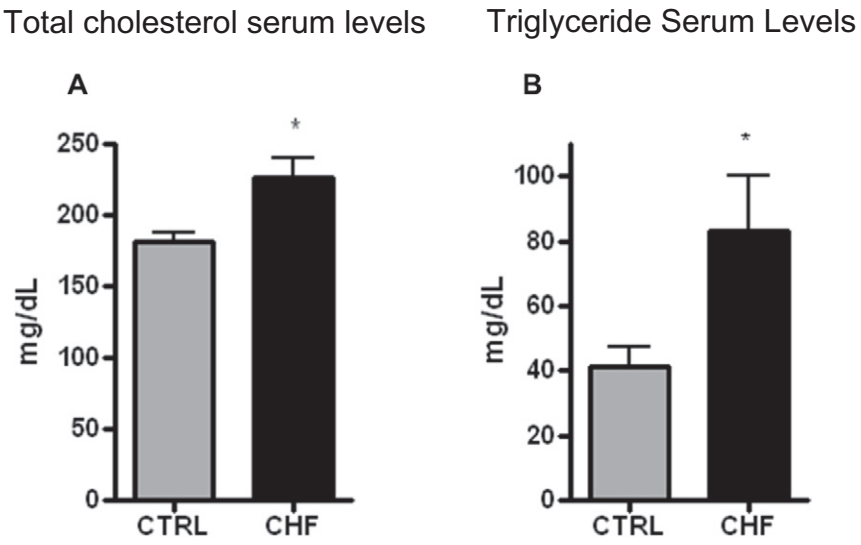

Fig. 1. Total cholesterol serum levels (A) and triglyceride serum levels (B) in control (CTRL) and Carioca High-conditioned Freezing (CHF) rats. For each parameter, $n \geq 10$. The results are expressed as mean ± SEM. $^{*}p < 0.05$, *vs.* CTRL group.

GRs with GR antagonists impaired normal feedback of the HPA axis [14]. Although we did not evaluate GR expression in the present study, Dias et al. studying CHF animals in fact did it [12], so we cannot discard an eventual relationship between hippocampal GR alterations and the high levels of serum corticosterone found here.

With regard to body composition, we found a two-fold increase in retroperitoneal and epididymal fat depots, although body weight and food intake were unaffected in CHF animals. A previous study that used animals obtained and bred for high emotionality also detected increased adiposity without any variation in body weight, food intake,

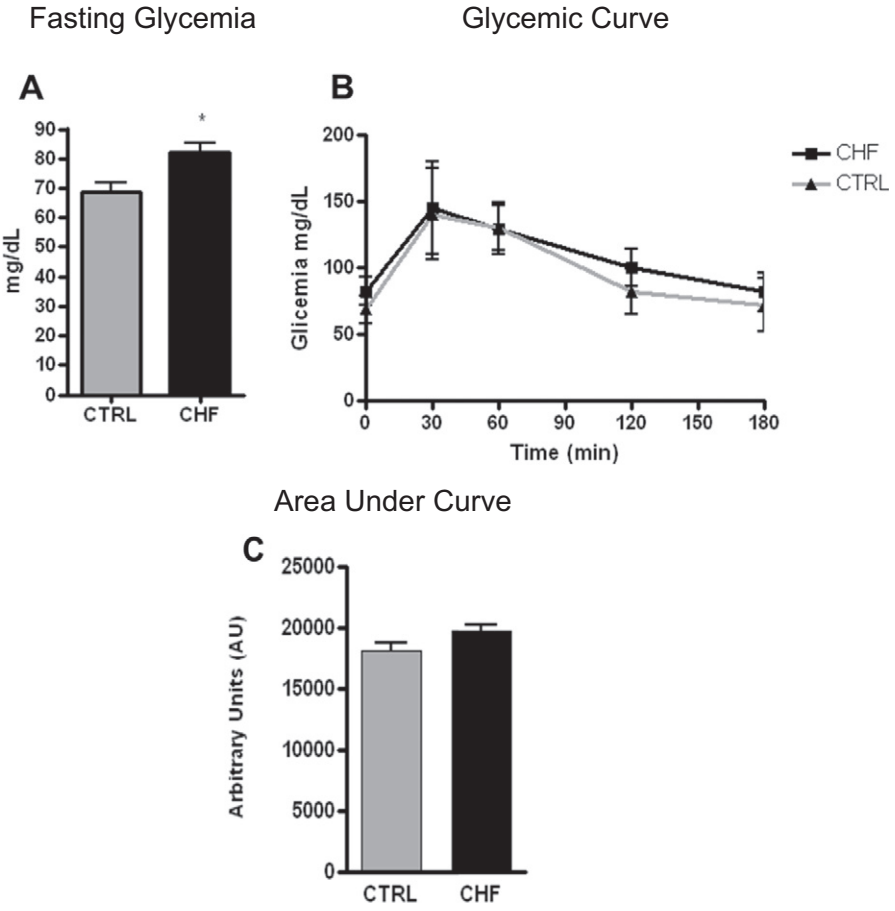

Fig. 2. Fasting glycemia (A), glycemic curve (B), and area under the curve (C) in control (CTRL) and Carioca High-conditioned Freezing (CHF) rats. For each parameter, $n = 11$. The results are expressed as mean ± SEM. $^{*}p < 0.05$, *vs.* CTRL group. Glycemia is represented as the area-under-the-curve in arbitrary units (C) in control (CTRL) and Carioca High-conditioned Freezing (CHF) rats.

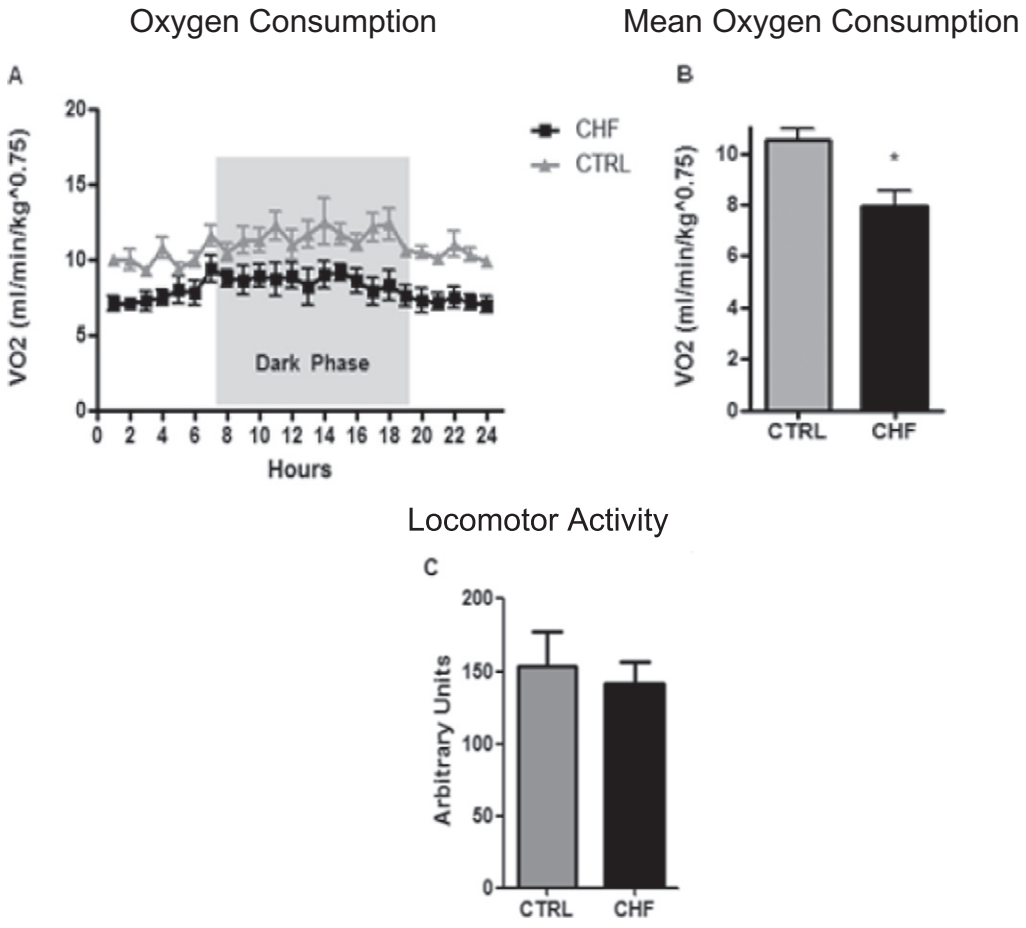

Fig. 3. Oxygen consumption over 24 h (A), mean oxygen consumption (B) over 24 h and locomotor activity (C) in control (CTRL) and Carioca High-conditioned Freezing (CHF) rats. $n = 5$. The results are expressed as mean ± SEM. $^{*}p < 0.05$, *vs.* CTRL group.

or lean mass [6], but the mechanisms involved are not completely understood. The present results suggest the involvement of a specific endocrine profile. Glucocorticoids play an important role in adipogenesis. Preadipocytes differentiate into adipocytes under the regulation of GCs in a dose-dependent manner [10], suggesting a role for GCs in preadipocyte differentiation and adipogenesis. Glucocorticoids also favor lipid storage in adipocytes by increasing the activity of lipoprotein lipase [3]. Furthermore, Farias-Silva et al. [13] reported an increase in serum GC levels induced by one daily footshock over 3 consecutive days, with a decrease in both β1 and β3 adrenoceptors. Such effects impaired the lipolysis induced by catecholamines. In the present study, the increase in corticosterone in CHF animals may be involved in the increases in retroperitoneal and epididymal fat depots by acting through different pathways of adipocyte cell physiology.

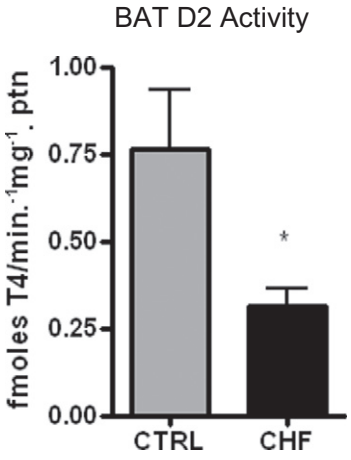

Fig. 4. Brown adipose tissue type 2 iodothyronine deiodinase (D2) activity in control (CTRL) and Carioca High-conditioned Freezing (CHF) rats. $n = 9$. The results are expressed as mean ± SEM. $^{*}p < 0.05$, *vs.* CTRL group.

Androgens are also important modulators of body fat distribution. Abdominal obesity is associated with low plasma testosterone levels [23]. Testosterone modulates adipocyte biology in different pathways. It is anti-adipogenic by inhibiting pre-adipocyte differentiation into mature adipocytes through molecular mechanisms that involve the nuclear accumulation of β-catenin, a pro-myogenic, anti-adipogenic stem cell regulatory factor [17]. Testosterone also influences lipid uptake by adipocytes. Castration significantly increased triglyceride accumulation in retroperitoneal fat, and this effect was reversed by testosterone treatment [27] and possibly attributable to higher lipoprotein lipase activity because testosterone decreases visceral lipoprotein lipase activity [32]. Castration also decreased catecholamine-induced lipolysis and the number of β-adrenoceptors. Testosterone replacement normalized these features [45]. Therefore, we also suggest a possible role for serum testosterone levels in the increase in adiposity detected in the present study. Khonicheva et al. [24] argued that serum testosterone levels are negatively correlated with anxiety traits. We detected lower serum testosterone levels in CHF animals than in CTRL animals. Therefore, the decrease in serum testosterone levels found in CHF animals could be related to an increase in adiposity.

In parallel with high retroperitoneal and epididymal fat depots, CHF animals exhibited an increase in serum triglycerides and total cholesterol levels. Boersma et al. [6] studied Roman Low and High

Avoidance rats and found an increase in total cholesterol in rats with high trait anxiety, but triglyceride levels were unchanged. Studies of patients with anxiety disorders reported a correlation between dyslipidemia and anxiety disorders [25,43]. Glucocorticoids play an important role in lipid metabolism. Some mutations in GR genes promoted an increase in the affinity between glucocorticoids and GRs, and these mutations were associated with increased serum triglycerides and total cholesterol levels [41]. A close relationship was also found between T3 and lipid metabolism. Hypothyroidism is associated with high levels of total cholesterol. The effect of T3 on lipid metabolism favors the catabolic lipoprotein pathway through modulation of the expression of low-density lipoprotein receptors in the liver and other tissues. In rats with hypothyroidism, a decrease in the hepatic level of low-density lipoprotein receptor mRNA was reported [38]. Thus, we suggest that the high levels of corticosterone associated with low serum T3 levels could be involved in the increase in serum triglycerides and total cholesterol levels detected in CHF rats.

We also found an increase in serum leptin levels and fasting glycemia in CHF animals, but basal serum insulin levels were not different between groups. In the glucose tolerance test, no difference in glycemia was found between groups. Insulinemia was not evaluated along the test. Boersma et al. clearly showed insulin resistance in animals with high emotionality. A close relationship exists between glucocorticoids and insulin resistance. Glucocorticoid treatment impairs glucose uptake by visceral adipose tissue [29], which could explain the alteration in glycemic homeostasis detected in the present study. Altogether, the increases in adiposity, serum total cholesterol, serum triglycerides, fasting glycemia, and serum leptin may suggest the development of a metabolic syndrome in CHF animals.

To our knowledge, this is the first study to report thyroid abnormalities in a model of rat anxiety disorder. Some human studies also suggested a relationship between anxiety disorders and thyroid abnormalities [33,36]. In the present study, we found lower T3 levels in CHF animals, whereas serum TSH and T4 levels remained unchanged in these animals. The secretion of thyroid hormones is regulated by the hypothalamic–pituitary–thyroid axis. Thyrotropin releasing hormone is released by neurons in the paraventricular nucleus of the hypothalamus, thus stimulating TSH production and secretion through thyrotrophs. Thyroid-stimulation hormone in the thyroid gland stimulates the synthesis and secretion of thyroid hormones. Glucocorticoid administration in subjects decreases the thyrotroph response to TRH and also basal TSH secretion [8]. However, our results showed lower T3 levels in CHF animals, without any alterations in TSH or T4, suggesting the altered peripheral metabolism of thyroid hormones.

The effect of T3 on energy expenditure is largely known. In humans, hyperthyroidism increases energy expenditure and induces weight loss. In hypothyroidism, energy expenditure is lower, with frequent weight gain. Lovejoy et al. [28] reported that administration of low doses of T3 in healthy subjects increased total energy expenditure. In rodents, BAT is an important tissue involved in energy expenditure, and the local production of T3 is essential for proper BAT function. In this tissue, T3 is generated through the activity of D2. We detected low BAT D2 activity in CHF animals, suggesting a decrease in T3 generation in BAT. CHF animals also exhibited low serum T3 levels. Altogether, these results may explain the lower oxygen consumption detected in these animals. The expression of D2 in BAT depends on β-adrenergic signaling, in which norepinephrine stimulates the expression of D2 in this tissue [4]. We suggest possible impairment in adrenergic signaling, at least with regard to BAT D2 activity and lower oxygen consumption in CHF animals, because both corticosterone and testosterone were altered and are related to β-adrenergic signaling. Furthermore, glucocorticoids inhibited the UCP1 gene response to adrenergic stimulation in a brown adipose cell line through transcriptional mechanisms [37]. These data suggest an additional role for glucocorticoids and thyroid hormones in the metabolic alterations observed in this model.

6. Conclusions

In summary, we observed impaired endocrine and metabolic function in CHF rats, an animal model of anxiety disorder. Although the precise mechanism involved in the CHF phenotype is not yet clear, our results show important modifications in the endocrine profile in CHF animals that are possibly associated with metabolic dysfunction. Additional studies should be conducted to better understand the relationships between anxiety and metabolic dysfunction.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

We are grateful for the technical assistance of Advaldo Nunes Bezerra, José Humberto Tavares de Abreu, Norma Lima de Araújo Faria, Wagner Nunes Bezerra, at the Laboratório de Fisiologia Endócrina Doris Rosenthal, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro and Flávia Candido Pereira, at the Laboratório de Neurociência Comportamental, Departamento de Psicologia, Pontifícia Universidade Católica do Rio de Janeiro.

This work was supported by grants from the Fundação Carlos Chagas Filho de Amparo `a Pesquisa do Estado do Rio de Janeiro (FAPERJ) (E26/110.609/2014) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (471454/2012-5).

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