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

F. Mousovich-Neto [a], A.L. Lourenço [a], J. Landeira-Fernandez [b], V.M. Corrêa da Costa [a,*]

[a] Laboratório de Fisiologia Endócrina Doris Rosenthal, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, CCS, Bloco G, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ 21949-902, Brazil

[b] Laboratório de Neurociência Comportamental, Departamento de Psicologia, Pontifícia Universidade Católica do Rio de Janeiro, Rua Marquês de São Vicente, 225, Rio de Janeiro, RJ 22453-900, Brazil

## 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

* Corresponding author at: Instituto de Biofísica Carlos Chagas Filho, CCS Bloco G, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ 21949-900, Brazil.
*E-mail address:* vmccosta@biof.ufrj.br (V.M. Corrêa da Costa).

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 ($1.83 \times 10^{-3}$ 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