The effect of fasting on local steroidogenesis in the brown anole, *Anolis sagrei*

Rachael Munoz and **H. Bobby Fokidis**

Department of Biology, Rollins College, Winter Park, Florida USA

Abstract

The stress response, or fight or flight response may be induced through the lack of food availability. The lack of food is clearly perceived as a stressor as it elevates GC levels and has drastic effects including suppressing reproduction and immune function, and the mobilizing of energy reserves. Although this response is known to be systemic, research has discovered the potential for local steroid production. In this study, 25 brown anoles were randomly placed in either control or fasted treatment groups. Their organs were then collected in order to measure Corticosterone (CORT) levels. CORT levels were elevated in the heart, liver, and adrenal glands of fasted anoles. Some tissues such as the kidneys showed elevated CORT in control groups.

Introduction

All animals face challenges in their environment and must cope with stressful situations. The hormonal stress response is an evolutionary adaptation enabling an animal's survival during stressful situations. A major part of this stress response is the activation of a hormone cascade, which ends with the synthesis and release of glucocorticoids (GCs), such as corticosterone (CORT) or cortisol, from the adrenal gland which then act on distant cells throughout the body. Traditionally, the stress response has been viewed as a systemic response, however more recent research suggests that different organs can also produce these hormones thus relying less on those from the adrenal. In this study, anole organs of both fasting and control groups were harvested to measure CORT levels.

Methods

1.Homogenization of Tissue

Following the experimental trial (1 week), blood and organ samples were collected. Tissues were then weighed and homogenized using zirconia beads in a bead ruptor with a mixture of 84% methanol solution. After centrifugation, the resulting supernatant was collected and prepared for solid phase extraction

2. Solid Phase Extraction

Steroid was extracted and purified from each organ sample using solid-phase extraction. The final purified solutions were collected with 5 mL of 90% methanol and dried in a speed vacuum (Thermo Fisher Scientific Inc., Pittsburgh, Pennsylvania, USA) at 40 ° C for 4 hours. The samples were then stored at 4° C until the assays were performed.

3. Cortisol Enzyme-linked Immunoasssay

Levels of cortisol were analyzed through cortisol enzyme-linked immunoassay from Arbor Assays, and determined through interpolation of the generated standard curve. The samples were read on a plate reader at 750 nm wavelength and data was analyzed using GraphPad Prism 6.

Results

The one week fasting experiment did result in substantial weight loss (Fig. 1)
As expected, increased CORT levels were observed in most organs from the fasted group when compared to controls.

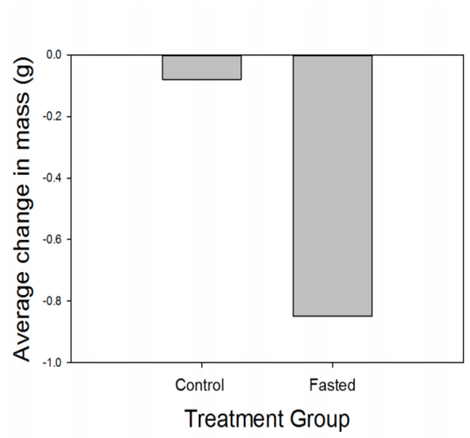

Figure 1. Average change in mass (g) of control and fasted anole groups.

.

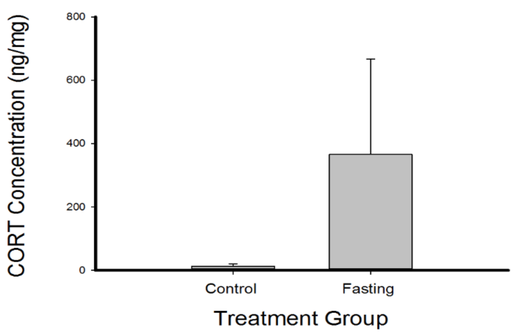

Figure 2. Average Corticosterone concentration (pg/mg) in the adrenal gland of control and fasted anoles.

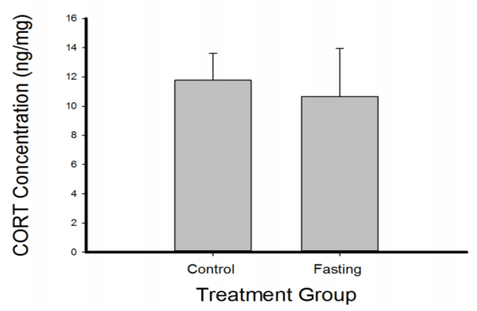

Figure 4.. Average Corticosterone concentration (pg/mg) in the plasma of control and fasted anoles.

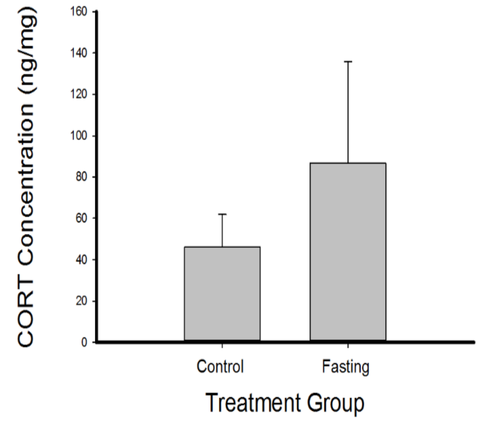

Figure 4. Average Corticosterone concentration (pg/mg) in the cardiac tissue of control and fasted anoles.

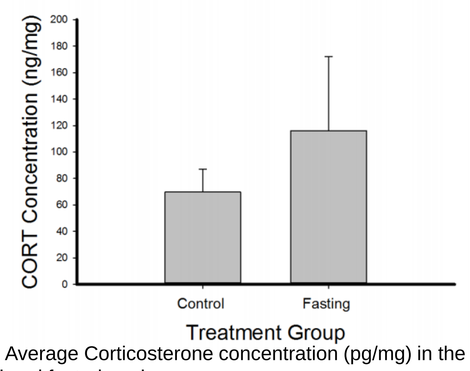

Figure 5. Average Corticosterone concentration (pg/mg) in the liver of control and fasted anoles.

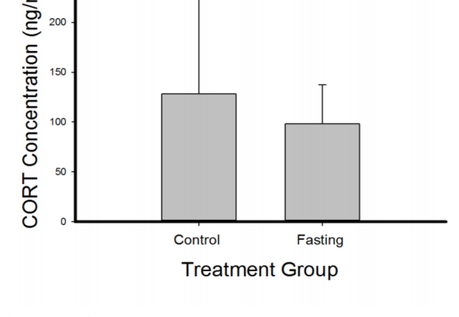

Figure 6. Average Corticosterone concentration (pg/mg) in the kidneys of control and fasted anoles.

Discussion

The fasting design used here was sufficient to induce chronic energetic stress in the male anole. As expected, compared to controls on a normal feeding regimen, the fasted anoles lost a substantial amount of weight (Figure 1). Fasting also caused physiological changes among most of the organs sampled. Compared to controls, fasted anoles had increased CORT concentrations in adrenal gland, heart, and liver (Figure 2,4,5). Surprisingly, plasma resulted in little to no change between the treatment groups (Figure 3), however we are currently examining whether undetectable values are minimizing this effect.

Acknowledgments

• This research was funded through the **John Hauck Foundation, the Office of the Dean of Arts and Sciences,** and the Department of Biology.

Future Direction A DHEA assay will also be conducted to determine if the fasting condition induce a physiological change, as CORT did. Following the assay data, the expression of steroidogenic enzymes will be tested via real-time polymerase chain reaction (RT-PCR). To begin, primers will be developed and hopefully altering expression levels will be observed in organs where the CORT and DHEA vary as well.