

## Morphological alterations in the heart and aorta of rats treated with glucocorticoids

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### Abstract

**Introduction:** The objective of this study was to analyze the morphological structure of the heart and aorta of rats treated with the synthetic glucocorticoid dexamethasone. **Material and Methods:** Male Wistar rats were divided into two groups: 08 control rats undergoing treatment with a 0.9% saline solution for 10 days and 08 rats treated for 10 days with dexamethasone (2mg/kg animal weight). **Results:** Histological analysis detected a mild cardiac hypertrophy and 15% reduction of collagen located in the aorta of animals treated with glucocorticoid when compared to the control group. **Conclusion:** We conclude that treatment with dexamethasone for a period of 10 consecutive days is able to promote morphological changes in the structure of the heart chamber and, impair morphological structure of aorta.

**Keywords:** glucocorticoids, artery, heart, rat.

### 1 Introduction

Dexamethasone is a synthetic glucocorticoid extensively used in the treatment and prophylaxis of various diseases and is approximately 30 times more potent than cortisol (ULLRICH, BERCHTOLD, RANTA et al., 2005). The cortisol is the major natural glucocorticoid circulating in humans (ANTI, GIORGI and CHAHADE, 2008), synthesized in the cortex of the adrenal medulla and released under the influence of adrenocorticotropic hormone (ACTH). It is a key hormone in the maintenance of electrolyte balance (balance of ions and water), in the regulation of the vascular system, metabolism of lipids and carbohydrates (QI, PULINILKUNNIL, AN et al., 2004; RAFACHO, ROMA, TABOGA et al., 2007; BAREL, PEREZ, GIOZZET et al., 2010).

The therapeutic use of glucocorticoids (GC) is due to its anti-inflammatory and immunosuppressive properties. The clinical response to these hormones is extremely variable, with adverse reactions according to the time of use, dose and type of corticosteroid used in the treatment (RAFACHO, MARROQUÍ, TABOGA et al., 2010). Cortisol excess or the prolonged usage of synthetic glucocorticoids contributes to the development of obesity, hypertension, insulin resistance, dyslipidemia (WHITWORTH, BROWN, KELLY et al., 1995; BREM, 2001; RAFACHO, MARROQUÍ, TABOGA et al., 2010), and can lead to muscle atrophy and the development of Cushing's syndrome (HOPKINS and MATTHEW, 2005; WALKER, 2007). Other studies show that chronic use of glucocorticoids may induce cardiac hypertrophy and reduction of collagen, thereby compromising cardiac function (DE VRIES, VAN DER LEIJ, BAKKER et al., 2002; LUMBERS, BOYCE, JOULIANOS et al., 2005; BAREL, PEREZ,

GIOZZET et al., 2010). Thus, these changes may result in an increased cardiovascular risk, such as myocardial infarction, affecting everything from morphology to physiology of heart chambers.

Despite this evidence, there are few studies reporting these changes in vascular channels. The aim of this study was to analyze the morphological structure of the aorta and heart of rats treated with dexamethasone after a period of 10 consecutive days.

### 2 Methodology

Wistar rats (250-300g) were provided by the Central Animal Laboratory of the Federal University of Sergipe, which received commercial feed (Labina) and water *ad libitum*. They were divided into 02 groups, control and treated, as described: 1) Rat Control (RC) - Male rats that received 0.9% saline intraperitoneally for 10 consecutive days; 2) Rats treated with glucocorticoids (RG) - male rats that received dexamethasone solution (EMS) at a rate of 2mg/kg of body weight of the animal (intraperitoneally) for 10 consecutive days. The project was approved by the Ethics Committee for Animal Experimentation of the Federal University of Sergipe (CEPA 54/2009 protocol).

On the tenth day, the animals were left for 12 hours food restriction, received intraperitoneally the body weight, anesthetized with sodium pentobarbital (45mg/Kg body weight) and, after being confirmed anesthetic effect, the infusion procedure was carried out to withdraw heart and aorta. A saline solution (0.9%) was used for washing the tissue

and heart weight measured in both groups. The cardiovascular structures were fixed with paraformaldehyde solution (4%) and subjected to histological procedures routine. The inclusion technique was used in paraffin, followed by microtome cuts of 5 $\mu$  and haematoxylin and eosin (HE) staining. Masson's Trichrome staining of artery cuts was used to demonstrate the collagen fiber and allow morphometric analysis as directed by Souza, Cordioli, Simões et al. (2003).

The histological sections images obtained were captured by optical microscope and analyzed. The areas of right and left ventricles of the heart chambers were quantified by specific software (Carl Weiss®) and the analysis of the arteries was performed by a specific program for morphometry (Imaje J - NIH, USA) using the point-counting method per equidistant points as described by Araujo, Santos, Souza et al. (2013).

### 3 Results

A mild cardiac hypertrophy was observed as shown on Table 1, especially the values of wet heart weight ratio to body weight of control (0.36  $\pm$  0.01) and treated animals (0.41  $\pm$  0.01) ( $p < 0.05$ ).

**Table 1.** Representative values of average absolute weights of the wet heart weight and the ratio between these two variables between animals in the control group and treated with dexamethasone.

	RC (n=8)	RG (n=8)
Wet Heart weight (g)	0.86 $\pm$ 0.024	0.8390 $\pm$ 0.024
(Wet heart weight) / (body weight) x 100	0.36 $\pm$ 0.01	0.41 $\pm$ 0.01*

Values are expressed as mean  $\pm$  standard (EPM) Error, the number of animals (n) of different groups used is expressed in parentheses. \*Represents statistical difference between the groups after Student's t test ( $p < 0.05$ ).

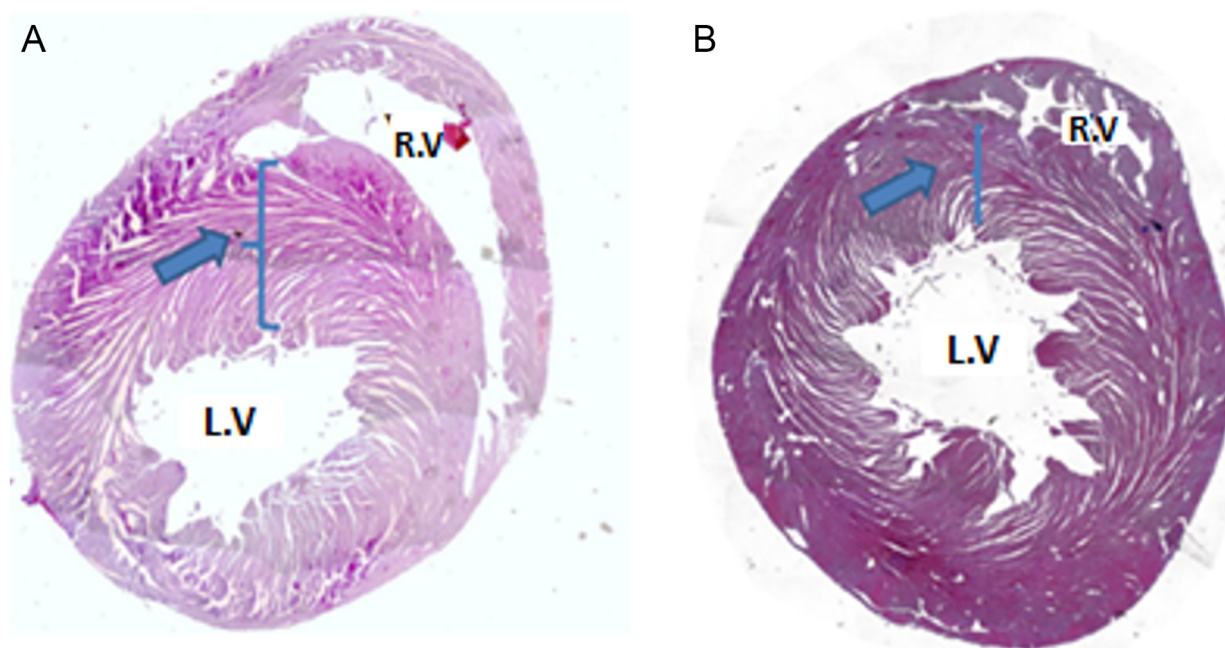
Figures 1A and 1B shows the cardiac tissue stained with haematoxylin - eosin (HE) from both groups of animals. In Figure 1B we observed a normal histological organization in the right and left ventricles from animals in the control group. In contrast, when we analyzed the histological sections of the heart of animals treated with dexamethasone (Figure 1A) observed spacing between the fibers of the right and left ventricular hypertrophy and cardiac fibers.

Aortas arteries from both groups were cut and stained with Masson's Trichrome which evidenced this in the arteries (image in brackets, Figure 2A and 2B) collagen. The morphometric analysis of the total cross-sectional area of the aorta in the group treated with dexamethasone showed a reduction of 15% when compared to the control group (Figure 3A). When evaluating the different layers that comprise the vascular bed, we did not detect significant differences in the middle layer (Figure 3B) layer. However, we observed a 25% decrease ( $p < 0.05$ ), the adventitial layer of the aorta of animals treated with dexamethasone compared to control (Figure 3C).

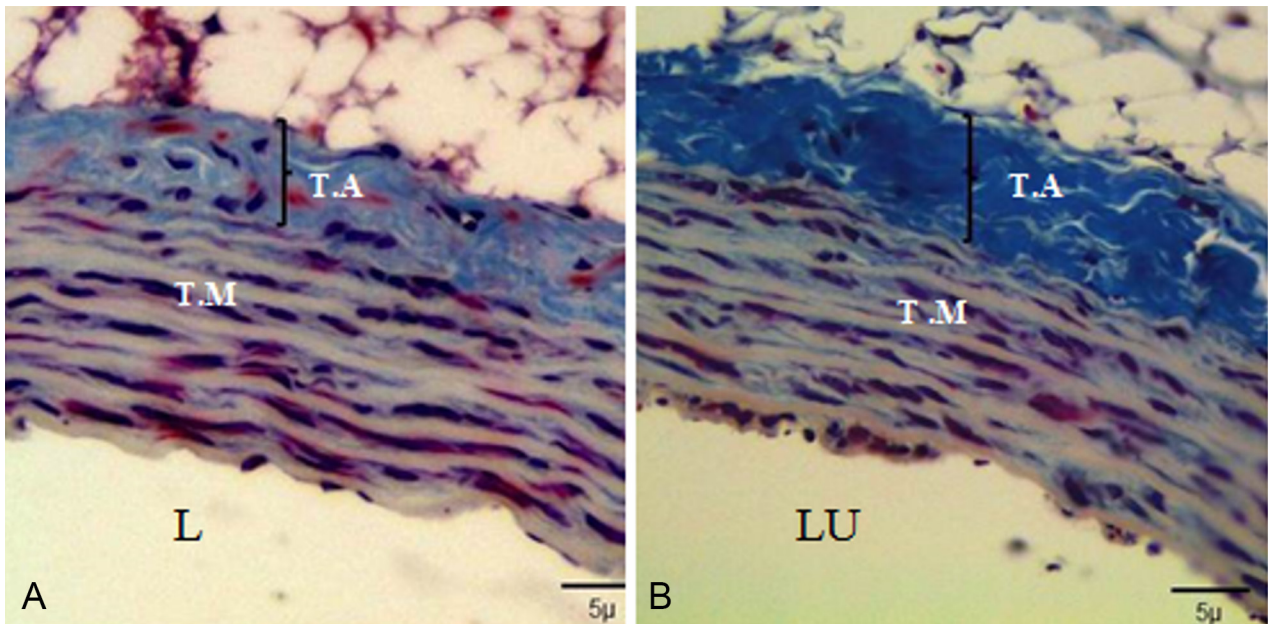
### 4 Discussion

The importance of this study is due to the relevance of the effects caused by treatment with dexamethasone in both fundamental components of the cardiovascular system, the heart and aorta. The arteries of rats treated with glucocorticoids have shown a reduction in the total area of collagen (15%), primarily in the adventitial layer region (25%).

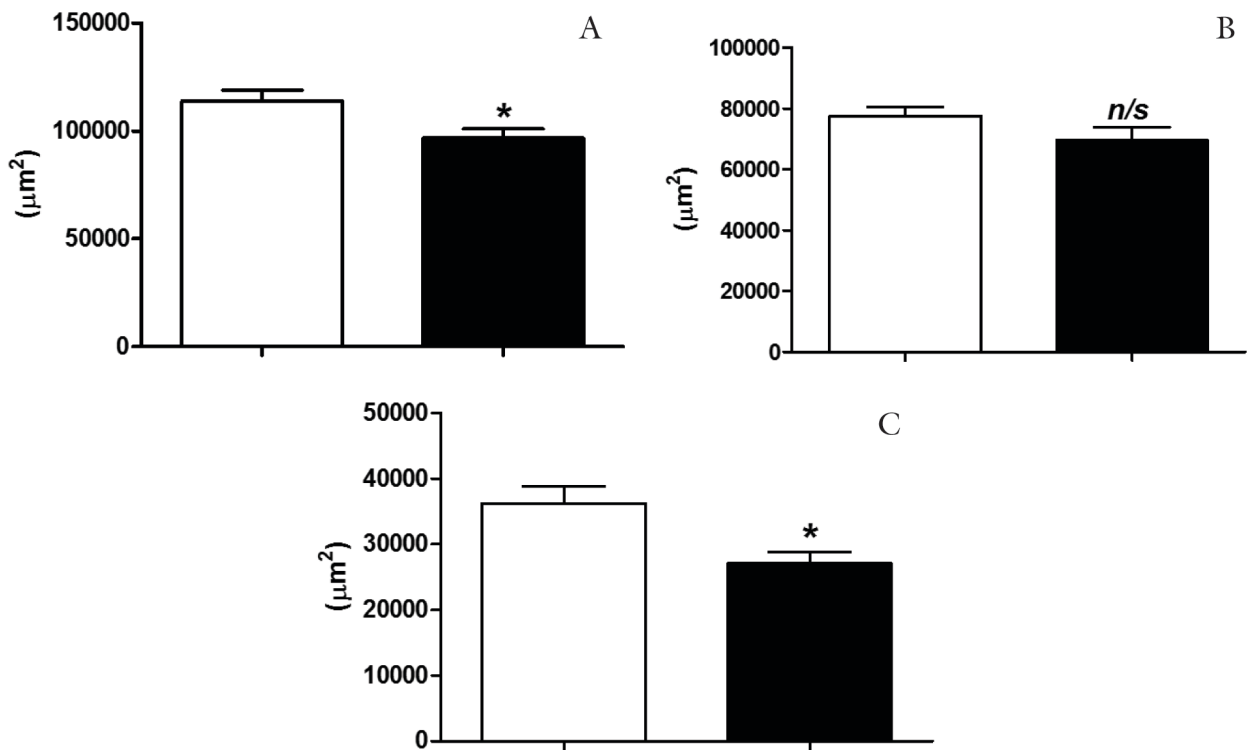
The aorta is the major conductance vessel of the body, possessing great distension capacity due to high concentrations of elastin in its wall cell, particularly in the tunica media. This strain is limited due to the presence of collagen, a key component of the interstitial matrix in the adventitia of arterial beds (BORG, RANSON, MOSLEHY et al., 1981; ROBINSON, COHEN-GOULD and FACTOR, 1983) and,



**Figure 1.** Haematoxylin and eosin staining heart cross section of rats treated with dexamethasone (A) and control group (B). The light blue arrows indicate the location of hypertrophy. In this section, one can observe the areas of the right ventricle (RV), left ventricle (LV) from rat control and treated with dexamethasone. (10x).



**Figure 2.** Artery of rats treated with dexamethasone (A) and control group (B). Cross- cutting and staining in Masson Trichrome. Between brackets is the tunica adventitia (TA), and can also be viewed in the tunica media or middle layer (TM) and the lumen (LU). (40x).



**Figure 3.** Morphometric analysis of the cross section of the aorta of the control group (open bars) and treated with dexamethasone (filled bars). Total area (A), middle layer (B) and adventitia (C). \*  $P < 0.05$  using the “t” test ( $n = 8$  for both groups) test.

among its functions is to prevent overstretching. However, the elastic distensibility of the aorta is very important for determining the level of blood pressure in the arterial system.

It is known that glucocorticoids are able to change both the circulating volume and vascular resistance (PLAVNIK, 2002; BREM, 2001; WHITWORTH, BROWN, KELLY et al., 1995), which may cause a frame of hypertension by modifying

the vascular actions of some vasoactive substances present in the circulation or local. Some studies with epidermal cells in culture also show that chronic treatment with glucocorticoids inhibits collagen synthesis by reducing mRNA expression of types I and III collagen in fibroblasts (OIKARINEN, PIHLAJANIEMI, HÄMÄLÄINEN et al., 1983; EHRlich, TARVER and HUNT, 1973). In addition, corticosteroids

can reduce the activity of IGF-1 (hormone responsible for promoting collagen formation) by inhibiting the enzyme activity of hydroxylation and glycosylation of pro-collagen (EUGENE and KUCHARZ, 1992). They also act as inhibitors of the growth factor (VEGF) expression, an important regulator of angiogenesis. Thus, treatment with dexamethasone can lead to deleterious effects on the cardiovascular system depending on concentration and period of treatment.

In the heart of animals treated with dexamethasone, we detected a mild left ventricular hypertrophy when compared to the control group. Cardiac hypertrophy is a chronic hemodynamic response of adaptation to a functional overload which in this case may have been caused by increased pressure volume normally observed in the use of glucocorticoids (BREM, 2001; BAREL, PEREZ, GIOZZET et al., 2010). Under physiological conditions, cardiac hypertrophy may result from physical exercise or pathological conditions or even after use of pharmacological substances such as administration of high doses of isoproterenol (DAVEL, FUKUDA, DE SÁ et al., 2008; MURAD and TUCCI, 2000).

## 5 Conclusion

The cardiac muscle hypertrophy observed refers to the acute effect of treatment with steroids, although widely used in medical practice as anti-inflammatory. Despite being an experimental study, the morphological effects observed demonstrate the importance of its use under medical supervision. It can be concluded that treatment with dexamethasone for 10 consecutive days induced a slight cardiac hypertrophy associated to a diminished total collagen content in isolated aorta of treated rats.

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