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Effects of Age on Mean Basal Cortisol and Glucose Levels in the Common African Toad *Bufo regularis*

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Abstract

Studies on the effects of ageing on basal cortisol level have been inconclusive. This study investigated the effects of ageing on basal cortisol and glucose levels in the common African toad *Bufo regularis*.

Seventy adult toads of either sex were used for the study. The toads were fasted for 24 hours and anaesthetized with sodium thiopentone (50mg/kg) intraperitoneally. Blood samples were collected from truncus arteriosus for determination of blood glucose and cortisol levels. Blood glucose was determined by modified glucose oxidase method while cortisol level was determined using cortisol assay kits. The ages of the toads were determined by modified skeletochronology technique. The fasting cortisol and glucose levels increased with age in the toads. There was positive correlation between basal cortisol and glucose levels with age in the toads.

In conclusion, the present study shows that mean basal cortisol and blood glucose levels increase with age in the common African toads *Bufo regularis*. The common African toad may be excellent animal model for studies on glucose metabolism and ageing.

Keywords: Cortisol, Blood glucose, Age, Skeletochronology technique, Common African toad

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INTRODUCTION

Ageing is associated with multi endocrine changes including changes in the structure and function of the adrenal gland. There are changes both in ACTH and cortisol secretion in normal ageing. For instance, the mean daily serum cortisol level has been reported to increase in the elderly and that the pattern of cortisol secretion by Zona fasciculata of the adrenal cortex undergoes modifications with age (Yiallouris *et al*, 2019). In humans and under basal conditions, cortisol secretion is intermittent with recurring pulses at 1 to 2-h intervals. The 24-h cortisol profile shows a morning maximum, declining levels late morning and afternoon, low concentrations at night, and an abrupt increase after first few hours of sleep (Van Cauter and Turek, 1994; Van Cauter *et al*, 1996). The levels of most hormones diminish with age while the mean cortisol levels increase (Piazza *et al*, 2010), with irregular patterns and flattened circadian profile (Nater *et al*, 2013, Gaffey *et al*, 2016). In rodents, cumulative exposure to glucocorticoids has been reported to cause degenerative changes in hippocampus, the brain region that inhibits glucocorticoid release. As a result, the ability to terminate glucocorticoid secretion at the end of stress is impaired, resulting in increased exposure to glucocorticoids and decreasing ability of the HPA axis to recover from a challenge (Sapolsky *et al*, 1986, Van Cauter *et al*, 1996). Cortisol, a catabolic hormone affects glucose metabolism. Previous studies reported association between high cortisol concentrations, insulin resistance, increased fasting glucose (Ward *et al*, 2003), and the risk of developing diabetes in older people (Schoorlemmer *et al*, 2009). A flatter diurnal slope of cortisol profile, a pattern found in older adults and related with type 2 diabetes has been reported (Hackett *et al*, 2014). Studies on the effects of ageing on basal cortisol levels are contradictory. Earlier studies in man reported that ageing is not associated with changes in the basal activity of the HPA axis or in its ability to respond to challenge (Blackman *et al*, 1994, Seeman and Robbins, 1994). However, these studies have reported changes in cortisol levels with age (Lupien *et al*, 1994, Bergendahl *et al*, 2000, Larsson *et al*, 2009, Barra *et al*, 2015). Studies on ageing and basal cortisol levels are not conclusive. This study was designed to investigate the effects of ageing on basal cortisol and glucose levels in the common African toads *Bufo regularis*.

MATERIALS AND METHODS

Seventy toads (70) were used in the study. The toads were randomly picked from wet bushes, slow moving streams, ponds around University of Ibadan environment**.** The toads were brought into laboratory after capture and kept inside the cage till following day. The toads were fasted for 24h before the start of the experiment but allowed access to water. Each animal was anaesthetized with sodium thiopental 50mg/kg body weight intraperitoneally. Each anaesthetized toad was placed on the dissecting board and the thorax opened. The truncus arteriosus was dissected and used for blood sampling. After 30 min stabilization, blood sample was collected from truncus arteriosus for cortisol and blood glucose determination. The two phalanges of each toad were collected and used for age determination of the animal. The age was determined by skeletochronology as described by Isehunwa et al (2020). Blood glucose was determined by modified glucose oxidase method Trinder (1969) while cortisol was determined using ELISA Kits.

RESULTS

The results of the study are shown in tables 1-2, figure 1 and plates 1-2 below:

Table 1 shows the different ages of the toads and the mean basal cortisol levels. The mean basal cortisol levels increased significantly with age in the toads. Table 2 shows the mean basal cortisol and fasting glucose levels for toads of different ages. There is positive correlation between the mean basal cortisol and fasting glucose levels. The fasting glucose and mean basal cortisol levels increased significantly with

age in the toads.

Figure 1 shows the fasting glucose levels for toads in different ages. The fasting glucose levels increased with age significantly. Plates 1 and 2 show the histology of the toad's phalanges. Each line of arrested growth (LAG) represents an annual ring and interpreted as one year.

Table 1: shows the mean basal cortisol levels with age in the common African Toads.

Age (Years)	2 n =7	*3 n =8*	4 n =6	5 n =6	6 n =5
Cortisol level (ng/ml)	13.14 ± 1.64	*18.86 ± 0.30*	13.92 ± 2.44	*21.58 ± 6.88	*20.30 ± 0.96

The mean basal cortisol levels increased significantly with age in common African toad *Bufo regularis* *P < 0.05

Table 2: Comparison of mean basal cortisol and fasting glucose levels in common African toad *Bufo regularis* in different age groups.

Age (Years)	2 (n =7)	3 (n =8)	4 (n =6)	5 (n =6)	6 (n =5)
Blood glucose (mg/dL)	38.71±3.67	59.63±2.56	67.50±6.65	77.50±5.93	78.40±4.42
Cortisol level (ng/mL)	13.14±1.64	18.86±0.30	13.92±2.44	21.58±6.88	20.30±0.96

Table 2 shows that, there is positive correlation between blood glucose and cortisol levels with age, the correlation value R= 0.830.

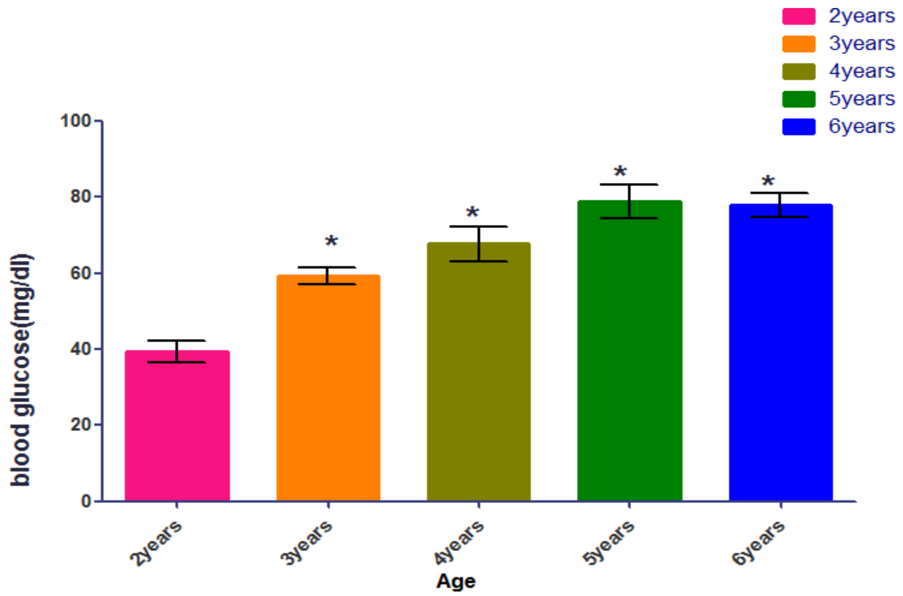

Figure 1 shows the effect of age on fasting glucose levels in the common African toad *Bufo regularis.*The blood glucose levels increased with age.

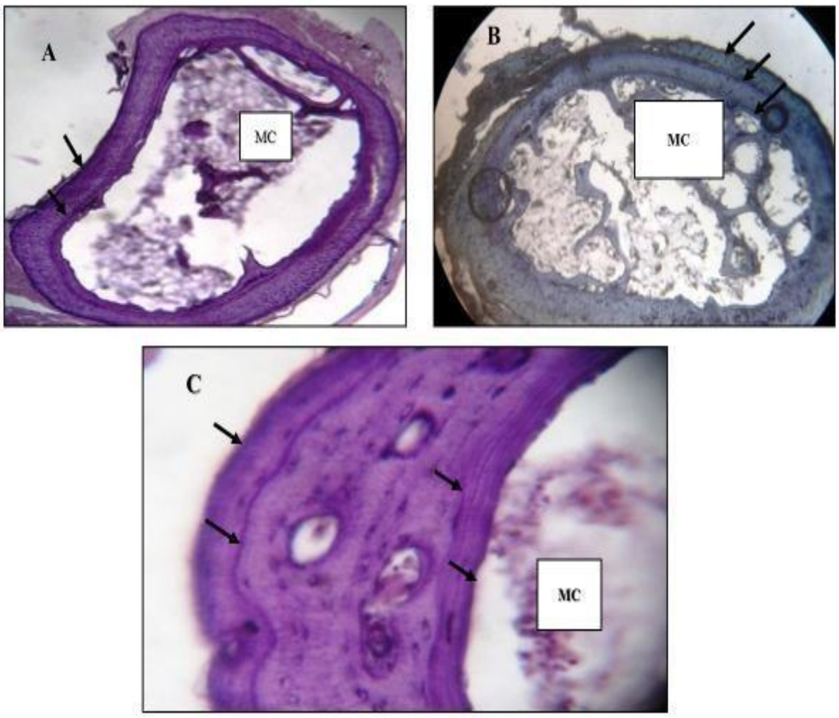

Plate 1: Histology of a phalanges A,B and C showing lines of arrested growth as indicated by arrows, which show the ages of the toads 2, 3 and 4 years old respectively. Marrow cavity (MC)

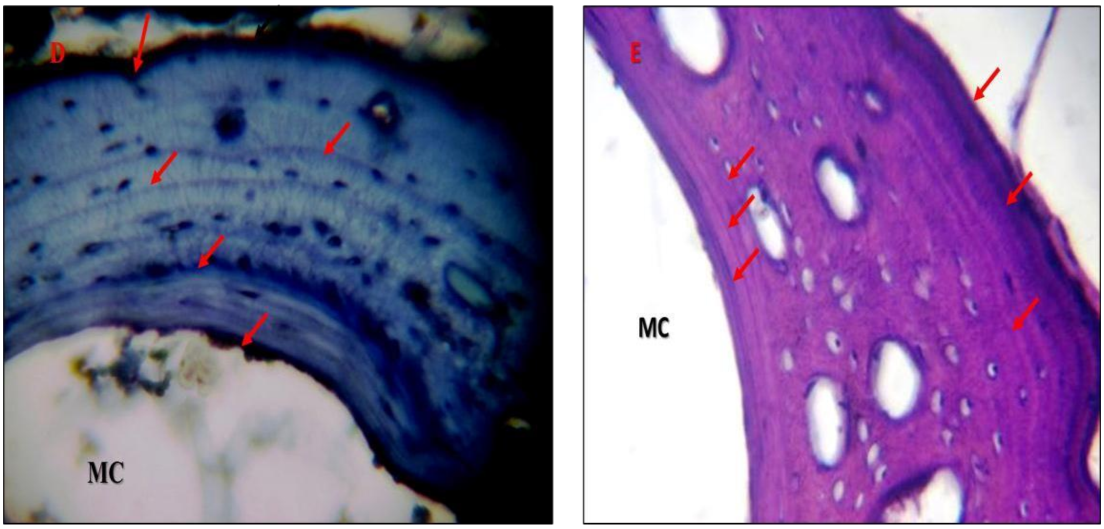

Plate 2: Histology of a phalanges D and E showing the lines of arrested growth, which are indicated by arrows that show the ages of the toads, 5 and 6 years old respectively. Marrow cavity (MC)

DISCUSSION

The results of this study further confirmed that blood glucose levels increased with age in the toads. This is consistent with studies in humans (Kutty *et al*, 2002, Ko *et al*, 2006, Salmon, 2012), Cynomolgus monkeys (Yue *et al*, 2016), rodents (Issa *et al*, 1990) and amphibians (Isehunwa *et al*, 2020). Ageing impairs fasting glucose level leading to dysfunction of glucose metabolism (Yue *et al*, 2016). It has been reported that ageing is associated with decline in insulin action (De Tata, 2014). The observed increase in glucose level in the ageing toads may be due probably to pancreatic beta cell dysfunction. Persistent hyperglycemia has been reported to impair glucose metabolism and alters expression of metabolic genes in pancreatic islets (Brereton *et al*, 2016).

The results of the present study showed that basal cortisol levels increased with age in the toads. This agrees with studies in humans (Lupien *et al*, 1998, Dmitrieva *et al*, 2013, Karlamangla *et al*, 2013, Barra *et al*, 2015) and rats (Issa *et al*, 1990, Lupien *et al*, 1998) which reported an increase in basal cortisol levels with age. The secretion pattern of cortisol by Zona fasciculata of the adrenal cortex has been reported to undergo changes with age ((Yiallouris *et al*, 2019)). In ageing, there is diminished negative feedback on the secretion of cortisol due to impaired sensitivity of the HPA axis (Ferrari *et al*, 2001). This age-related attenuation of axis negative feedback may explain partly the increase in mean basal cortisol levels seen during ageing. The observed increase in basal cortisol levels in the present study may account partly for the increased glucose levels in ageing toads. This is in agreement with the study of Lee et al, (1999) that cortisol plays role in age-associated hyperglycemia. However, some human studies have reported that basal corticotropic function was not affected by ageing (Urban and Veldhuis, 1988; Blackman *et al*, 1994; Seeman and Robbins, 1994).

Chronic stress has been reported to accelerate ageing (Lavretsky and Newhouse, 2012). The ability to terminate stress response is impaired in the elderly and the hypothalamic-pituitary-adrenal (HPA) axis is the primary player in the stress response (Herman *et al*, 2016). Insulin sensitivity has been reported to decrease with age (Broughton and Taylor, 1991, Ferrannini *et al*, 1996). The study of Kamba et al (2016) reported association between higher cortisol levels and decreased insulin secretion. The observation of the present study in which the blood glucose and cortisol levels increased with age in the toads may be due partly to decreased insulin secretion. Cerf (2013) reported an association between beta cell dysfunction, insulin resistance and persistent hyperglycemia. The results of the present study agrees with studies in humans and rodents in relationship to fasting glucose and cortisol levels during normal ageing (Lee *et al*, 1999).

In conclusion, the results of the study suggest that in the common African toad *Bufo regularis*, the basal cortisol and glucose levels increase with age. The results showed also a positive correlation between fasting blood glucose and cortisol levels in ageing toads. The common African toad may be excellent animal model for research in glucose metabolism during ageing.

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