



Research Article

Effects of Age on Fasting Blood Glucose, Liver and Muscle Glycogen Levels in the Common African Toad *Bufo regularis*

*Isehunwa, G. O; Adebajji, O and Alada A. R. A

Department of Physiology, College of Medicine, University of Ibadan, Nigeria.

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Abstract

Ageing is associated with elevated blood glucose levels, insulin resistance and pancreatic islet dysfunction. The age-related dysfunction of glucose metabolism has been well studied in humans and rodents. However, there is paucity of information on ageing and glucose metabolism in amphibians. This study was designed to investigate the effect of ageing on fasting blood glucose, liver and muscle glycogen levels in the common African toad, *Bufo regularis*. Toads of different sizes were randomly picked and used for the study. They were fasted for 24 hours and anaesthetized with sodium thiopentone (50mg/kg) given intraperitoneally. Blood samples were collected from truncus arteriosus for blood glucose determination while the liver and muscle glycogen levels were determined using anthrone reagents methods. Ages of toads were determined using modified skelectochronology technique. The fasting glucose level increased with advancing age while liver and muscle glycogen levels decreased with age in *Bufo regularis*. The results suggest that glucose metabolism slows down with age in the toads and demonstrated similarities between the toads, humans and rodents in relation to age-related changes in glucose and glycogen metabolism

Key Words: Blood glucose, Liver glycogen, Muscle glycogen, Age, Common African Toad

INTRODUCTION

Ageing has been reported to affect tissue and cellular functions associated with glucose metabolism. Studies in humans and rodents showed that ageing is characterized by a decrease in capacity for glucose disposal and metabolism, elevated glucose levels and chronic hyperglycemia that can lead to morbidity and mortality (Suji and Sivakami, 2004; Park *et al.*, 2006). Ageing has been reported to impair glucose uptake at the muscle due to post receptor defect in insulin action which caused decrease in number of transporters and maximum velocity at which glucose is transported. Ageing has been reported to decrease expression of skeletal muscle Glut 4 levels (Houmar *et al.*, 1995). The increase in insulin resistance and decrease in insulin secretion that occurs during ageing process is believed to underlie age-related hyperglycemia (DeFronzo, 1981; Chang and Halter, 2003). Blood glucose metabolism is regulated primarily by insulin secreted by the pancreatic islet β -cells. There are reports of pancreatic beta cell dysfunction due to ageing that resulted in impaired oral glucose tolerance, decreased glucose stimulated insulin release and abnormal glucose metabolism (Gu *et al.*, 2012; Kalyanic and Egan, 2013). Insulin sensitivity decreased with age (Stout, 1984; Basu *et al.*, 1986; Broughton and Taylor, 1991, Ferrenin *et al.*, 1986). Cortisol has been reported to play role in the age-associated hyperglycemia (Lee *et al.*,

1999). Most of the studies on ageing and glucose metabolism have been on humans and rodents. There is limited information on ageing and glucose metabolism in the toads.

Glycogen is an important energy substrate that supports skeletal muscle activity in both humans (Holloszy and Kohrt, 1996; Shulman and Rothman, 2001) and exercising rats (Goldfarb *et al.* 1989; Ivey and Gaesser 1987; Richter *et al.*, 1982). The key enzyme in glycogenesis is glycogen synthase (Kollberg *et al.*, 2007; Manchester *et al.*, 1996; Pederson *et al.*, 2004) while glycogen phosphorylase enzyme controls glycogenolysis activity (Lucia *et al.*, 2008). Muscle glycogen metabolism has been reported to be affected by age-related changes (Montori-Grau, 2009). Resting muscle glycogen concentration has been found not to change in old rats but reduced in untrained older humans. Exercise training was found to increase muscle glycogen levels of older people (Cartee *et al.*, 1994).

Previous studies on ageing and glycogen content are conflicting. For instance, the studies of (Poland *et al.*, 1984; Meynial-Derus *et al.*, 2005) reported no difference in muscle glycogen content of soleus lateralis and gastrocnemius muscles of aged (22-24 months) compared with young adult (4-8 months) rats. Khandelwal *et al.*, (1984) reported age-associated deficit in liver glycogen metabolism as a result of reduction in the activities of liver glycogen synthase and phosphorylase in aged rats.

The age-related dysfunction of glucose metabolism has been well-studied in humans and rodents. However, there is paucity of information on ageing and glucose metabolism in the toads. The present study was therefore, designed to investigate age-related changes in fasting blood glucose, liver and muscle glycogen levels in the Common African toad *Bufo regularis*.

MATERIALS AND METHODS

Toads of different sizes were randomly picked and used in the study. The toads were brought into the laboratory after capture and kept in a plastic wire-gauged cage. The toads had free access to water and were fasted 24hours before the start of the experiment. Each animal was anaesthetized with sodium thiopentone 50mg/kg given intraperitoneally. The thorax was opened and the truncus arteriosus was dissected free from surrounding connective tissues for blood sample collection. Each animal was allowed 30mins to stabilize after surgery. After stabilization, blood sample was taken from truncus arteriosus for glucose determination. The glucose level was determined immediately blood sample was taken by modified glucose oxidase (Trinder, 1969).The liver and gastrocnemius muscle were removed under anesthesia for glycogen determination. The glycogen content was determined by anthrone reagents methods (Jermyn, 1975 and Seifter et al., 1950) as previously described by Isehunwa et al, (2013). The phalanges of the toads were removed for age determination.

Age Determination in Toad: The age of the toads was determined using modified skeletochronology technique (Khonsueetai, 2000; Kumbar and Pancharatna, 2004). This involves counting the lines of arrested growth (LAGS) in the cross-sections of phalanges (Castanet and Smina, 1990).The surrounding tissues of the phalanges were cleared. The third phalanges of the hind limbs were removed, washed in running water and fixed in 10% formalin. The phalanges were decalcified for 48hours in 5% nitric acid. Thereafter, all samples were washed in running water for 24hours to remove all traces of the decalcifying agent. The samples were then stained using Harris’ haematoxylin for 3hours. Subsequently, the stained bones were dehydrated by running them step by step through 70% ethanol for 1 hour, 80% ethanol for 1hour, 95% eosin-alcohol dye liquor for 3min, 95% ethanol for 1hour, 100% ethanol for 30min, 1:1 xylene-alcohol mixture for 20min,100% xylene for 30min and paraffin wax in Automatic Tissue processor until the tissues were infiltrated completely. Then, the impregnated phalanges were embedded in paraffin wax using Embedding system (Leica EG 1160).They were thereafter sectioned with microtome at 4 microns. The sections were then floated on water using water bath at 45 degree Celcius and then picked on frosted end slide. The slides were fixed on hot plate for about 30 minutes. The sections were then stained by haematoxylin and Eosin and viewed through a microscope (Olympus CH) with an attached digital camera (Casio Exilim with 14.1 megapixels), growth rings were apparent.

Statistical analysis

All values given are mean + S.E.M of the values measured. Values between two groups were compared using student t-test while One-way analysis of variance (ANOVA) was used to compare mean values in multiple groups. P values of 0.05 or less were taken as statistically significant.

RESULTS

Table 1 shows the effect of different ages on fasting glucose levels. The fasting glucose level increased significantly with advancing age. The table also shows the different ages of the toads and their fasting liver and muscle glycogen levels. There was significant reduction in the liver and muscle glycogen content with age. As the glucose level increased significantly with advancing age, there was significant reduction in liver and muscle glycogen content with age. Plates 1 and 2 show histology of the phalanges. Each line of arrested growth (LAG) represents an annual ring and interpreted as one year

Table 4: Comparison of the effects of age on blood glucose, liver and muscle glycogen levels in *bufo regularis*

Age (Years)	2	3	4	5
Blood glucose (mg/dl)	57.0 ± 3.8	69.9 ± 3.3*	84.6 ± 5.6*	87.4 ± 6.3*
Liver glycogen (mg/100g tissue)	81.1 ± 3.9	67.5 ± 1.8*	48.4 ± 4.2*	46.4 ± 4.4*
Muscle glycogen (mg/100g tissue)	62.6 ± 1.7	60.6 ± 3.9*	38.8 ± 3.3*	37.7 ± 5.6*

Statistically significant at *P < 0.05 compare with age (2 years).

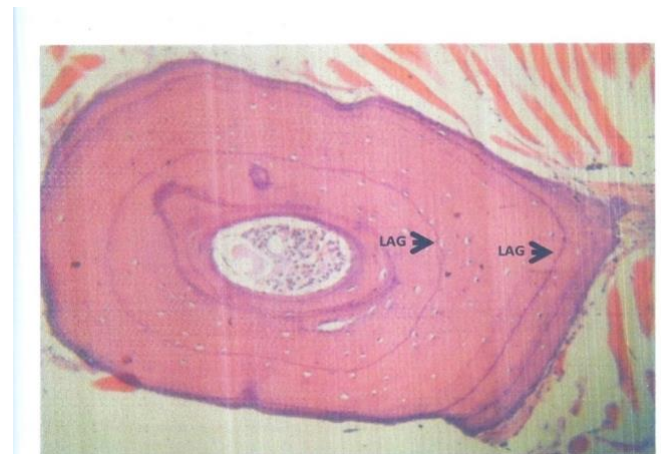


Plate 1 Cross section of the middle third phalanx of *Bufo regularis* showing five lines (5years) of arrested growth (LAG) which are interpreted as annual rings. M×200 H&E

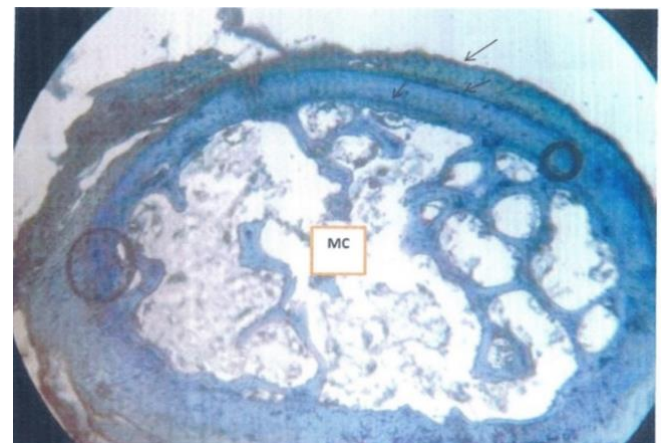


Plate 2 Histology of a phalanges showing 3 lines of arrested growth, this indicates that the toad is 3 years old. Marrow cavity (MC)

DISCUSSION

The results of the present study show that the glucose level of the toads rises with age. This is consistent with studies in humans (Roberts *et al.*, 1982; Kutty *et al.*, 2002; Ko *et al.*, 2006; Khan *et al.*, 2012, Kalyani and Egan, 2013). Cynomolgus monkey (Yue *et al.*, 2016), Rodents (Wu *et al.*, 2009; Gu *et al.*, 2012) which reported elevated plasma glucose level with advancing age. The increase in glucose level with age is multi-factorial. There is limited information on relationship between age and blood glucose level in the toads. The observed rise in glucose level with age in the present study may probably be due to decrease in insulin secretion as a result of pancreatic beta cell dysfunction. Pancreatic beta cell dysfunction has been reported to contribute to abnormal glucose metabolism with advancing age (Gu *et al.*, 2012, Kalyani and Egan, 2013) or increase in insulin level with advancing age (Chang and Halter, 2003). The study of Gu *et al.*, (2012) reported a decrease in glucose stimulated insulin release due to Islet beta cell dysfunction caused by ageing in rats. The results of this study suggest that like humans and rodents, toads can develop diabetes with age. And that, age is an independent factor for impaired fasting glucose level in the toads which is in agreement with previous studies in humans and rodents.

The rise in glucose level in older toads may also be due to increase in cortisol level. Cortisol has been reported to play significant role in age-associated hyperglycemia (Lee *et al.*, 1999). The findings of the present study showed that the liver and muscle glycogen levels decreased with age in the toads. This is in agreement with the studies in rats (Poland *et al.*, 1982; Khandelwal *et al.*, 1984) which reported age-associated decrease in muscle and liver glycogen levels respectively. The reduction in liver and muscle glycogen levels may be due to reduction in the activities of liver glycogen synthase (Khandelwal *et al.*, 1984; Montori-Grau, 2009). Poland *et al.* (1993) reported an impaired muscle glycogenesis in old rats compared with the young rats. Muscle glycogen metabolism is susceptible to age-related changes (Montori-Grau, 2009).

In conclusion, similar to humans, cynomolgus monkeys and rodents the glucose levels increased with advancing age in the toads while liver and muscle glycogen levels decreased with age. Thus, suggesting that glucose metabolism slows down with advancing age in the common African toad *Bufo regularis*. The results also revealed that the common African toad can be an excellent model for studying age-related changes in glucose and glycogen metabolism

REFERENCES

Basu, R. E., A. L. Breda, C. C. Oberg, C. Powell., A. Dalla Man., Basu , *et al.* 2003. Mechanism of the age-associated deterioration in glucose tolerance: Contribution of alterations in insulin secretion, action, and clearance. *Diabetes* 52: 1738-48 Medline: 12829641.

Broughton, D. L., R. Taylor. 1991. Review: deterioration of glucose tolerance with age: the role of insulin resistance. *Age Ageing* 20:221-5 Medline: 1853796

Cartee, G. D., D. A. Young., M. D. Sleeper, J. Zierath., H. Wallberg-Henriksson, and J. O. Holloszy. 1989. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am. J. Physiol.* 256: E494– E499.

Castanet, J. and F. Smirina. 1990. Introduction to skeletochronological method in amphibians and reptile. *Annales Des science Naturelles Comprenant la Zoologie*; 13:191-196

Change, A. M, and J.B. Halter. 2003. Aging and insulin secretion *Am. J. Physiol. Endocrinol Meta.* 284 E7 -12 medline: 12485807.

Defronzo, R. A. 1981. Glucose Intolerance and aging. *Diabetes care* 4:493-501 (Pub Med: 7049632).

Ferrannini, E, A. Natali., B. Capaldo., M. Lehtovirta., S. Jacob., H. V. Yki-Jar. 1996. Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. European group for the study of insulin resistance, (EGIR). *Diabetes* 45: 947-53 Medline: 8666147.

Gu, Z., Y. Du., C. Li., Y. Liu, L. Ma., L. Li, Y. Gong., H. Tiam., and C. Li. 2012. Effect of aging on islet beta cell function and its mechanisms in Wistar rats *Age*: 34: 1393 – 1403.

Houmard, J. A., M. D. Weidner., P. L. Doland., N. Leggett-Frazier., K. E. Gavigan., M.S. Hickey, *et al.* 1995. Skeletal muscle Glucose protein concentration and aging in humans. *Diabetes* 44:555-60, DOI: 10, 2337/Diabetes, 44.5.555.

Isehunwa, G.O., O. T. Olaniyan., and A. R. A. Alada. 2013. The role of alpha and beta adrenergic receptors in cortisol-induced hyperglycemia in the common African toad *Bufo regularis*. *Afri. J. Biotech.* 12:(36).5554 – 5558.

Ivey, P. A., G. A. Gaesser. 1987. Postexercise muscle and liver glycogen metabolism in male and female rat. *J. Appl. Physiol.* 62:1250-1254

Jackson, A R. 1990. Mechanism of Age-Related Glucose Intolerance. *Diabetes care*; 13(Suppl. 2):9-19

Jermyn, M. A, (1975). Increasing the sensitivity of the anthrone method for carbohydrate. *Anal. Biochem.* 68:322-335

Kalyani, R. R. and J. M. Egan. 2013. Diabetes and Altered glucose metabolism with aging. *Endocrinol. Metab.* 42(2): 333-347.

Khan S. H., U. Mashood., M. S. Hanif., S. O. R. S. Bokhari., M. J. Khan. 2012. Effect of age and gender on blood lipids and glucose. *Rawal Med. J.* 37 (4): 344- 347.

Khandelwal, R. L., T. L. Enno., N. Narayanan. 1989. Effect of age on glycogen synthase and phosphorylase activities in rat liver. *Mach Ageing Dev.* 28(1):13-22

Khonsue, W., M. Matsui., and Y. Misawa, Y. 2000. Age determination by skeletochronology of *Rana Nigrovittat*, a frog from tropical forest of Thailand. *Zool. Sci.* 17:253-257.

Ko, G. T. C., H. P. S. Wal., J. S. F. Tang. 2006. Effect of age on plasma glucose levels in non-diabetic Hong Kong Chinese. *Croat. Med. J.* 47: 709-13.

Kollberg, G., M. Tulinius., T. Gilljam., I. Ostman-Smith., G. Forsander., P. Jotorp., A. Oldfors., E. Holme. 2007. Cardiomyopathy and exercise intolerance in muscle glycogen storage disease. *New Eng. J. Med.* 357:1507– 1514. [PubMed: 17928598]

Kumber, S. M. and K. Pancharatna. 2004. Annual formation of growth marker in a tropical amphibian. *Herpetol. Rev.* 35:35-37.

Kutty, V. R., C. R. Soman., A. Joseph., K. V. Kumar., and R. Pisharody. 2002. Random capillary blood sugar and coronary risk factors in a South Kerala population. *J. Cardio. Risk.* 9: 361-7 Medline: 12478206.

- Lee, Z. S., J. C. Chan., V. T. Yeung, C .C. Chow., M. S. Lau., G. T. Ko. *et al.*1999. Plasma insulin, growth hormone, cortisol and central obesity among young Chinese type 2 diabetic patients. *Diabetes Care* 22:1450-7. Medline; 10480508.
- Lucia, A., G. Nogales-Gadea., M. Pérez., M. A. Martín., A. L. Andreu, J. Arenas. 2008. McArdle disease: what do neurologists need to know? *Nat Clin. Pract. Neurol.* 2008; 4:568–577. [PubMed: 18833216]
- Manchester, J., A. V. Skurat., P. Roach., S. D. Hauschka., J. C. Jr. Lawrence. 1996. Increased glycogen accumulation in transgenic mice overexpressing glycogen synthase in skeletal muscle. *Proc. Natl Acad. Sci. USA.* 93:10707–10711. [PubMed: 8855244]
- Montori-Grau, M., R. Minor., C. Lerin., J. Allard., C. Garcia-Martinez., R. de-Cabo, and A. M. Gomez-Foix. 2009. Effects of aging and calorie restriction on rat skeletal muscle glycogen synthase and glycogen phosphorylase. *Exp. Gerontol* : 44(6-7): 426 – 433.
- Pederson, B. A., H. Chen., J. M. Schroeder., W. Shou., A. A. DePaoli-Roach., P. J. Roach. 2004. Abnormal cardiac development in the absence of heart glycogen. *Mol. Cell Biol.* 2004; 24:7179–7187. [PubMed: 15282316]
- Richter, E. A., L. P. Garetto., M. N. Goodman., N. B. Ruderman. 1982. Muscle glucose metabolism following exercise in the rat: increased sensitivity to insulin. *J. Clin. Invest.* 69:785–793. [PubMed: 6804492]
- Seifter, S., S. Dayton., B. Novic., E. Muntwryler. 1950. Isolation and purification. The estimation of glycogen with anthrone reagent. *Archiv. Biochem.* 25: 191-199
- Stout, R. W. 1994. Glucose tolerance and ageing. *J. R. Soc. Med.* 87: 608-9 Medline: 7966111
- Suji, G., K. S. Siva. 2004. Glucose, glycation and aging. *Biogerontology* 5:365-73, DOI: 10.1007/s10522-004-3189-0.
- Trinder, E. 1969. Determination of blood glucose using 4 amino phenazone as oxygen acceptor. *J. Chem. Pathol.* 22:246-248.
- Wu, M., D. M. Desai., S. K. Kakarla., A. Katta., S. Paturi., A. K. Gutta., K. M. Rice., E. M. Walker., and E. R. Blough, 2009. A Cetamoniphen prevents aging – associated hyperglycemia in aged rats: Effect of aging – associated hyper-activation of p38 – MAPK and ERK1/2. *Diabetes Metab. Res. Rev.* 25: 279 – 286 doi: 10.1002/dmrr. 932.
- Yue F., G. Zhang., R. Tang., Z. Zhang., L. Teng., Z. Zhang. 2016. Age and sex-related changes infanting plasma glucose and Lipo protein in Cynomolgus Monkeys lipids in Health and Disease. 15:111-121

