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Serum glucose levels in captive Sri Lankan elephants

(Elephas maximus maximus)

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Abstract

Serum glucose level of the Sri Lankan elephant (*Elephas maximus maximus*), a subspecies of the Asian elephant. has not been reported. The aim of this study was to determine the random serum glucose level of captive adult Sri Lankan elephants to be used as reference base line data. The blood was collected from 16 individuals and serum glucose levels were determined using an enzymatic procedure (glucose hexokinase UV method) which is reliable, accurate and specific for glucose. The results show that the mean random glucose level of the Sri Lankan adult elephant was (mean±SEM) 115.02±7.3mg/dL (range: 64.2-186.15mg/dL). The mean random serum glucose level of the males (132.49±6.7mg/dL, range 88-186.2mg/dL) was significantly (Mann- Whitney U-test, $P<0.05$)higher than the females (97.55±6.7mg/dL,range: 64.2-128.7mg/dL). There was no significant difference amongst the random serum glucose leves of the three male phenotypes (Atha:129.95mg/dL, Aliya:121.0±12.3mg/dL and Pussa: 148.5±18.9mg/dL) and two female phenotypes (Athinna: 95.3±18.5mg/dL and Alidena: 98.8±4.8mg/dL). Further more, the random serum glucose level was not age related (using Spearman correlation $r^2= 0.033$, $P>0.05$).

Key words: *Elephas maximus maximus*, elephant, blood glucose, sri lanka

1. Introduction

Mean serum glucose level and its range in the Sri Lankan elephant (*Elephas maximus maximus*), is not scientifically documented, possibly due to logistic problems involved. However data has been reported for the Indian elephant *(Elephas maximus indicus)*. Data on blood glucose level is very useful in evaluating the status of health of Sri Lankan elephants, and in diagnosis and treatment of their diseases. For example, it is generally known among the vetenarians that in instances where captive elephants fall into sternal and lateral recumbence, intravenous dextrose administration is effective in regaining their normal posture. Further, such data may be useful in taxonomic studies as there are some striking differences already reported with some physiological parameters in the Sri Lankan elephant with the other subspecies of the Asian elephant (Lincolon, *et al.*, 1996;Ratnasooriya, 1995; Ratnasooriya, *et al.*, 1993; Ratnasooriya, *et al.*, 1992a; Ratnasooriya, *et al.*, 1992b and Ratnsooriya, *et al.*, 1992c). Above all, such information would be most valuable for the future scientists as reference data since the Sri Lankan elephant is an endangered animal which is predicted to be extinct in the early part of the 21st century (see Ratnasooriya, 1990).

In this study we report, for the first time, random blood glucose level (using an enzymatic procedure) of captive adult Sri Lankan elephants based on 16 individuals which were brought to Colombo from various parts of the country to participate in the Navam Perahera (a cultural pageant) in February, 1998.

2. Materials and methods

Sixteen apparently healthy adult captive elephants (males 08 and females 08) who were brought to Colombo to participate in Navam Perahera, 1998, were used in its study.

The animals were made to assume a position of lateral recumbence gradually at the command of their respective mahouts. Blood samples (1.0-2.0 ml) were collected from a vein and or artery from the posterior side of either ear [using aseptic precautions into 2.0 ml microcentrifuge tubes between 9.00 to 14.00 hours on 09.02.98 using a butterfly needle (18 gauge)]. The bleeding procedure lasted 1.0-1.5 min and was done without being seen by the elephant. At the time of collection of blood and on the day before kitul logs (*Caryota urens* L.) and coconut fronds (*Cocus nucifera* L.) were freely available to the elephants. After the collection of blood the sex of the animal was noted and their phenotype(the "Atha" or tusker, an "Aliya"

or male with tushes, the"Pussa" or male without tusks or tushes, "Athinne" or female with tushes and "Alidena" or female without tushes was recorded as described by Deraniyagala (1995). The approximate ages of the elephants were obtained from the mahouts and/or owners.

Blood was allowed to clot at room temperature (28-30^0 C) and the samples were kept cold (4-6^0 C) and the serum was separated within 3.5 hours of collection by centrifugation using a microcentrifuge (5415C, Eppenddorf, Hamberg, Germany) at 10,000 r.p.m. for 2.0 min. Only unhemolysed sera were used for glucose analysis. The serum was stored at 4^0 C until glucose levels were determined (within 20 hours). The serum glucose level has been found to be stable up to 72 hours at 2-8^0 C (Teitz, 1986). Glucose levels were determined using a commercial reagent kit (DMA products, Texas, USA) which is based on glucose-hexokinase (Slein, 1963 and Varley, 1969).

In this procedure, hexokinase catalyses the phosphorylation of glucose-6-phosphate in the presence of glucose 6-phosphate dehydrogenase. Glucose 6-phosphate is then oxidized to 6-phosphogluconate and NAD reduced to NADH.

Hexokinase reagent (1.0 ml) was mixed with 5 µl of serum or standard glucose solution (200 mg/dL) and incubated 3 mins at 37 ^{0}C. The absorbance was measured at 340 nm by UV spectrophotometer (Cecil 2000-Cecil instruments Limited, Cambridge, UK) against a reagent blank. All readings were taken within 20 mins after incubation.

The data are represented as mean ±SEM. Statistical comparisons were made using Mann Whitney U-test and Spearman linear correlation. Singificance level was set at $p<0.05$.

3. Results

Of the 16 elephants, 50% were males and 50% were females. Of the females, 01 was an "Atha" (12.5%) 04 were "Aliyas" (50%) and 03 were "Pussas" (37.5%). Amongst the females, 05 were"Alidenas" (62.5%)and 03 were "Athinnas" (37.5%).

All the serum samples were almost colourless and were devoid of any haemolysis. The coefficient of variation was 2.5%.The results obtained are summarized in Table 1. As shown, the random mean glucose level adult captive elephants (n=16) was 115.02+7.27 mg/dL. However, 7out of 8 males (87.5%) and 1 of 8 females (12.5%) had serum glucose levels

>120mg/dL. The mean random serum glucose level of the males was significantly ($p<0.05$) higher than the females (by 35%). Amongst the male phenotypes, the highest serum glucose level wes found in "Pussas" (148.5±18.9mg/dL) and lowest in "Aliyas" (121.0±12.3mg/dL). But this difference was not significant ($p>0.05$). Amongst the two female phenotypes, the difference in serum glucose level was extremely slight (3.5mg/dL). Further, there was no significant correlation evident between age and serum glucose levels of the elephants (males $r^2=0.34$, females $r^2= 0.41$; $p>0.05$) In man, fasting serum blood glucose level is 70-115mg/dL and the normal threshold for glucose is 180mg/dL (Long *et al.* and Pagana,1994).

4. Discussion

This study, for the first time, reports the random serum glucose levels of adult captive Sri Lankan elephants. The serum glucose levels were determined using an enzymatic procedure utilizing hexokinase and glucose-6-phosphate dehydrogenase (Slein,1963 and Varley,1969). This assay system is reliable, sensitive and specific to glucose and is used widely for the quantitative determination of glucose in serum, plasma and cerebrospinal fluid (Young, 1990). Furthermore, in this technique, linearity for glucose extends upto 500 mg/dL (as claimed by DMA products, Texas, USA). In this study, all the serum samples were devoid of haemolysis, glucose estimations were performed within a short time of collection of blood and the recorded glucose levels were well below 500 mg/dL. This indicates that the values obtained reflect normal or near normal values. Stress (pain, fear or anger) elevates serum glucose level through the release of adrenaline and cortisol (Long *et al.*, 1993), resulting in false positive values. We attempted to minimize this effect by requesting the mahouts to make elephants to assume lateral recumbence gradually,using proper sized butterfly needles, collecting the blood samples rapidly (within 1.0-1.5min) without being seen by the elephants. Clinically, five tests that measure serum glucose levels are available (Long *et al.*, 1993 and Pagana,1994): fasting (kept at least 8 hours without food, usually, from midnight before the test), random (with no reference to meals), postprandial (2 hours after meals), preprandial (1/2 to 01 hour before meals) and upto 5 hours following an ingestion of 100g of glucose (oral glucose tolerance test). Elephants whether wild or captive consume a great deal of food often spending 12-18 hours in feeding each day (Eltringham,1991). The passage time of food in the gut of an elephant is between 24-50 hours (Eltringham, 1991) and they defaecate approximately 13 times per day (Ratnasooriya, *et al.*,1994). Considering these factors, we thought that it is more appropriate to measure random serum glucose levels rather than fasting levels. The other tests that monitor serum glucose levels are impracticable for elephants.

The mean random serum glucose level of elephants in this study was 115mg/dL and the range was 64-186 mg/dL. In Sri Lanka, captive elephants are usually given a fixed menu consisting of 3 main items: kitul logs (*Caryota urens* L.), coconut fronds (*Cocus niucifera* L)and jak branches with leaves (*Artocarpus integrifolia* L). *Artocarpus integrifolia* leaves posses hypoglycemic activity (Thabrew, *et al.*, 1994) and these leaves were not given to elephants in this study, on the day of collection of blood and on the previous day. Therefore, it may be possible that the actual random serum glucose levels of captive elephants may be slightly lower than that is recorded here. The mean random serum blood glucose level and the range recorded in this study are in general agreement with that (mean, 110 mg/dL: range 82-173 mg/dL) reported by Lewis (1974) for performing Indian elephants (*Elephas maximus indicus*) which were kept in the USA. However, in this study, the time of collection of blood was not stated and therefore presumed to be random. On the other hand, the mean serum glucose level recorded in this study is about 51-62% higher than those reported by Nirmalan and Nair (1969) [mean 56 mg/dL; range, unspecified; time of collection: in the morning before feeding]and Simon (1961) [mean, 43 mg/dL; range, 28-56 mg/dL; time of collection unspecified for Indian elephants kept in working camps in the Kerala state of India. This difference is unlikely to be due to subspecies variation since the mean glucose values of our study for Sri Lankan elephants (*Elephas maximus maximus*) is in close agreement with those of Lewis (1974) reported for Indian elephant *(Elephas maximus indicus)*; differences in several physiological parameters between the Sri Lankan elephant and the Indian elephant exist (Lincolon,*et al.*, 1996; Ratnasooriya, *et al.*, 1995; Ratnasooriya, *et al.*, 1996; Ratnasooriya *et al.*, 1992a; Ratnasooriya, *et al.*, 1993; Ratnasooriya, *et al.*, 1992b and Ratnasooriya, *et al.*, 1992c).It is possible that the low mean serum glucose values reported in these two studies (Nirmalan, *et al.*, 1969 and Simon, 1961) may have resulted from not taking proper precautions to inhibit glycolysis after collection of blood, due to inherent errors encountered in Somogyi blood glucose estimaton method (Mukherjee, 1988) and also due to long time gap between collection and estimation of blood levels: glucose levels decrease in whole blood rapidly on standing (Varley, 1969). In humans, a random blood glucose level of <150 mg/dL is considered normal if the person had not taken a meal within the last hour (Long,1993). Taken together,the results of our study and that of Lewis (1970) suggest that a similar relationship may hold true for Sri Lankan elephants as well. Indeed, there are several physiological phenomena in elephants which are comparable with man (Eltringham, 1991).

In the Sri Lankan elephant, the mean random serum glucose level of males was significantly higher than the females (by 35%) as was reported previously for their total serum cholesterol level (Ratnasooriya *et al.*, 1995) Such a gender difference between serum glucose and cholesterol levels are

not evident in the Indian elephant (Nirmalan, *et al.,* 1969; Simon, 1961 and Ratnasooriya, *et al.*, 1995). This may be yet another physiological difference between the Indian and Sri Lankan subspecies of elephants. In humans too, a gender difference in serum glucose levels is not reported (Long 1993). In this study, a significant difference in serum glucose levels amongst the different phenotypes was not evident possibly due to small sample size: with a bigger sample size a significant difference in serum cholesterol levels are reported between the two female phenotypes of Sri Lankan elephants, namely Athinne and Alidena (Ratnasooriya, *et al.*, 1995). Further, in this study, a significant linear relationship between serum glucose level and age was not evident. This could be also due to small sample size or due to the fact that the age given by the mahouts and or owners were not correct. In humans an increment in serum glucose level of about 2 mg/dL per decade is generally evident (Long,*et al.*,1993).

In conclusion, this study, reports for the first time, the random serum glucose level of captive adult Sri Lankan elehants. This can be used as a reference baseline data, However, the values may be higher for Sri Lankan elephants in the wild where enormous variety of plant materials are consumed (Mckey, 1973).

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Table 1. Mean serum glucose level of captive Sri Lankan elephants *(Elephas maximus maximus)*

		n	Mean±SEM (mg/dL)	Range (mg/dL)	Median mg/dL)
Entire Group		16	115.02±7.27	64.20-186.15	114.47
Males		8	132.49±9.69	88.00-186.15	129.80
	Atha	1	129.95		
	(with tusks)				
	Aliya	4	121.00±12.3	88.00-146.60	124.70
	(with tushes)				
	Pussa	3	148.50±18.9	127.30-186.10	132.10
	(Without tusks)				
Females	or tushes) Females	8	97.55±6.76	64.20-128.65	98.50
	Athinna	3	95.30±18.50	64.20-128.30	93.30
	(with tushes)				
	Alidena	5	98.94±4.74	82.70±109.20	102.80
	(without tushes)				