Lipid profile of captive Sri Lankan elephants

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Abstract The lipid profile of the Sri Lankan elephant (*Elephas maximus maximus*) is hitherto not scientifically documented. The aim of this study was to determine the normal lipid profile of adult captive Sri Lankan elephants. Blood was obtained from 78 individuals and lipid levels were determined using enzymatic techniques. The results show that the mean total serum cholesterol level was 44.28±15.52 mg/dl (mean ±SD) (males: 46.06±13.59 mg/dl, females: 42.83 ± 13.44 mg/dl), HDL-cholesterol was 41.49±9.19 mg/dl (males: 44.94±8.05 mg/dl, females: 38.54±9.33 mg/dl) and triglycerides was 25.28 ± 10.69 mg/dl (males: 25.49 ± 8.54 mg/dl, females: 25.14 ± 11.99 mg/dl) the mean total cholesterol / HDL cholesterol ratio was 1.22±0.36 (males: 1.17±0.42, females: 1.28±0.28). LDL-cholesterol was not detectable and therefore LDL/HDL-cholesterol ratio could not be computed. Further, there was no significant gender difference amongst these parameters. This is the first study to record base line data on lipid profile of Sri Lankan elephants.

Key words: *Elephas maximus maximus* Sri Lankan elephant, lipid profile, cholesterol, HDL-cholesterol, LDL-cholesterol. triglycerides

Introduction

Very few studies have been conducted on the haematology, blood biochemistry or genotyping (based on blood) of *Elephas maximus maximus*, the elephant of Sri Lanka, which is critically endangered and included in the Appendix I of the Convention of International Trade on Endangered Species of wild Fauna and Flora (CITES). Regrettably, currently, there are only between 3000-4000 wild elephants and 186 captive elephants in Sri Lanka (Kurt & Mar, 2003). These studies include investigations of blood counts (Ratnasooriya *et al.*, 1990; Ratnasooriya *et al.*, 1993; Silva & Kuruwita, 1993a; Silva & Kuruwita 1993b), osmotic fragility of red blood cells (Silva & Kuruwita, 1994), blood hormonal levels (Ratnasooriya *et al.*, 1992; Ratnasooriya *et al.*, 1993; Lincolin & Ratnasooriya 1996; Poole *et al.*, 1997), blood levels of some ions (Kuruwita, 1993a; Silva & Kuruwita 1993b), enzymes (Kuruwita, 1993a; Silva & Kuruwita 1993b), glucose (Ratnasooriya *et al.*, 1999), triglycerides (Kuruwita, 1993a; Silva & Kuruwita 1993b), proteins (Kuruwita, 1993a; Silva & Kuruwita 1993b), total cholesterol (Ratnasooriya *et al.*, 1995) and mitochondrial haplotypes (Vandebona *et al.*, 2002). However, as yet, a full blood lipid profile (including HDL- cholesterol and LDL-cholesterol) of the Sri Lanka elephant is not scientifically documented. Such data is quite useful directly in diagnosis, treatment, breeding and general welfare and indirectly in the long term conservation and sound management of our elephant. In addition, zoologically, documentation of whatever data on the Sri Lankan elephant is important in view of its endangered status. The aim of this study was to investigate the serum lipid profile of Sri Lankan elephants. This was done on elephants brought to Colombo from various parts of the country to participate in the Navam Perahara (a cultural pageant) in February 1997 and 1998 and elephants from the Pinnawala elephant orphanage.

Methods

A total of 78 adult (35 males and 43 females), apparently healthy elephants, who participated in the Navam Perahera, in 1997 and 1998, and Pinnawela elephant orphanage were the subjects of this study. No attempt was made to differentiate these elephants into the five morphotypes.

Blood samples (5-10 ml) were collected (in standing position) from a vein or artery on the posterior side of either ear (using aseptic precautions) without using a sedation, (between 7.00 h - 12.00 h), using a butterfly needle (18 gause) connected to a plastic 10 ml syringe. The entire bleeding procedure lasted 1.0 - 1.5 min.

Blood was allowed to clot at room temperature (28 - 31°C) and the serum was separated within three hours of collection by centrifugation at 500g for 20 min. The serum was stored at -70°C until the total serum lipid profiles were made. Total serum cholesterol was assayed by an enzymatic procedure using a commercial reagent kit (Randox, Ireland). In this procedure cholesterol esters are hydrolysed by cholesterol esterase to cholesterol and fatty acids. The cholesterol is oxidized by cholesterol oxidase to cholestene3-one and hydrogen peroxide. The

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Table 1. Mean (±SD) of serum lipid and lipoprotein levels of adult Sri Lankan elephant (Elephas maximus maximus). Ranges are giving in parenthesis.

Parameter	Entire Group	Males	Females
Total Cholesterol (mg/dl)	44.28 ± 15.52 (28.20 – 85.88)	46.06 ± 13.59 (18.20 – 85.88≠)	42.83 ± 13.44 (18.20 – 73.14)
HDL – Cholesterol (mg/dl)	41.49 ± 9.19 (23.45 – 58.65)	44.94 ± 8.05 (28.59 – 58.65)	38.54 ± 9.33 (23.45 – 53.60)
LDL – Cholesterol (mg/dl)	Non Detectable	Non Detectable	Non Detectable
Total Cholesterol / HDL – Cholesterol (mg/dl)	1.22 ± 0.36 (0.68 – 2.01)	1.17 ± 0.42 (0.68 – 2.01)	1.28 ± 0.28 (0.98 – 1.69)
LDL / HDL Cholesterol (mg/dl)	Cannot be Computed	Cannot be Computed	Cannot be Computed
Triglycerides (mg/dl)	25.28 ± 10.69 (12.24 – 55.90)	25.49 ± 8.54 (12.24 – 47.16)	25.14 ± 11.99 (11.19 – 55.90)

Table 2. The mode, median and variance of total cholesterol, HDL cholesterol and LDL-cholesterol in the serum of the Sri Lankan (Elephas maximus maximus) population sampled.

		Mode	Median	Variance
Total cholesterol	Male	-	42.63	184.75
	Female	-	40.08	180.76
	Entire group	62.45	41.92	182.78
HDL cholesterol	Male	-	45.75	64.82
	Female	37.44	38.15	87.07
	Entire group	37.44	42.63	84.38
Triglyceride	Male	-	26.63	72.86
	Female	11.64	21.68	143.86
	Entire group	47.16	24.33	114.37

chromophore quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase (Richmond, 1973). Ten microliters of the serum sample or standard cholesterol solution (200 mg/dl) were incubated with 1000 microliters of the reagent mix at room temperature (28°C) for a period not less than 10 minutes. Absorbance was measured at 500 nm using a Shimadsu double beam spectrophotometer (UV - 21005, Schimadzu Crop., Kyoto, Japan) against a reagent blank. The readings were completed within one hour of incubation. The test is linear up to a cholesterol concentration of 750 mg/dl.

HDL-cholesterol was determined using Randox Test kits. In this procedure low density lipoproteins [LDL (low density lipoprotein) & VLDL (very low density lipoprotein)] and chylomicron fraction are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ion. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant was determined as follows. The serum sample (200 μl) was mixed with the precipitant (500 μl) and allowed to sit for 10 minutes at room temperature. The mixture was centrifuged for 10 minutes at 4,000 rpm. and the clear supernatant was separated within two hours. The HDL-cholesterol content in the supernatant was determined by mixing the supernatant (100μl) with the reagent (1000μl), incubating for 5 minutes at 37°C, and measuring the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) at 500 nm against the reagent blank within 60 minutes. (The Concentration of HDL cholesterol in the supernatant = A_{sample}/A_{std} x concentration of standard.) LDL-cholesterol was determined using the formula:

LDL cholesterol (mg/dl) = Total cholesterol (mg/dl) - Triglycerides 5 (mg/dl) - (HDL - cholesterol) (mg/dl).

The serum triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The sample was mixed (10μl) with the reagent (1000μl) and incubated for 5 minutes at 37°C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured at 500 nm, against the reagent blank within 60 minutes.

[Triglyceride concentration = ($A_{sample}/A_{standard}$) x 200 (mg/dl)]

The test is linear up to a triglyceride concentration of 150 mg / dl. Two preassyed quality control sera (Randox, Ireland) were used as positive controls to monitor accurately. The results are represented as means ± SD. Statistical analysis were made using Mann-Whitney U-test. Significance was set at $P < 0.05$.

Results

All the serum samples made in this study were free of any marked haemolysis and of yellow colouration on visual examination. The results of the blood lipid parameters monitored or computed are summarized in Tables 1 and 2. LDL-cholesterol was either absent or found below the detectable level of the assay kit used. Although, the males generally had a slightly higher level of the individual parameters monitored, none was significantly different ($P > 0.05$).

Discussion

This study records for the first time the random lipid level profile of captive Sri Lankan elephant (*Elephas maximus maximus*). Sequential and fasting blood samples were not collected due to logistic problems. However, according to some, the lipid profile following a meal may be a more important indicator of coronary heart disease risk than the fasting level (Ryu *et al.*, 1992). Further, we have previously determined blood glucose (Ratnasooriya *et al.*, 1999) and cholesterol levels (Ratnasooriya *et al.*, 1995) of Sri Lankan elephants under random conditions rather than in fasting states. Blood samples were collected from apparently healthy animals always in a single day, in a standing position between 7.00-12.00 hours, without applying any pressure to the blood vessels of the ear to minimize variations in lipid profile due to posture, time of the day, and physical state or stress (Dart *et al.*, 1990). Lipid profiles were monitored using enzymatic procedures which are claimed to be sensitive and reliable (Richmond, 1973) and which are widely used in humans. In this study, the lipid profile was determined in 78 individuals, which is a sizable number to provide meaningful data: the current number of captive elephants in Sri Lanka is reported to be 186 (Kurt & Mar, 2003). Collectively, these procedures used allows the data obtained to be regarded as representative and to be considered as reference base line data for captive Sri Lankan elephants as all these captive elephant had their origin in the jungles. It is generally recognized that for each country a base line data on any biological parameter is a must. The overall total cholesterol level reported in this study is in close agreement to what has been reported by us previously using a lesser number of animals (Ratnasooriya *et al.*, 1995).

Both the blood cholesterol and triglyceride level were low in comparison with humans an omnivore (Hahn & Payne, 1997) and presumably in carnivores. This hypolipdaemia is likely to be related to the herbivorous diet of the elephant. Captive Sri Lankan elephants are mostly fed with a monotonous menu: logs of *Caryota urenus* Linn., fronds of *Cocus nucifera* Linn. and leaves and twigs of *Artocarpus heterophyllus* Linn. (Illangakoon., 1993; Godagama, 1999) which are rich in both soluble (pectins, gum and mucilage) and insoluble (cellulose, lignin and insoluble non cellulose polysaccarides) fibre. Soluble fibre is known to induce hypolipidaemia (Bhattacharya & Bhattacharya, 1994) and this may account for low levels of cholesterol and triglycerides in elephants. Increase clearance of these may also precipitate hypolipidaemia.

The most striking observation in this study is the undetectable level of blood LDL-cholesterol (bad cholesterol) and desirable level of HDL-cholesterol (good cholesterol). Lack of LDL-cholesterol is yet another possible cause for a low level of blood cholesterol as it is the main carrier of cholesterol in the blood serum (Holum., 1990; Vance & Van den Bosch., 2000). A high level of HDL and low level of LDL-cholesterol makes the elephants less prone to atherosclerosis and eventually lowers risk of cardiovascular diseases and stroke. This notion is supported by the low cholesterol: HDL ratio and zero LDL: HDL ratio, which are considered as atherogenic indices (Loke *et al.*, 1991; Agarawal *et al.*, 1998), evident in this study. Interestingly, high levels of LDL and low levels of HDL-cholesterol is positively linked with arterial plaque formation (Holum., 1990; Vance & Van den Bosch, 2000) and are reported to be protective against astherosclerosis and cardiovascular diseases (Holum, 1990; Vance & Van den Bosch., 2000). High HDL level in elephants may be due to a high fiber diet, exercise (as several were working elephants) or due to presence of a metabolite/s which acts as fibrate group of lipid regulating drugs (Anon, 2000). Alternatively, it could be due to some hereditary influence, as in humans, whose high HDL-cholesterol levels are determined partially by inheritance (Holum, 1990).

Similarly, several mechanisms may be responsible for the undetectable LDL-cholesterol level in elephants. Presence of a large number of LDL receptors in the liver and/or having high affinity LDL receptors to cholesterol (Vance & Van den Bosh., 2000) in the liver are two striking possibilities: Patient with familial hypercholesterolemia have a defect in the function of LDL receptor and/or low LDL receptors (Holum, 1990). Enhanced physical activity is another mechanism (Hahn & Payn., 1997). Alternatively, metabolites may be present in the elephant liver which may competitively inhibit 3-hydroxy-3-methylglutrayl coenzyme-A (HMG Co-A) as statins, a class of potant lipid lowering drugs (Anon, 2000). Obviously, additional experiments are required to elucidate these potential mechanisms. Understaking such studies is important as it could lead to development of new lipid regulating drugs.

In this study, of the lipid parameters monitored or computed, a significant difference was not evident between males and females. In contrast, in a previous study of ours (Ratnasooriya *et al.*, 1995) a slight but significantly higher level of cholesterol was evident in male elephants. This could be due to the small sample size of the previous study. In conclusion, this study reports for the first time the lipid profile of the Sri Lankan elephant.

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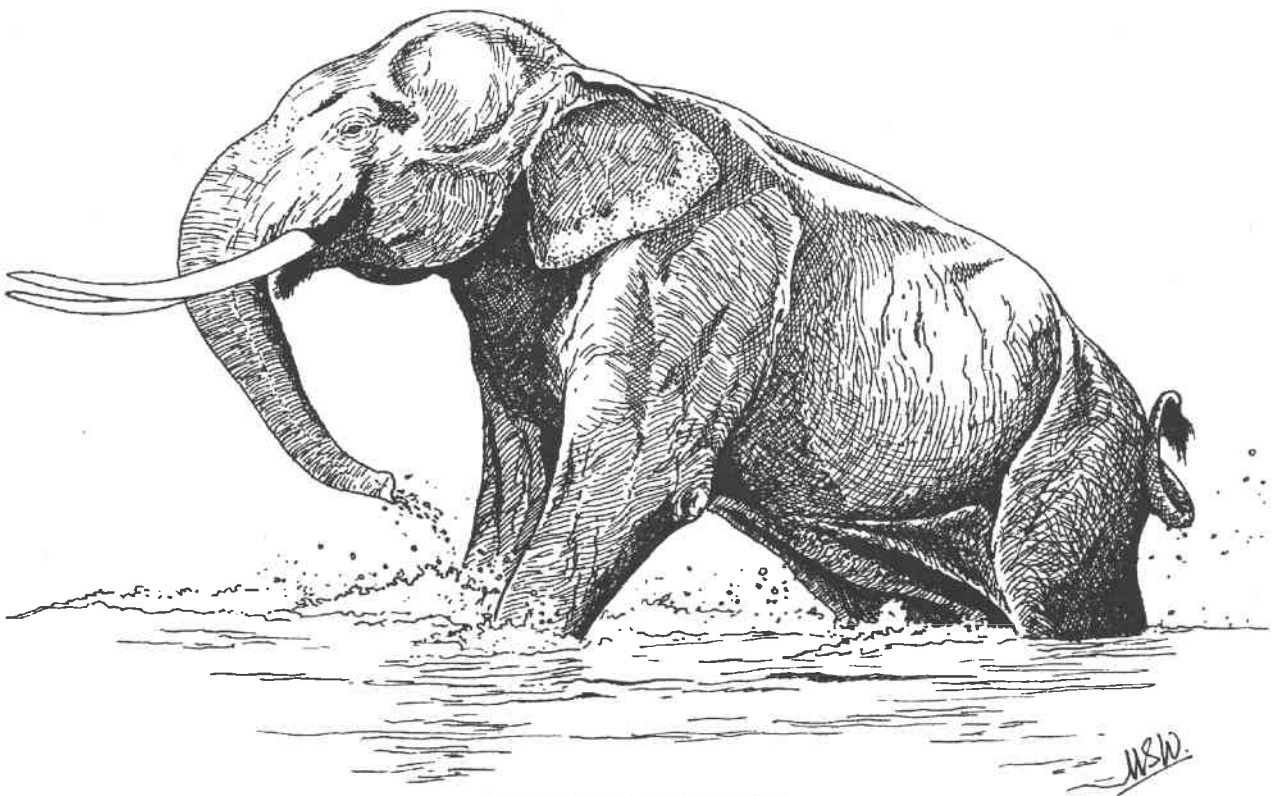

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