

Seasonal Variation in Serum Ascorbic Acid and Serum Lipid Composition of Free-Living Baboons (*Papio ursinus*)

J. P. KOTZÉ, W. A. DE KLERK, M. J. WEIGHT, I. V. MENNE, M. J. A. WEIGHT,
L. P. HORN, N. F. LAUBSCHER

SUMMARY

Two surveys were conducted in the Kruger National Park in which 205 baboons were captured. The first survey was done during late summer and the second during late winter. Serum ascorbic acid, serum cholesterol and serum phospholipids were determined. Baboons of both sexes and various ages were captured.

This work was undertaken to establish serum ascorbic acid, serum cholesterol and serum phospholipid values for baboons under free-living conditions. A seasonal variation was found, and the serum ascorbic acid, serum cholesterol and serum phospholipid values were significantly higher during winter than during summer.

S. Afr. Med. J., 48, 1700 (1974).

Most of our work on cholesterol metabolism and the significance of captivity stress on primates has been done on baboons (*Papio ursinus*).¹⁻³ All the baboons used in these experiments were captured in the Kruger National Park. A survey was conducted in this game reserve to determine the serum ascorbic acid and serum lipid composition of the baboons under natural environmental conditions. We consider these data important as they enable us to draw comparisons with the data obtained after the animals have been placed in experiments.

We are also concerned with the adaptation of baboons (*Papio ursinus*) and monkeys (*Cercopithecus aethiops*) to captivity, since thousands of these animals are captured annually in South Africa for research purposes. De Klerk *et al.*⁴ showed that the serum ascorbic acid levels of baboons and monkeys drop dramatically during the first days of captivity. Kotzé *et al.*⁵ found that baboons on a daily ascorbic acid intake of 16 mg/kg body weight showed

a drop in serum ascorbic acid over the first few weeks of captivity. Supplementation of their diet with ascorbic acid needs serious consideration, since it plays an important role in their health maintenance. Honjo *et al.*,⁶ for instance, showed that *Cynomolgus* monkeys (*Macaca irus*) on an ascorbic acid-deficient diet were more susceptible to relatively low doses of *Shigella flexneri* 2a.

MATERIALS AND METHODS

The baboons were immobilised with phencyclidine hydrochloride (Sernylan; Parke Davis). This was done by darting them with Cap-Chur projection gas pistols, after luring them to our vehicles with fruit and vegetables. As this method was not always successful, we also made use of very small baboons to attract the attention of the troops—baboons usually attempt to rescue a baby baboon when it is held outside a vehicle for them to see. They were then darted as they closed in.

Baboons of both sexes and various weights were captured and released again after weighing and taking of blood samples. A number was tattooed onto the chest of each baboon for later identification, should the animal be caught again. The majority were caught in the Skukuza, Lower Sabie, Pretoriuskop and Tshokwane areas of the park.

The surveys were performed during February 1972 (late summer) and August (late winter) of the same year.

Serum ascorbic acid levels were determined according to the 2,4-dinitrophenyl hydrazine method of Roe and Kuether.⁷ Thio-urea was used instead of Norit as described by Schaffert and Kingsley.⁸

Serum cholesterol values were determined according to the method of Zak and Resser⁹ and phospholipid values according to the method of Bartlett.¹⁰

The data were statistically analysed to determine whether significant differences existed between the two sexes and between the two seasons in which the surveys were done for each of the 4 variables, i.e. body mass, serum ascorbic acid, serum cholesterol and serum phospholipids. Means and standard deviations were computed (Table I) as well as all correlation coefficients between the 4 variables (see Table II). To eliminate the possible effect that body mass might have on the other 3 variables, partial correlations were determined with the effect of body mass eliminated (see Table III).

A two-way analysis of variance¹¹ was done to determine the effect of sex as well as season on the 4 variables. The two hypotheses used were that neither

National Research Institute for Nutritional Diseases of the South African Medical Research Council, Pretoria

J. P. KOTZÉ (Present address: National Food Research Institute, CSIR, Pretoria)

W. A. DE KLERK (Present address: National Food Research Institute, CSIR, Pretoria)

M. J. WEIGHT

I. V. MENNE

M. J. A. WEIGHT

L. P. HORN

National Research Institute for Mathematical Sciences, CSIR, Pretoria

N. F. LAUBSCHER

TABLE I. MEANS AND STANDARD DEVIATIONS FOR BODY MASS, SERUM ASCORBIC ACID, SERUM CHOLESTEROL AND SERUM PHOSPHOLIPIDS OF BABOONS CAPTURED DURING SUMMER AND WINTER

	Body mass (kg)		Serum ascorbic acid (mg/100 ml)		Serum cholesterol (mg/100 ml)		Serum phospholipids (mg/100 ml)	
	Average	SD	Average	SD	Average	SD	Average	SD
Males first survey (summer) (N = 65)	23,8	10,8	1,41	0,33	84	23	118	42
Females first survey (summer) (N = 29)	13,3	5,3	1,30	0,40	87	15	133	38
Males second survey (winter) (N = 79)	24,3	10,1	1,73	0,38	95	21	170	37
Females second survey (winter) (N = 32)	15,0	4,5	1,79	0,48	106	27	179	52

sex nor season has an influence on the means of the variables.

From Table II it can be seen that serum cholesterol is reasonably well correlated with body mass. Hence we attempted to explain differences in serum cholesterol in terms of differences in body mass. To this effect, a two-way analysis of covariance¹¹ was computed on serum cholesterol, using cubic regression on body mass.

Since 5 variables were under consideration, 10 significance tests were computed. The results are presented in Table IV. In order to guarantee an over-all 5% probability of type I error, individual tests were performed at the 0,005 significance level, an approximation which is based on the Bonferroni inequality.¹²

RESULTS AND DISCUSSION

A total of 205 baboons were captured during the two seasons, 94 during February (69% were males and 31% females) and 111 during August (72% were males and 28% females). The summer of 1971-1972 had an above-average rainfall and availability of food and general conditions were in the favour of the baboons, while the winter of 1972 was particularly severe for this game reserve which has a subtropical climate.

The baboons caught during the summer had an average serum ascorbic acid level of 1,37 mg/100 ml, and those caught during the winter had a higher average of 1,74 mg/100 ml. The average serum cholesterol value of 84,9 mg/100 ml obtained in summer was also lower than the average of 98,2 mg/100 ml obtained during winter. The winter average value for serum phospholipids, i.e. 172,6 mg/100 ml was also higher than the summer average of 122,6 mg/100 ml.

The higher serum ascorbic acid levels found during August (late winter) were probably due to differences in diet during the two seasons.

It is clear from Table I that the phospholipid concentrations in the serum were higher than those of cholesterol. The ratio of serum cholesterol/serum phospholipids was 0,71 for males in summer and decreased to 0,56 in winter. For females this ratio was 0,65 in summer and it decreased to 0,59 in winter.

The cholesterol/phospholipid ratios in this study were also lower than the ratios of 0,95, 0,90 and 0,83 reported by Kotzé *et al.*²³ for baboon very-low-density lipoproteins,

TABLE II. CORRELATION COEFFICIENTS OF THE 4 VARIABLES

	Survey		Serum ascorbic acid	Serum cholesterol	Serum phospholipids
	No.	Sex			
Body mass	1	M	-0,15	-0,73	-0,42
		F	-0,06	-0,46	-0,23
	2	M	-0,20	-0,47	-0,31
		F	-0,18	-0,47	-0,44
Serum ascorbic acid	1	M		0,18	0,15
		F		0,45	0,21
	2	M		0,15	0,16
		F		-0,21	-0,03
Serum cholesterol	1	M			0,61
		F			0,58
	2	M			0,55
		F			0,54

TABLE III. PARTIAL CORRELATIONS—EFFECT OF BODY MASS ELIMINATED

	Survey		Serum cholesterol	Serum phospholipids
	No.	Sex		
Serum ascorbic acid	1	M	0,10	0,10
		F	0,47	0,20
	2	M	0,06	0,14
		F	-0,15	0,05
Serum cholesterol	1	M		0,79
		F		0,55
	2	M		0,48
		F		0,43

low-density lipoproteins and high-density lipoproteins, respectively.

The baboon cholesterol/phospholipid ratios found by us were considerably lower than those reported for humans by Katz and Stammler,²⁴ who found ratios of 0,80 to 0,76 for normal human males and females, respectively. These workers maintain that there is no correlation between cholesterol/phospholipid ratios and the incidence and severity of atherosclerosis.

Cherkovich and Usunian¹⁵ determined serum cholesterol values in 153 rhesus monkeys and 158 sacred baboons, during spring, summer, autumn and winter over a period of two years, and found a seasonal variation. In summer the serum cholesterol values were higher (rhesus monkeys

TABLE IV. ANALYSIS OF VARIANCE—TWO HYPOTHESES TESTED AT AN OVER-ALL 5% SIGNIFICANCE LEVEL

Hypotheses	Body mass	Serum ascorbic acid	Serum cholesterol	Serum phospholipids	Serum cholesterol with body mass seen as a covariate
Hypothesis 1: Sex has no influence on the means	Rejected	Not rejected	Not rejected	Not rejected	Rejected
Hypothesis 2: Season in which survey was done has no influence on the means	Not rejected	Rejected	Rejected	Rejected	Rejected

157,5 ± 7,8 mg/100 ml; sacred baboons 133,7 ± 5,6 mg/100 ml) than in winter (rhesus monkeys 135,0 ± 2,9 mg/100 ml; sacred baboons 119,9 ± 3,4 mg/100 ml). These workers also supply evidence that daily blood sampling of rhesus monkeys resulted in a gradual drop in serum cholesterol levels as the animals adapted to handling.

St Clair *et al.*,¹⁶ reporting on the serum cholesterol values of squirrel monkeys (*Saimiri sciureus*), found that the serum cholesterol rose significantly—from 116 mg/100 ml at time of capture in the jungle to a value of 194 mg/100 ml at the time of their arrival at the laboratory. They suggest that this rise in serum cholesterol may reflect the squirrel monkeys' reaction to captivity stress. They also feel that the serum cholesterol values for squirrel monkeys of between 175 and 200 mg/100 ml found by several other laboratories are not typical for free-ranging animals and might in fact represent hypercholesterolaemic levels. We agree with this and feel that testing free-living non-human primates is the most reliable method for obtaining biological normals.

Van der Watt *et al.*¹⁷ performed cholesterol determinations on 74 samples of sera obtained from 43 male and 31 female free-living baboons (*Papio ursinus*) caught in the Northern Transvaal (Tshipise district) and the Skukuza area of the Kruger National Park. They found that the serum cholesterol levels had a mean value of 104 mg/100 ml in the males and 99 mg/100 ml in the females.

The correlation coefficients between serum ascorbic acid and serum cholesterol and between serum ascorbic acid and serum phospholipids are, in general, low. The correlation between serum cholesterol and serum phospholipids is reasonably high, which confirms the relationship between these two variables.

Partial correlations (eliminating the effect of body mass) between serum ascorbic acid on the one hand, and serum cholesterol and serum phospholipids on the other, do not indicate that differences in body mass are responsible for these relatively low correlations.

There are significant differences in body mass between males and females, but on the average, males and females do not differ significantly from the first survey to the second. While the average serum ascorbic acid, serum cholesterol and serum phospholipid levels do not differ significantly between males and females, very highly significant differences are indicated from survey 1 to

survey 2 (compare mean values given in Table I).

The covariance analysis of serum cholesterol on body mass as predictor shows that highly significant differences exist both between sexes and surveys. This, together with the analysis of variance result (comparing males and females and finding no significant difference), indicates that differences in body mass account for differences in serum cholesterol levels between males and females.

CONCLUSION

The higher serum ascorbic acid values found during winter might be due to differences in the diet of the baboons during the two seasons. This aspect needs further investigation. Our surveys and experiments have shown that certain variables such as serum ascorbic acid and serum cholesterol can differ markedly in primates under different conditions. Serum ascorbic acid levels drop and serum cholesterol values rise in captivity. We agree with other workers that in order to obtain normal values, it is best to test free-living animals.

The statistical analysis indicates that differences in body mass, and not sex, account for the differences in serum cholesterol levels between male and female baboons.

We thank Dr U. de V. Pienaar for permission to work in the Kruger National Park and for making laboratory facilities available to us.

REFERENCES

- De Klerk, W. A., Kotzé, J. P., Weight, M. J., Menne, I. V., Matthews, M. J. A. and McDonald, T. (1973): *S. Afr. Med. J.*, **47**, 1503.
- Kotzé, J. P., Matthews, M. J. A. and De Klerk, W. A. (1973): *Lancet*, **1**, 610.
- Weight, M. J., Kotzé, J. P., De Klerk, W. A. and Matthews, M. J. A. (1974): *Int. J. Biochem.* (in press).
- De Klerk, W. A., Du Plessis, J. P., Van der Watt, J. J., De Jager, Annemarie and Laubscher, N. F. (1973): *S. Afr. Med. J.*, **47**, 705.
- Kotzé, J. P., De Klerk, W. A. and Weight, M. J. Unpublished data.
- Honjo, S., Takasaka, M., Fujiwara, T., Imaizumi, K. and Ogawa, H. (1969): *Jap. J. Med. Sci. Biol.*, **22**, 149.
- Roe, J. M. and Kuether, C. A. (1943): *J. Biol. Chem.*, **147**, 399.
- Schaffert, R. R. and Kingsley, G. R. (1955): *Ibid.*, **212**, 59.
- Zak, B. and Ressler, N. (1955): *Amer. J. Clin. Path.*, **25**, 433.
- Bartlett, G. R. (1959): *J. Biol. Chem.*, **234**, 466.
- Scheffé, H. (1959): *The Analysis of Variance*. New York: John Wiley & Sons.
- Miller, R. G. (1966): *Simultaneous Statistical Inference*. New York: McGraw-Hill.
- Kotzé, J. P., Neuhoff, J. S., Engelbrecht, G. P., Van der Merwe, G. J., Du Plessis, J. P. and Horn, L. P. (1974): *Atherosclerosis*, **19**, 469.
- Katz, L. N. and Stammler, J. (1953): *Experimental Atherosclerosis*, p. 92. Springfield Ill.: C. C. Thomas.
- Cherkovich, G. M. and Usunian, L. A. (1967): *Z. Versuchstierk.*, **9**, 236.
- St Clair, R. W., McNinch, J. E., Middleton, C. C., Clarkson, T. B. and Lofland, H. B. (1967): *Lab. Invest.*, **16**, 828.
- Van der Watt, J. J., Kotzé, J. P., Kempff, P. G., Du Plessis, J. P. and Laubscher, N. F. (1973): *J. Med. Prim.*, **2**, 25.