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Abstract: To date, no information is available on the fatty acid composition of cheetah liver and brain, nor have any comparisons been made between captive-fed and wild cheetah. The fatty acid compositions of the tissues of other members of the family Felinae have been published previously. However, the cheetah does not belong to the same genus as those species, and thus the fatty acid pattern may be different. For many years, it has been known that the plant derived polyenoic fatty acids, linoleic acid and  $\alpha$ -linolenic acid, are essential dietary components for mammals. It has been shown that at least two species of the Carnivora (the lion - Panthera leo and the domestic cat - Felis cattus) lack the enzyme 6-desaturase and thus, the ability to further desaturate these two fatty acids to produce their metabolically important derivatives. However, the polyenoics produced are required by both species. In order to satisfy the requirement for these fatty acids, other animals must be consumed, hence these animals are true obligate carnivores. The cheetah (Acinonyx jubatus) exhibits many adaptive differences from both the above species, and many show other differences from the rest of the Felinae other than the obvious anatomical ones. Thus, the possibility exists for an active 6-desaturase enzyme in cheetahs, although tentative evidence to the contrary does exist. In this study, the livers and brains of 2 adult male cheetahs were obtained after the animals had been culled in SWA/Namibia. The liver of 1 aged male cheetah from de Wildt, Transvaal also became available. The animals from SWA/Namibia had not been in captivity as far as was possible to ascertain, thus it was assumed that their diet had consisted predominantly of nondomesticated prev. It has been shown that wild cheetah only eat freshly killed animals and do not return to a kill, thus the lipids obtained are completely fresh and the chances of fatty acid degradation are minimal. The de Wildt animal, however, had been fed in captivity for a long period of time, in fact most of its life, and thus had subsisted on lipids from tissues in a state of partial degradation. The possibility of fatty acid deterioration must therefore be considered in relation to this animal.

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# THE FATTY ACID COMPOSITION OF THE LIVER AND BRAIN OF SOUTHERN AFRICAN CHEETAHS

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## INTRODUCTION

To date, no information is available on the fatty acid composition of cheetah liver and brain, nor have any comparisons been made between captive-fed and wild cheetah. The fatty acid compositions of the tissues of other members of the family Felinae have been published previously.<sup>1</sup> However, the cheetah does not belong to the same genus as those species, and thus the fatty acid pattern may be different.

For many years, it has been known that the plant derived polyenoic fatty acids, linoleic acid  $(18:2\omega 6)$  and  $\alpha$ -linolenic acid  $(18:3\omega 3)$ , are essential dietary components for mammals. It has been shown that at least two species of the Carnivora (the lion—*Panthera leo*,<sup>5</sup> and the domestic cat—*Felis cattus*<sup>6</sup>) lack the enzyme  $\Delta 6$ -desaturase and thus, the ability to further desaturate these two fatty acids to produce their metabolically important derivatives. However, the polyenoics produced are required by both species. In order to satisfy the requirement for these fatty acids, other animals must be consumed, hence these animals are true obligate carnivores.

The cheetah (Acinonyx jubatus) exhibits many adaptive differences from both the above species,<sup>8,9</sup> and may show other differences from the rest of the Felinae other than the obvious anatomical ones. Thus, the possibility exists for an active  $\Delta 6$ -desaturase enzyme in cheetahs, although tentative evidence to the contrary does exist.<sup>2</sup>

In this study, the livers and brains of 2 adult male cheetahs were obtained after the animals had been culled in SWA/Namibia. The liver of I aged male cheetah from de Wildt, Transvaal also became available. The animals from SWA/Namibia had not been in captivity as far as was possible to ascertain, thus it was assumed that their diet had consisted predominantly of non-domesticated prey. It has been shown that wild cheetah only eat freshly killed animals and do not return to a kill,<sup>8,9</sup> thus the lipids obtained are completely fresh and the chances of fatty acid degradation are minimal. The de Wildt animal, however, had been fed in captivity for a long period of time, in fact most of its life, and thus had subsisted on lipids from tissues in a state of partial degradation. The possibility of fatty acid deterioration must therefore be considered in relation to this animal.

## MATERIALS AND METHODS

Fatty acid methyl esters for use as standards were obtained from Sigma Chemical Co., St. Louis, MO. All other reagents were of analytical reagent grade.

Samples (5 g) of each tissue were homogenized in chloroform/methanol (2:1, v/v + 0.01% 2,6-di-tert-butyl-*p*-cresol as antioxidant) and the lipids were extracted by the method of Folch *et al.*<sup>3</sup> The lipids were separated by thin layer chromatography into ethanolamine phosphoglyceride (EPG), choline phosphoglyceride (CPG), and sphingo-myelin (SM) fractions. Development was with chloroform/methanol/water (65:25:4, v/v/v), and the bands located by spraying with 0.2% 2,7-dichlorofluorescein in ethanol, and visualized under U.V. light (366 and 254 nm). The bands were scraped off the plates and the lipids eluted with 5 ml of chloroform/methanol (4:1, v/v). The fatty acids of each fraction were transmethylated using boron trifluoride in methanol (14% BF<sub>3</sub>)<sup>4</sup> and the methyl esters were extracted into petroleum ether (40-60°C). These were characterized by

TABLE 1. The Comparison of the Fatty Acid Profiles of Liver and Brain EPG, CPG and SM from Wild and Captive Cheetahs\*

Fatty acid	EPG					CPG					SM				
	Ll	L2	L3	<b>B</b> 1	B2	LI	L2	L3	B1	<b>B</b> 2	L1	L2	L3	<b>B</b> 1	B2
14:0	0.5	nd	0,4	0.2	0.3	nd	0.4	0.5	1.1	1.5	nd	nd	nd	0.8	nd
16:0	17.5	16.9	18.7	3.9	4.6	13.4	12.2	14.5	18.2	20.3	10.5	10.8	10.0	8.6	8.3
18:0	16.9	19.9	17.5	8.7	10.7	18.3	16.8	19.1	9.1	9.8	13.5	12.4	13.3	10.1	9.7
24:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.6	1.2
16:1w9	0.8	nd	0.6	2.7	3.4	nđ	nd	nd	2.2	2.4	0.8	0.6	0.9	0.6	1.1
18:1w9	19.8	21.7	14.8	28.9	30.4	15.9	16.8	13.6	28.8	28.0	19.9	20.8	22.5	20.2	20.2
20:1w9	2.5	1.8	3.4	10.4	8.8	1.8	- 2.0	2.3	3.3	3.5	5.1	4.9	5.4	5.2	6.1
24:1w9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.9	1.1
18:2w6	14.5	15.9	20.6	3.0	2.8	17.5	18.0	18.6	4.5	3.6	11.6	13.1	13.9	13.6	11.9
20:2 <i>w</i> 6	0.2	nd	0.1	0.1	0.9	nd	nd	nd	nd	nd	nd	nd	0.8	nd	0.9
20:3w6	0.5	nd	nd	2.3	1.7	nd	nd	nd	2.2	2.5	nd	nd	nd	nd	nd
20:4w6	13.0	12.6	20.7	20.0	19.3	19,4	20.1	18.4	9.5	10.7	30.7	29.3	28.0	28.5	29.1
22:4006	1.8	1.7	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
22:5ω6	0.9	0.7	0.2	0.4	nd	nd	nd	nd	nd	0.1	nd	nd	nd	0.4	0.3
18:3w3	2.5	2.1	nđ	nd	nd	1.8	2.1	1.2	nd	nd	nd	nd	nd	nd	nd
20:3w3	2,8	2.2	0.9	2.1	1.6	1.6	1.8	1.7	6.2	5.8	nd	nd	nd	nd	nd
20:5ω3	nd	nd	nd	0.4	0.5	nd	nd	nd	0.6	0.5	nd	nd	nd	0.4	0.6
22:5 <i>w</i> 3	2.4	1.4	0.2	5.4	5.5	4,4	4.6	6.0	2.4	6.1	6.2	6.3	4.3	4.6	6.0
22:6w3	3.4	2.9	1.5	11.5	9.5	5.9	5.4	4.5	11.9	9.4	1.7	1.8	0.9	4.5	3.5

\*All results are expressed as percentage total area, and are the means of 3 analyses. The differences as described in the results section were significant at p < 0.05; but these have been omitted from the Table for the sake of clarity.

L1 = liver from 1<sup>st</sup> wild cheetah, L2 = liver from 2<sup>ad</sup> wild cheetah, L3 = liver from captive cheetah, B1 = brain from 1<sup>st</sup> wild cheetah, and B2 = brain from 2<sup>ad</sup> wild cheetah. nd = not detectable.

gas liquid chromatography using a Varian 3400 gas chromatograph with autosampler. The  $30 \text{ m} \times 0.2 \text{ mm}$  ID 10% OV351 column was run with temperature programming from  $180-220^{\circ}$ C at 2°C per min. The peaks were quantitated by means of a Varian 4390 integrator.

### RESULTS

Table 1 shows the results of the analyses of the methyl esters from the EPG, CPG, and SM fractions of the livers and brains of the wild cheetahs and the liver from the captive animal. If one compares the liver EPG fatty acids of the wild to those of the captive animal, then several differences become apparent. In the captive animal, the levels of  $18:1\omega 9$ ,  $22:4\omega 6$ ,  $18:3\omega 3$ ,  $20:3\omega 3$ ,  $22:5\omega 3$ , and  $22:6\omega 3$  were all lower than those of the wild, while the reverse is true of  $18:2\omega 6$  and  $20:4\omega 6$ . The CPG fraction of the liver of the captive animal exhibited levels of  $18:1\omega 9$ ,  $20:4\omega 6$  and  $22:6\omega 3$  that were lower than those of the wild animals, while the level of  $22:5\omega 3$  was higher. In the liver SM fraction, the captive animal showed lower  $20:4\omega 6$ ,  $22:5\omega 3$  and  $22:6\omega 3$  levels than those of the wild animals, while the level of  $18:1\omega 9$  was higher. The results of the analyses of the brain EPG, CPG, and SM fractions showed a high degree of similarity between the two wild animals, with no significant differences being detectable.

Major differences were detected between the liver and brain EPG and CPG fractions but only slight differences were noted between the SM fractions from the two tissues. Within the EPG, the liver levels of 16:0, 18:0, 18:2 $\omega$ 6, 22:4 $\omega$ 6 and 18:3 $\omega$ 3 were higher than those of the brains, while the levels of 16:1 $\omega$ 9, 18:1 $\omega$ 9, 20:1 $\omega$ 9, 20:3 $\omega$ 6, 20:4 $\omega$ 6, 22:5 $\omega$ 3 and 22:5 $\omega$ 3 were lower. Within the CPG, the levels of 18:0, 18:2 $\omega$ 6, 20:4 $\omega$ 6, 18:3 $\omega$ 3 and 22:5 $\omega$ 3 were higher in the livers than in the brains, while those of 16:0, 16:1 $\omega$ 9, 18:1 $\omega$ 9, 20:1 $\omega$ 9, 20:3 $\omega$ 6, 20:3 $\omega$ 3 and 22:6 $\omega$ 3 were lower. In the SM fractions, the differences were fewer and much less marked than in the other two fractions. The levels of 16:0 and 18:0 were higher in the livers than those of the brains while 24:0, 24:1 $\omega$ 9 and 22:6 $\omega$ 3 were present at lower levels.

#### DISCUSSION

The results presented (Table 1) represent the first stage in the evaluation of the lipid status of the cheetah both in captivity and in the wild. The two wild cheetahs showed very similar patterns of fatty acids within the liver EPG, CPG, and SM fractions, and these results are comparable to those published on the lion, leopard, and hyena.<sup>1</sup> The captive cheetah exhibited a liver fatty acid profile in the EPG, CPG, and SM fractions largely in agreement with those of the two wild animals, but the levels of 18:1 $\omega$ 9 (oleic acid), 18:3 $\omega$ 3 ( $\alpha$ -linolenic acid), and all the C22 fatty acids were lower in the captive animal, while those of 18:2 $\omega$ 6 (linoleic acid) and 20:4 $\omega$ 6 (arachidonic acid) were higher. The differences observed may reflect membrane phosphoglyceride changes resulting from either the advanced age of the captive animal, or arise from the dietary regime fed in captivity. It has not proven possible at present to quantify any differences resulting from the degree of freshness of the diets. However, if the results do reflect dietary differences, then the lower levels of desaturase reaction products, other than those provided in large quantities by meat, may be circumstantial evidence for desaturase deficiency in the cheetah.

The brains of the two wild cheetahs showed a high degree of consistency of the fatty acids within the EPG, CPG, and SM fractions. These results were similar to those published for lion, leopard, and hyena,' and other mammals,' showing once again the extremely conservative nature of the mammalian brain.

It is probable that this species does require lipids of animal origin, and thus fulfills that criterion for obligate carnivore status. Further work using radiolabeled fatty acids is planned to clarify the situation, and must be carried out before definite conclusions can be drawn.

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### REFERENCES

- 1. CRAWFORD, M. A. and SINCLAIR, A. J. In Lipids, Malnutrition and the Developing Brain pp. 268-292, CIBA Foundation Symposium, 1971.
- 2. DAVIDSON, B. C., CANTRILL, R. C. and VARADAY, D. S. Afr. J. Zool. 21, 161-164 (1986).
- 3. FOLCH, J., LEES, M. and SLOANE-STANLEY, G. H. J. Biol. Chem. 226, 497-509 (1957).
- 4. MOSCATELLI, E. A. Lipids 7, 268-271 (1972).
- 5. RIVERS, J. P. W., HASSAM, A. G., CRAWFORD, M. A. and BRAMBELL, M. R. FEBS, Lett. 67, 269-270 (1976).
- 6. RIVERS, J. P. W., SINCLAIR, A. J. and CRAWPORD, M. A. Nature 258, 171-173 (1975),
- 7. SINCLAIR, A. J. Proc. Nutr. Soc. 34, 287-291 (1975).
- 8. SMITHERS, R. H. N. In *The Mammals of Africa: An Identification Manual*, pp. 1-10, Smithsonian Institution Press, Washington, D.C., 1975.
- SMITHERS, R. H. N. In The Mammals of the Southern African Subregion, pp. 364-369, University of Pretoria Press, Pretoria, South Africa, 1983.