The reversal of essential fatty acid deficiency symptoms in the cheetah

B.C. Davidson, R.C. Cantrill and D. Varaday

Department of Medical Biochemistry, University of the Witwatersrand Medical School, Johannesburg and Cheetah Sanctuary, Damwal

Two members of the Order Carnivora (the lion and the domestic cat) are known to be Δ-6-desaturase deficient. Two anoestrous 8-year-old female cheetahs exhibiting symptoms consistent with essential fatty acid (EFA) deficiency were treated with encapsulated natural oils as supplement to their normal meat diet. The condition of both animals improved markedly, they came into full oestrus, mated, became pregnant, and have since produced healthy litters of cubs. This may be the first indication of a possible requirement for Δ-6-desaturase reaction products in this species.

S. Afr. J. Zool. 1986, 21: 161 – 164

Dit is bekend dat twee lede van die Orde Carnivora (die leeu en die gewone huiskat) aan 'n Δ-6-desaturasegebrek ly. Twee anestreuse agt jaar oue vroulike jagluiperds wat simptome van essensiële vetsuurgebrek vertoon het, is behandel met kapsules van natuurlike olies, as aanvulling by hulle vleisdieet. Hulle kondisie het merkbaar verbeter, hulle het in volle estrus gekom, gepaar, dragtig geword en het sedertdien gesonde werpsels gehad. Hierdie mag die eerste indikasie wees van 'n moontlike behoefte vir die produkte van die Δ-6-desaturasereaksie in hierdie spesie.

S.-Afr. Tydskr. Dierk. 1986, 21: 161 – 164

B.C. Davidson* and R.C. Cantrill
Department of Medical Biochemistry, University of the Witwatersrand Medical School, Johannesburg, 2001 Republic of South Africa

D. Varaday
Cheetah Sanctuary, Damwal, Republic of South Africa

*To whom correspondence should be addressed

Received 4 September 1985; accepted 26 November 1985

For many years it has been known that mammals deprived of the plant-derived polyenoic fatty acids, linoleic acid (*cis*-C18:2ω6) and α-linolenic acid (*cis*-C18:3ω3) show certain characteristic symptoms (Crawford & Sinclair 1972; Rivers & Davidson 1974; Sinclair, Fiennes, Hay, Watson, Crawford & Hart 1974). The symptoms in monkeys, rats, and mice were skin lesions with associated hair loss, anal sores, dullness of the eyes, self mutilation, sperm abnormalities in males, and loss of oestrus in females. These are now considered to be the classic symptoms of essential fatty acid (EFA) deficiency. In experimental animals (rats, mice, and monkeys) these symptoms are reversible by administration of linoleic and α-linolenic acids (Rivers & Davidson 1974; Sinclair *et al.* 1974).

It has been shown that at least two species of the Carnivora, the domestic cat (*Felis cattus*) (Rivers, Sinclair & Crawford 1975) and the lion (*Panthera leo*) (Rivers, Hassam, Crawford & Brambell 1976) lack the ability to further desaturate the polyenoic fatty acids linoleic acid (*cis*-C18:2ω6) and α-linolenic acid (*cis*-C18:3ω3). They apparently do not possess an active form of the enzyme Δ-6-desaturase. This is the first enzyme in the desaturase cascade, which converts linoleic and α-linolenic acids into the longer chain more unsaturated fatty acids. These moeities are vital for normal cell membrane structure and also act as substrates for the prostaglandin synthetase enzymes. Thus, the lack of the ability to produce these long chain polyunsaturates, imposes the necessity for a carnivorous life-style on these two species. The presence or absence of active forms of Δ-5 and Δ-4-desaturase (the other two enzymes in the desaturase cascade) has not been thoroughly investigated, although some tentative evidence for the absence of Δ-5-desaturase in domestic cats has been put forward (Frankel & Rivers 1978). Other mammals have no such limitations (Brenner 1971).

The cheetah (*Acinonyx jubatus*) belongs to a different genus to both the lion and the domestic cat, and exhibits many adaptive differences from the above two species, as characterized by Smithers (1975, 1983). The species may show differences other than the anatomically obvious ones, and thus the possibility exists for an active Δ-6-desaturase enzyme in cheetahs. The importance of this possibility, when considering the composition of the diet of captive cheetahs, cannot be too highly stressed. If the animal is a true obligate carnivore (like the lion and domestic cat), then the provision of more than just plant- and meat-derived fatty acids is vital for the health and breeding of this species. These fatty acids are highly unstable and rapidly deteriorate on storage. In the wild these fatty acids would be provided fresh by the offal and other parts of the prey, in captivity these components of the diet

are unlikely to be fresh, and thus the nutritional value will have been reduced. Under conditions of dietary stress, such as repeated pregnancy and lactation, females may well be drained of their reserves of such fatty acids and thus exhibit essential fatty acid deficiency symptoms. The genetic depauperate nature of the cheetah has been demonstrated (O'Brien, Wildt, Goldman, Merril & Bush 1983; O'Brien, Roelke, Marker, Newman, Winkler, Meltzer, Colly, Evermann, Bush & Wildt 1985), and the high incidence of spermal abnormalities shown (Coubrough, Bertschinger, Soley & Meltzer 1976; Coubrough, Bertschinger & Soley 1978). These abnormalities are similar to those demonstrated in essential fatty acid deficient capuchin monkeys and baboons (Sinclair *et al.* 1974), and thus the fatty acid status of the animals may have been impaired.

The opportunity arose to investigate the apparent poor health of two adult female cheetahs at a private cheetah breeding station at Damwal in the Transvaal, South Africa. The two females were both eight years old, and had both previously produced three litters of cubs. The latest litter was born in July 1983, they had both been anoestrous since then, and had shown steadily deteriorating general condition.

Materials and Methods

The animals were fed a diet consisting of 90% mammal carcasses (70% beef, 10% horsemeat, 10% donkey), and 5% intact poultry carcasses. The balance of the diet consisted of a mixture of minerals, vitamins, and bran as fibre. This regime was fed for six out of every seven days, and was the only diet fed to the animals during the period of the oil supplementation regime.

Natural oil capsules of two types, one rich in the immediate precursor of dihomo-γ-linolenic acid (DGLA) and the other rich in eicosapentaenoic acid (EPA), were made available, and were utilized as a supplement to the diet of the affected animals. The oils were in the form of evening primrose oil (EPO) and a fish oil (FO). The capsules contained 500 mg of oil in each case and were dosed orally, by inclusion in the rations, at the rate of four EPO and two FO every second day. The fatty acid analyses of beef (calf), chicken, EPO, and FO are shown in Table 1.

The fatty acids were extracted according to the method of Folch, Lees & Sloane Stanley (1957), and the fatty acid methyl esters produced by transesterification of the fatty acids with 14% boron trifluoride in methanol (Moscatelli 1972). The samples were analysed utilizing a Varian 3400 gas chromatograph with a 30 m × 0,25 mm ID 10% OV351 column. The oven was temperature programmed from 180–220°C, and the peaks quantitated by means of a Varian 4270 integrator.

Table 1 The fatty acid profiles of the diet and supplements

Fatty acid	Beef	Chicken	EPO	FO
16:0	26,9	26,7	6,1	8,5
18:0	13,0	7,1	4,5	3,5
16:1ω9	6,3	7,2	tr	12,3
18:1ω9	42,0	39,8	10,3	28,0
20:1ω9	tr	0,6	nd	6,8
18:2ω6	2,0	13,5	71,2	4,5
18:3ω6	nd	nd	7,9	nd
20:2ω6	nd	nd	nd	nd
20:4ω6	1,0	0,5	nd	3,9
18:3ω3	1,3	0,7	nd	1,3
20:5ω3	tr	0,2	nd	17,2
22:6ω3	nd	1,0	nd	8,4

All results expressed as % total fatty acid methyl esters. nd = not detectable; tr = trace (< 0,1%).

Results

The symptoms, as witnessed by us early in January 1985, were dull, drab coat with stiff bristle-like hair; poor pigmentation of coat, i.e. loss of both yellow and black colouration; dull staring eyes; dry, scaly skin with encrustation sores at both anus and nostrils; and no oestrus cycle. The animals had been treated with antibiotics for suspected kidney infection but no improvement in the above symptoms had been observed, and in fact the animals' condition had continued to deteriorate.

The symptoms were tentatively interpreted as indicative of essential fatty acid (EFA) deficiency, and thus a supplementation regime as described above was commenced early in February 1985. After one month of supplementation the animals showed considerable improvement in their condition. One animal had shown complete reversal of all the external symptoms, whilst the other had shown considerable regression of the symptoms and its condition was still improving.

The two females were at this time very close to the end of their breeding life, and had not exhibited any signs of oestrus since the birth of their last litters nearly two years previously. Both of the females have since come into full oestrus, mated, become pregnant, and produced litters, with one litter of three cubs and the other of two cubs. All the cubs appear healthy and seem to be following the same growth pattern as litters produced by other females at the reserve.

Discussion

The basic diet is rich in meat and provides more than sufficient arachidonic acid (McCance & Widdowson 1960; Crawford, Gale, Woodford & Casped 1970) to fulfil the requirement for this fatty acid as the 2 series eicosanoid precursor (Crawford 1983). The diet is, however, deficient in both the 1 series and 3 series eicosanoid precursors, DGLA and EPA as shown in Table 1. Neither of these fatty acids are provided by oils derived from plants, and thus the supplementation of diets with, for example sunflower seed oil, would not relieve deficiency symptoms in animals incapable of the utilization of plant EFA's. It has been shown that arachidonic acid exhibits inhibitory effects on the desaturation of the parent fatty acids of both the ω6 and ω3 series fatty acids, linoleic and α-linolenic acids, respectively (Irvine 1982); thus the high levels of this fatty acid provided by the diet may inhibit the enzyme Δ-6-desaturase, and this may be especially important if its activity is low to start with and substrate availability is restricted.

The EPO-rich supplement provides a fatty acid, γ-linolenic acid (GLA, *cis*-C18:3ω6) which is the product of the Δ-6-desaturase reaction, and this can be chain-elongated to provide the precursor of the 1 series of prostaglandins. The FO supplement contains both EPA, the 3 series prostaglandin precursor, and its metabolite docosahexaenoic acid (DHA, *cis*-C22:6ω3), thus providing the products of both Δ-5 and Δ-4-desaturases. Therefore any deficiency of the ω3 series fatty acids resulting from the absence of these enzymes may be circumvented. The ω6 product of Δ-5-desaturase, arachidonic acid (*cis*-C20:4ω6), was provided in large amounts in the meat diet as described above. Thus the only desaturase products not provided by the diet and the supplement was the Δ-4-desaturase product of the ω6 series of fatty acids, docosatetraenoic acid (*cis*-C22:4ω6), found in large amounts in adrenal

tissue. The general sources of the different series of polyenoic fatty acids and their metabolic interrelationships are shown in Figures 1 and 2.

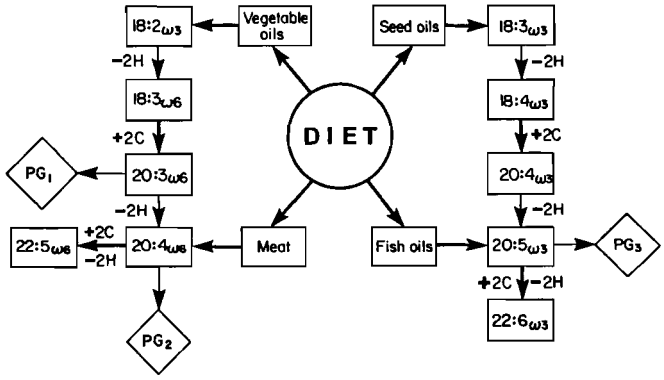

Figure 1 The generalized pathway from the plant dietary essential fatty acid source to the long chain polyenoic derivatives.

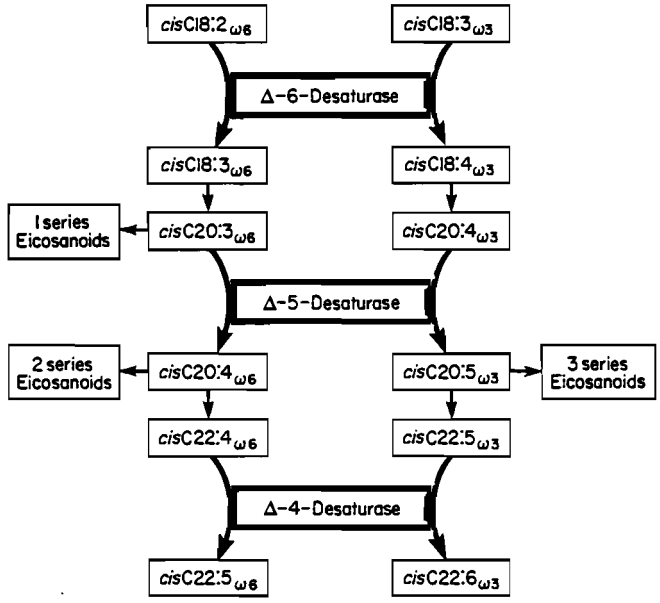

Figure 2 The desaturase enzyme cascade in detail.

The oil supplementation had apparently reversed all the EFA deficiency symptoms in these animals, thus probably validating the interpretation of the symptoms, and provided sufficient polyenoic fatty acids of each series to allow the pregnancies to proceed to full term, and for subsequent lactation. The high dietary levels of arachidonic acid, and the dearth of other metabolically important polyenoic fatty acids, may well have produced an eicosanoid imbalance in these two females, which the polyenoic supplementation has redressed. The fatty acid profile of the membrane phosphoglycerides in these animals may have been distorted by the dietary deficiencies, and this was exacerbated during previous pregnancies by the drain on the bodily stores of polyenoics to provide for the foetus through the placenta, and the suckling cub through the milk. The reversal of both membrane and hormonal associated symptoms may reflect the redressing of imbalances in both membrane phosphoglyceride and eicosanoid profiles, by the provision of sufficient polyenoics to restore the normal state.

Conclusion

In relation to its undoubted carnivore nature the cheetah would appear to resemble its relatives the lion and the domestic cat in being a true obligate carnivore. Thus it may well be dependent on the consumption of animal lipid to maintain a normal fatty acid balance in both its membrane phosphoglycerides and its eicosanoid metabolism. The results presented above are the first evidence of such a requirement in the cheetah. In most cases of animal husbandry the provision of plant-derived essential fatty acids is sufficient to satisfy the requirement of the animal for EFAs. If the status of the animal is in doubt with regard to its ability to utilize such fatty acids (especially carnivores), then the supplementation of the dietary regime with at least a fish oil should be considered carefully.

Acknowledgements

The authors would like to thank Efamol Ltd., London and National Chemical Products, South Africa for the provision of the oil supplements.

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