Comparative Fatty Acids of the Dolphin and the Herring

G. WILLIAMS, B.C. DAVIDSON, P. STEVENS and **M.A. CRAWFORD,**
Department of Biochemistry, Nuffield Institute of Comparative Medicine,
Zoological Society of London, Regent's Park, London, NW1 4RY, England

ABSTRACT AND SUMMARY

The tissue samples from two bottlenosed dolphins (*Tursiops truncatus*) became available, and it was decided to examine the fatty acid compositions of the phospholipid fractions. The food chain of the dolphin provides a vast preponderance of ω3 series fatty acids compared to ω6, while the land food chain provides a more even balance between the two series. The ancestors of the dolphin had evolved on the land with the terrestrial ω3:ω6 balance, but the dolphin now lives in an ω3-rich environment. The investigations were carried out to discover whether the dolphin reflected its marine environment or alternatively its evolutionary history. The results showed that the fatty acids of the dolphin bear a much closer resemblance to those of land mammals than to those of other marine vertebrates.

INTRODUCTION

The investigation of the phosphoglyceride fatty acids in the liver, muscle, and brain of 32 different species of land mammals has shown that the ω3:ω6 series of fatty acids are present in the ratio of between 1:1 and 1:4 (1,2). The bottlenosed dolphin (*Tursiops truncatus*) which lives in the Carribean feeds largely on fish and the occasional squid (3), while the porpoise (*Phocoena phocoena*) living in the North Sea eats mainly herring (*Clupea harengus*) and other clupeoids (4). All these fish are rich in ω3 fatty acids relative to ω6, the herring showing ratios of 30:1 (Table I) while some tropical fish show ratios of the order of about 3:1 (Crawford and Munhambo, unpublished data).

TABLE I

% Composition of Fatty Acid Methyl Esters in Herring Muscle and Liver

	Muscle		Liver	
	EPG[a]	CPG[b]	EPG	CPG
14:0	0.0	0.9	0.0	1.4
16:0	17.0	25.6	17.4	21.6
18:0	3.4	5.2	3.4	4.2
20:0	1.0	0.1	0.1	0.9
22:0	0.0	0.0	0.0	0.0
Total saturates	21.4	31.8	20.9	28.1
16:1	0.6	0.9	2.3	2.4
18:1	5.3	3.6	12.4	11.0
20:1	0.1	0.0	1.4	0.0
22:1	0.7	0.0	0.7	0.0
Total monoenes	6.7	4.5	16.8	13.4
18:3 ω3	0.1	0.1	1.3	0.1
20:5 ω3	4.6	15.8	7.3	17.5
22:5 ω3	0.9	0.8	1.3	0.7
22:6 ω3	62.8	43.9	46.3	37.9
Total ω3	68.4	60.6	56.2	56.2
18:2 ω6	0.2	0.2	0.7	0.2
20:2 ω6	0.0	0.0	0.0	0.0
20:3 ω6	0.0	0.0	0.0	0.0
20:4 ω6	0.5	2.1	0.6	1.7
22:4 ω6	0.2	0.0	0.0	0.0
22:5 ω6	0.5	0.0	0.0	0.0
Total ω6	1.4	2.3	1.3	1.9

[a]EPG = ethanolamine phosphoglyceride.
[b]CPG = choline phosphoglyceride.

The tissues of two bottlenosed dolphins became available to us. One had died shortly after being transported from the U.S.A. and the other had been in captivity at Whipsnade Park for nearly two years. Both animals were caught in the Gulf of Mexico. The first animal gave us the opportunity to analyze the total phospholipids (PL) in the liver and the ethanolamine phosphoglycerides (EPG) in both the liver and the brain, together with the lipids of the adipose fat (Table II). The results were so interesting that when a second animal became available, we carried out a more detailed analysis and analyzed the EPG and choline phosphoglyceride (CPG) in the liver, brain, and muscle, and again the total lipids of the fat (Table III).

The land food chain provides both ω3 and ω6 fatty acids, with green foods rich in ω3 and seed foods rich in ω6, while the marine food chain is rich in ω3 (5). Because of the extreme differences between the food chemistry on land and in the sea, it was suspected that the dolphin liver, muscle, and brain might be different from those of terrestrial mammals (6). The results of our investigations did not appear to support the hypothesis.

MATERIALS AND METHODS

The liver, brain, muscle, and subcutaneous fat of the two bottlenose dolphins were examined and the results compared with samples from herring and mackerel. The lipid material was extracted by macerating the tissue with chloroform-methanol (2:1 v/v) containing 2:6 di-t-butyl-p-cresol (0.1% w/v) as antioxidant, in an MSE top drive homogenizer and the extracts filtered and washed with physiological saline (20% v/v) as described by Folch (7). All solvents used were obtained from British Drug Houses Ltd., Poole, England and were of Analar grade unless otherwise specified. The whole lipid extracts were concentrated under vacuum in an all glass rotary evaporator (Buchi Rotovator "R" Orme Scientific Ltd., Manchester, England) and were then made to 100 cm^3 with chloroform. 2 x 1 cm^3 aliquots were pipetted into preweighed vials, blown to dryness under a stream of nitrogen, and then dessicated under vacuum over P_2O_5 for 2 hr and reweighed. The weight of lipid extracted per unit weight of tissue was then calculated. The remaining lipid was then treated on thin layer chromatography (TLC), all of the plates (20 cm x 20 cm) being coated with Silica Gel G by Merck (Anderman & Co., London). The plates were spread with a gel giving a thickness of 0.5 x 10^{-3}m. The lipids were separated and identified with the aid of TLC standards (Sigma Ltd., London) into phosphatidyl choline (CPG) and phosphatidyl ethanolamine (EPG) fractions, the plates being developed in chloroform-methanol-water (60:30:4 v/v). The neutral lipids were separated from total phospholipids by development in petrol ether-diethyl ether-glacial acetic acid-methanol (65:15:2.5:1 v/v). The lipid bands were located by spraying lightly with 2:4 dichlorofluoroscein (DCF) and viewing under ultraviolet light (250 x 10^{-9} m). The bands containing the lipids were transferred directly into sealable screw cap vials, to which were added 5 cm^3 of sulphuric acid in methanol (5% v/v). The mixture was heated at 70 C for 180 min in which time derivatization of the fatty acids to methyl esters was accomplished. The samples were removed from the heat, cooled, distilled water

TABLE II

% Composition of Fatty Acid Methyl Esters from Tissues of Dolphin Which Died in Transit

	Liver		Brain	Fat
	EPG[a]	Total PG[b]	EPG	Total FMEs[c]
14:0	0.0	0.0	0.0	9.1
16:0	7.5	10.1	8.2	10.5
18:0	23.3	29.8	22.0	1.0
20:0	0.0	0.0	0.0	0.0
22:0	0.0	0.0	0.0	0.0
Total saturates	30.8	39.9	30.2	20.6
16:1	15.1	8.1	2.8	10.9
18:1	17.1	18.6	11.2	23.4
20:1	0.8	1.4	0.2	13.6
22:1	0.0	0.0	0.0	13.1
Total monoenes	33.0	28.1	14.2	61.0
18:3 ω3	0.5	0.4	1.4	0.5
20:5 ω3	6.5	5.7	0.2	2.4
22:5 ω3	1.4	2.3	2.2	1.9
22:6 ω3	6.6	9.0	26.1	8.0
Total ω3	15.0	17.4	29.9	12.8
18:2 ω6	1.9	3.1	0.6	1.4
20:2 ω6	0.1	0.0	0.3	0.0
20:3 ω6	0.1	0.8	0.1	0.1
20:4 ω6	13.2	7.8	6.5	0.1
22:4 ω6	0.9	0.1	5.2	0.1
22:5 ω6	0.4	0.2	0.5	0.1
Total ω6	16.6	12.0	13.2	1.8

[a]EPG = ethanolamine phosphoglyceride.
[b]PG = phosphoglyceride.
[c]FME = fatty acid methyl ester.

TABLE III

% Composition of Fatty Acid Methyl Esters from Tissues of Dolphin Held Captive for 22 Months

	Liver		Brain		Muscle		Fat
	EPG[a]	CPG[b]	EPG	CPG	EPG	CPG	Total FMEs[c]
14:0	0.0	0.0	0.0	0.0	0.0	0.0	9.1
16:0	6.2	14.2	3.0	38.6	3.1	9.5	10.1
18:0	35.0	23.5	21.3	16.4	20.5	7.9	1.0
20:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total saturates	41.2	37.7	24.3	55.0	23.6	17.4	20.2
16:1	4.2	9.8	2.0	1.2	5.2	21.9	10.5
18:1	9.2	26.2	6.4	32.0	10.5	34.3	23.1
20:1	1.1	1.5	0.2	0.0	0.1	0.0	13.8
22:1	0.0	0.0	0.0	0.0	0.0	0.0	12.2
Total monoenes	14.5	37.5	8.6	33.2	15.8	56.2	59.6
18:3 ω3	0.1	0.3	0.9	0.0	0.2	0.1	1.0
20:5 ω3	9.8	3.7	0.8	0.0	12.3	8.8	2.2
22:5 ω3	1.3	1.3	2.3	0.4	0.0	0.0	1.5
22:6 ω3	15.3	8.4	28.4	3.3	18.6	1.9	9.3
Total ω3	26.5	13.7	32.4	3.7	31.1	10.8	14.0
18:2 ω6	1.6	3.8	0.8	0.0	2.9	1.5	1.8
20:2 ω6	0.0	0.0	0.9	0.8	0.0	0.0	0.2
20:3 ω6	0.0	0.0	0.9	0.1	0.0	0.0	0.1
20:4 ω6	11.7	3.3	7.2	3.3	6.7	5.3	0.3
22:4 ω6	0.0	0.0	2.3	0.6	0.0	0.0	0.2
22:5 ω6	0.0	0.0	0.2	0.3	0.0	0.0	0.2
Total ω6	13.3	7.1	12.3	5.1	9.6	6.8	2.8

[a]EPG = ethanolamine phosphoglyceride.
[b]CPG = choline phosphoglyceride.
[c]FME = fatty acid methyl ester.

(10 cm^3) added, and the methyl esters extracted by shaking with 3 x 10 cm^3 of petroleum ether (bp 40-60 C). The pooled petrol extracts were washed once with water (10 cm^3) to remove excess acid and reduced in volume under a stream of nitrogen, with gentle heating.

The fatty acid methyl esters were separated by gas liquid chromatography (GLC) using two columns in parallel. Column No. 1 (2.1 m x 4 mm ID) contained polyethylene glycol adipate (PEGA), 10% w/w on diatomite C 100-120 mesh. Column No. 2 (2.1 m x 4 mm ID) contained ethylene glycol succinate silicon-X (EGSS-X) 10% w/v on diatomite C 100-120 mesh. Both the diatomite C and the PEGA were obtained from Pye Unicam Ltd. and the EGSS-X obtained from Applied Science Ltd. (Field Instruments Ltd., London). The GLC system used was a Pye Unicam Gas Chromatograph series 104 having flame ionization detectors. The samples were run at 188 C oven temperature, with a carrier gas (argon) flow of 70 cm^3/min and both injection port and detector heaters were set at 250 C. The theoretical plate value (Ref C18:0) for PEGA was 2000 and for EGSS-X 3500. Identification of fatty acid methyl esters were made using the retention volumes of known GLC standards (Sigma Ltd.). Quantitation of the peaks were made using an Infotronics Series CRS-208 automatic digital integrator.

RESULTS AND DISCUSSION

The results shown in Tables I, II, and III are expressed as

TABLE IV

Ratios of Saturates and Monoenes to Total Polyunsaturates (PUFA), and Ratios of ω3 to ω6 within Polyunsaturate Fractions

	Tissue and fraction	Saturates + monoenes:PUFA	ω3:ω6
Herring	Liver EPG[a]	0.7:1	43.2:1
	Liver CPG[b]	0.7:1	29.6:1
	Muscle EPG	0.4:1	48.9:1
	Muscle CPG	0.6:1	26.3:1
22 Months dolphin (2)	Liver EPG	1.4:1	1.9:1
	Liver CPG	3.6:1	1.9:1
	Brain EPG	0.7:1	2.6:1
	Brain CPG	10.0:1	0.7:1
	Muscle EPG	1.0:1	3.2:1
	Muscle CPG	4.2:1	1.6:1
	Adipose	4.8:1	5.0:1
	Fat total FMEs[c]		
Transit dolphin (1)	Liver EPG	2.0:1	0.9:1
	Liver total PG	2.3:1	1.5:1
	Brain EPG	1.0:1	2.3:1
	Adipose	5.6:1	7.1:1
	Fat total FMEs		

[a]EPG = ethanolamine phosphoglyceride.
[b]CPG = choline phosphoglyceride.
[c]FME = fatty acid methyl ester.

a percentage of the total C14-C22 fatty acids, thus enabling comparison with published data from land mammals (9,10). Table IV shows the ratios of saturates and monoenes to polyunsaturates (PUFA) and ω3 to ω6 values.

The liver of the second animal shows higher levels of ω3 PUFA, whereas the brain and adipose tissue of the two animals show a high degree of similarity in spite of their different dietary histories.

The ratio of saturates plus monoenes to PUFA in the herring was 0.7:1 indicating that the fish has a highly unsaturated system. It was also shown that the ratio of ω3:ω6 fatty acids was high, indicating that the availability of ω6 fatty acids, relative to ω3, was low. Examination of the dolphin tissue shows a very different pattern in most cases.

The saturates plus monoenes to PUFA ratios were in most cases the opposite to those of the herring, that is, the dolphin tissue was richer in saturate and monoene fatty acids. Except for its adipose tissue, the dolphin ω3:ω6 ratios were markedly different from those of the herring in that there were considerably lower levels of ω3 fatty acids. The fatty acid composition of herring was reflected in the dolphin adipose tissue, which was mainly ω3 fatty acids.

Herring is a rich source of lipid (20 g/100 g fish) (8) compared with meat (2 g - 5 g/100 g) (8,9), thus although the ω6 is low relative to the ω3, the absolute levels of ω6 would be adequate for the dolphin, and it could afford to be selective as to the type of fatty acids incorporated into its structural lipids. Excess fatty acids, predominantly of the ω3 series, would probably be utilized for energy.

Although there is the possibility of another source of ω6 fatty acids being available to the dolphin, we consider this to be unlikely, due to the absence of a rich primary source of the parent ω6 acid (18:2 ω6, ω9) in the marine environment.

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