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GREY SEAL (HALICHOERUS GRYPUS) DAM-PUP TRANSFER OF FATTY ACIDS

by

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INTRODUCTION

This laboratory has shown (Eaton, et al., 1977) that the hearts of several species of seals of diverse ages and both sexes are essentially free of a type of myocardial lesion ascribed to docosenoic acid (22:1) in the diet of animals, especially rats (Christophersen et al., 1976). As part of a continuing study of the relationship between diet, depot fat and cardiac triglycerides in various animals, we have compared selected fatty acids of the blubber (depot) fat and cardiac triglycerides in a number of grey seals (*Halichoerus grypus*) collected on Sable Island in January of 1974.

EXPERIMENTAL

Six dam-pup pairs were collected. Length, girth, sex and blubber thickness over the sternum were observed in the field (Table 1). Hearts were excised and transferred to Halifax. Blubber sections were collected from the mid-dorsal region and were also studied for organochlorine residues (Addison and Brodie, 1977). The total lipid recovered from the tissues by  $\text{CHCl}_3$ -MeOH extraction was recorded (Table 1). The triglyceride fraction of the cardiac lipid was isolated and determined gravimetrically with a polystyrene-divinylbenzene gel column (Sipos and Ackman, 1968). Fatty acid methyl esters analyses by gas-liquid chromatography (Tables 2 and 3) followed procedures published earlier (Ackman et al., 1972, 1974).

RESULTS

Blubber

Seal milk is notoriously rich in fat (> 50%) and therefore fat transfer from dam to pup is a very rapid process (Jangaard and Ke, 1968; Ackman et al.

1972; Addison and Brodie, 1977). The pups apparently show increases in blubber thickness and in blubber fat content for stages 1 through 3, and the dams may show a corresponding reduction in blubber thickness although not in blubber fat content (Table 1).

The seal pups have more saturated acids in the blubber than do their dams, (Table 2) averaging 22.0% versus 16.1%. The proportions of 16:1, possibly derived in part from 16:0 in the pup (Ackman et al., 1972), are also higher in the pup blubber than in the dam blubber (averaging 20.1%, versus 15.9%) and the difference is probably more extreme in stage 1 than later. The 18:1 is not as consistent, with more in the blubber of pups than of dams in stages 1 and 3, the reverse generally is found in the stage 2 pups, but differences are modest.

For the 20:1 and 22:1 the pattern is clear and consistent with half as much or less of these acids in pup blubber as in dam blubber. The pup blubber average is 0.7% 22:1 versus 0.73% in the single animal of age 3 months examined earlier, (Ackman and Hooper, 1974). Clearly, the fatty acid composition of grey seal pup blubber is different from that of the pups of harp seals which contains 2-4% 22:1 (Ackman et al., 1971). This species difference extends to adults, grey seals, average 2.6%; harp seals, average 9.2% (unpublished data). Overall total monoethylenic acids do not differ systematically between pup and dam. Therefore to effect the difference described above for saturated acids, the polyunsaturated acids have lower totals in the pup blubber (20.0%) than in the dam blubber (26.5%). This relationship is non-selective among the polyunsaturated fatty acids with the exception of the minor acid 20:4 $\omega$ 6, which is consistently about twice as important, in the blubber of the pup than in that of the dam. The average ratio of 20:5 $\omega$ 3 to the successor acid 22:6 $\omega$ 3 is interesting, with 0.52 for dam blubbers, and 0.64 for pup blubbers. The ratio of 20:5 + 22:5 to 22:6 is consistent for both dams, 0.95 and pups, 1.1, whereas this ratio for harp seal commercial oils is 1.7 (Ackman et al., 1971).

### Cardiac Lipids

The cardiac lipid percentage values (Table 1) are comparable to values obtained earlier for one grey and two harbour (*Phoca vitulina*) seals (Ackman and Hooper, 1974) and the differences between these lipids and the cardiac lipids from other species, including terrestrial animals is, therefore, consistent.

Overall the percentages of saturated acids in the cardiac triglyceride (Table 3) are variable, ranging from 15.6% to 33.1%, but the averages of totals for pups (22.5%) and dams (23.2%) are very similar. Possibly there is a trend from lower 16:0 in the pup than in the dam at stage 1 to more 16:0 in the pup at stage 3.

The latter relationship is quite apparent with the 16:1 which can be either derived from 16:0 in the pup, or more probably is preferentially transferred with the milk. The percentage of 16:1 is twice as high in

stage 3 pups than in dams, whereas in stage 1 the converse relationship is observed although the difference is less marked.

The 18:1 may also follow this relationship in the cardiac lipid triglyceride and it must be noted that a substantial portion ca 25% (unpublished data) of total 18:1 is the *cis*-vaccenic isomer 18:1 $\omega$ 7 accompanying the more common 18:1 $\omega$ 9 or oleic acid. The 18:1 $\omega$ 7 is necessarily derived from 16:1 $\omega$ 7. Totals for 16:1 + 18:1 (not shown) proceed from dam > pup to dam < pup in a systematic way with stage of development. The 20:1, like the 18:1, shows a trend from similar levels in the pup and dam cardiac triglycerides at stage 1, to dam levels twice those of the pup at stage 3. A similar, although more pronounced relationship is evident for 22:1. Total monoethylenic acids are consistent throughout with dam > pup in all samples of cardiac triglyceride.

The polyunsaturated acids of the cardiac triglyceride differ from the respective blubber lipids in overall composition. In contrast to the blubber the total of these acids and hence the calculated iodine values are slightly higher for the pups than for dams. However, there is a definite difference for both pups and dams of less, 20:5 $\omega$ 3 relative to 22:5 $\omega$ 3 and 22:6 $\omega$ 3, in the cardiac than in the blubber triglyceride. The latter two acids do not differ very systematically in the cardiac triglyceride from either dam or pup blubber levels, but there is an exclusion, with stage of development, of 20:5 $\omega$ 3 (and probably of the related minor components 18:4 $\omega$ 3 and 20:4 $\omega$ 3) in the dams which must reflect selective transfer of 20:5 $\omega$ 3 to the pups. The observation of percentages of 20:4 $\omega$ 6 in the pup cardiac triglyceride being greater than those in the dam, is however parallel to the observation in the blubbers.

#### DISCUSSION

The conclusions of principal interest in these comparisons of triglyceride of blubber and cardiac tissue, and of dam and pup, are that although the total monoethylenic acids in the pup cardiac triglyceride are approximately the same as in the corresponding blubber, there is 4 to 5 times as much 22:1 in the cardiac triglyceride. It cannot therefore be argued that the grey seal pup myocardium is not exposed to the 22:1, which is the presumed cause of myocardial lesions in rats. The triglycerides of the hearts of the dams also are of interest since the average percentage of 22:1 rises sharply during lactation, stage 1 (3.6%) through stage 2 (6.0%) to stage 3 (11.4%). It is believed that this lipid should reflect circulating fatty acids so that during the nursing period the mobilization of depot fat in the dam must result in a selective transfer of certain fatty acids (e.g. 16:0, 16:1) but not of others (e.g. 20:1, 22:1) through the milk to the pup. Milk analyses carried out by Jangaard and Ke (1968) tend to confirm this in harp and hooded (*Cystophora cristata*) seals. The particular fatty acids in the circulating blood lipids of the dam should rapidly influence the fatty acids (Table 3) of the small amount of triglyceride in the myocardial lipid but the corresponding changes in the much larger mass of the blubber (depot) fat would be difficult to detect during this period of rapid fat depletion for energy and milk fat production.

It is also of interest to consider, from the the fatty acid trends, that the turnover of cardiac triglyceride is quite rapid in pups and also in dams. There is little or no data of this type for other animals of comparable size. One study on rat heart lipids showed very rapid response to variations in dietary fat status (Phuc-Canh Quan and Le Breton, 1974). Others have shown rapid adaption to high 22:1 diets with elimination of transient lipidoses (Beare-Rogers et al., 1971). The myocardium of the grey seal evidently can cope with naturally fluctuating levels of 22:1 from birth without eventual development of the necrotic lesions which the rat and some other species develop when exposed to 22:1. Since primates fed 22:1 at high levels in high fat diets also do not develop an excess of lesions over those found in control groups also fed high fat, low 22:1 diets (Ackman and Loew, 1977; Loew et al., 1977), the seal offers an interesting comparison for further study of the question of whether the convincing adverse effects of high levels of 22:1 on the rat myocardium (Beare-Rogers et al., 1972; Teige and Beare-Rogers, 1973) can be extrapolated to other species, including man.

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TABLE 1. Biological data for Sable Island grey seals and certain lipid data.

	Pup Sex	Development <sup>a</sup> Stage	Length <sup>a</sup> (cm)	Girth <sup>a</sup> (cm)	Blubber <sup>a</sup> Thickness (mm)	% Blubber Lipid	% Cardiac Lipid	% Triglyceride in Cardiac Lipid
Dam - 1	-	-	195	157	40	80	1.5	34.5
Pup - 1	M	1	105	75	18	71	1.6	23.0
Dam - 2	-	-	203	156	37	ND <sup>b</sup>	1.6	20.7
Pup - 2	F	1	104	66	10	60	2.0	25.0
Dam - 5	-	-	192	142	40	87	1.9	31.3
Pup - 5	M	2	98	83	27	80	2.0	24.4
Dam - 6	-	-	199	137	34	83	1.4	10.6
Pup - 6	M	2	116	80	25	75	1.5	21.3
Dam - 3	-	-	205	150	35	85	2.5	30.2
Pup - 3	F	3	102	92	45	86	1.4	16.5
Dam - 4 <sup>c</sup>	-	-	199	130	35	85	1.7	36.0
Pup - 4	M	3	107	90	27	83	1.8	36.9

<sup>a</sup>cf. Addison and Brodie, 1977

<sup>b</sup>ND, not determined

<sup>c</sup>Attributed relationship not confirmed.

TABLE 2. Major and particular fatty acids of triglycerides isolated from blubber of Sable Island grey seals with calculated fatty acid iodine values.

Fatty Acid <sup>a</sup> (w/w %)	SEAL PAIRS											
	1		2		5		6		3		4	
	Dam	Pup	Dam	Pup	Dam	Pup	Dam	Pup	Dam	Pup	Dam	Pup
14:0	3.9	4.4	4.9	5.7	5.0	5.4	4.5	4.4	5.0	4.2	3.9	5.6
16:0	7.5	12.7	11.4	17.5	7.2	9.9	6.8	15.9	9.7	11.0	6.2	13.7
18:0	1.2	1.3	1.8	1.4	0.9	1.1	0.8	1.4	1.5	1.2	1.1	1.5
Total Sat.	15.5	20.6	20.8	26.5	15.5	19.0	13.7	24.0	18.4	18.9	12.7	22.9
16:1	13.0	18.1	14.4	23.8	15.3	18.7	18.8	19.2	17.2	20.7	16.5	20.3
18:1	29.4	32.5	21.7	27.3	34.1	31.7	32.9	29.7	20.7	30.4	22.6	26.9
20:1	8.4	4.2	11.2	4.0	10.0	7.1	7.0	4.9	13.3	5.1	10.3	8.6
22:1	1.5	0.4	4.2	0.8	1.8	0.9	1.1	0.5	4.5	0.5	2.6	1.3
Total Mono.	55.0	56.6	52.9	58.2	63.0	60.2	62.0	55.5	57.2	58.5	54.1	59.1
18:2 $\omega$ 6	1.2	1.0	1.4	1.0	1.3	1.1	1.3	1.1	1.2	0.9	1.4	1.2
18:3 $\omega$ 3	0.7	0.3	0.4	0.3	0.6	0.5	0.3	0.2	0.5	0.4	0.5	0.3
18:4 $\omega$ 3	0.9	0.6	1.5	0.8	0.8	1.1	0.5	0.6	1.3	0.8	1.1	1.1
20:4 $\omega$ 6	0.4	0.8	0.4	0.8	0.4	0.6	0.4	0.4	0.3	0.9	0.3	0.5
20:4 $\omega$ 3	0.7	0.5	0.5	0.3	0.6	0.4	0.3	0.2	0.3	0.4	0.5	0.3
20:5 $\omega$ 3	7.0	6.6	5.1	2.9	4.7	4.9	4.8	4.3	6.2	5.7	7.0	4.1
22:5 $\omega$ 3	5.2	3.8	3.6	2.5	3.9	2.8	4.1	3.1	4.2	4.1	6.1	2.8
22:6 $\omega$ 3	11.9	7.7	11.6	5.4	9.0	8.0	12.1	10.0	8.7	7.4	14.5	6.1
Total Poly.	29.5	22.8	26.3	15.3	21.5	20.8	24.3	20.5	24.4	22.6	33.2	18.0
Calc. I.V.	163	137	147	109	138	132	151	130	143	138	178	120

<sup>a</sup>Total saturated, monoethylenic and polyethylenic fatty acids include minor acids not listed.

TABLE 3. Major and particular fatty acids of triglycerides isolated from cardiac lipid of Sable Island grey seals with calculated fatty acid iodine values.

Fatty Acid <sup>a</sup> (w/w%)	SEAL PAIRS											
	1		2		5		6		3		4	
	Dam	Pup	Dam	Pup	Dam	Pup	Dam	Pup	Dam	Pup	Dam	Pup
14:0	3.6	2.2	5.1	3.3	4.1	2.8	3.5	2.9	3.2	3.7	2.2	3.5
16:0	11.4	10.5	16.1	16.7	10.9	13.8	19.1	13.7	11.9	14.1	8.3	12.7
18:0	2.4	5.3	3.8	4.5	2.4	5.0	8.0	4.5	2.8	4.2	3.5	3.7
Total Sat.	19.4	20.2	27.9	26.5	19.8	23.3	33.1	22.1	19.3	24.9	15.6	22.1
16:1	10.4	7.6	13.6	11.8	10.4	6.3	7.8	8.0	8.6	13.1	5.0	12.0
18:1	33.0	32.6	24.8	26.0	29.1	29.6	26.0	27.3	23.9	30.0	19.0	23.6
20:1	10.3	11.4	9.8	9.5	14.7	15.7	14.5	12.7	19.3	10.6	29.7	15.4
22:1	2.8	2.1	4.4	3.0	6.1	3.9	5.8	2.9	10.2	2.3	12.6	4.6
Total Mono.	57.5	54.8	53.9	51.4	61.0	55.8	54.7	51.2	63.0	57.3	66.8	57.2
18:2 $\omega$ 6	1.8	2.0	1.9	1.9	1.5	1.8	1.7	2.5	1.7	1.5	1.8	2.1
18:3 $\omega$ 3	0.6	0.3	0.6	0.3	0.4	0.2	0.2	0.2	0.3	0.2	0.3	0.3
18:4 $\omega$ 3	0.5	0.3	0.6	0.4	0.5	0.2	0.1	0.2	0.3	0.3	0.2	0.4
20:4 $\omega$ 6	0.7	1.9	0.7	1.2	0.6	0.5	0.6	0.9	0.3	1.3	0.5	0.9
20:4 $\omega$ 3	0.4	0.2	0.3	0.2	0.3	<0.1	0.1	0.1	0.1	0.1	0.1	0.2
20:5 $\omega$ 3	3.7	3.9	2.9	2.4	2.7	1.2	0.8	1.7	2.1	2.5	1.1	2.5
22:5 $\omega$ 3	4.7	4.9	2.4	3.8	3.4	2.9	1.5	4.2	3.9	3.0	3.9	3.5
22:6 $\omega$ 3	9.5	10.3	7.5	10.3	8.4	13.8	6.7	16.6	8.2	7.0	9.1	9.8
Total Poly.	23.1	25.0	18.2	22.1	19.2	20.9	12.2	26.7	17.7	17.8	17.6	20.7
Calc. I.V.	136	141	114	128	124	130	91	149	119	115	121	126

<sup>a</sup>Total saturated, monoethylenic and polyethylenic fatty acids include minor acids not listed.