Hepatic Fatty Acids in Wild Rockhopper (*Eudyptes crestatus*) and Magellanic (*Spheniscus magellanicus*) Penguins before and after Moulting

Laurence S. Harbige[1], Keb Ghebremeskel[2], Glynne Williams
and Michael A. Crawford[2]

[1]*Institute of Zoology, Zoological Society of London, Regents Park, London
NW1 4RY, UK., and [2]The Institute of Brain Chemistry and
Human Nutrition Hackney Hospital, Homerton High
Street, London E9 6BE, UK.*

ABSTRACT—Intra- and inter-species hepatic differences for wild rockhopper (*Eudyptes crestatus*) and magellanic (*Spheniscus magellanicus*) penguin fatty acids were compared both pre-and post-moult. Linoleic (18:2n-6) and arachidonic (20:4n-6) acid composition were significantly higher and palmitic (16:0) acid significantly lower in pre moult rockhopper penguins than in comparable magellanics. Post-moult magellanics had significantly more palmitoleic (16:1), gadoleic (20:1n-9) and erucic, (22:1n-9) and less arachidonic and eicosapentaenoic (20:5n-3) percent fatty acids than post-moult rockhoppers. In both species moulting resulted in a significant reduction in eicosapentaenoic and docosapentaenoic (22:5n-3), and a significant increase in linoleic acid (18:2n-6) percent. In rockhoppers, post moult was associated with an increase in the proportion of palmitic (16:0) and a decrease in palmitoleic (16:1) acid. In the post-moult magellanics, however, there was a decrease in stearic (18:0) and an increase in gadoleic (20:1n-9) and erucic (22:1n-9) fatty acid composition.

INTRODUCTION

Successful breeding, migration, and moulting in birds are closely linked to nutritional factors [1–4]. Scarcity of food and thus poor nutrient deposition prior to these events can result in failure to breed, poor health and mortality [1–6], especially in species such as pengiuns which abstain from feeding while breeding and moulting. In penguins moulting lasts between two and five weeks depending on species, during which time body weight losses of 23–60% have been recorded [7–11]. Before breeding and moulting penguins build up body reserves mainly in the form of lipid. These adaptive responses result in body weight increases of 5–33% depending on species and sex [10–12]. Lipids have diverse biological roles; neutral lipids provide important energy reserves, whilst phospholipids have membrane structural functions [13–15]. The polyunsaturated fatty acid components of phospholipids provide substrates for the cell regulatory molecules the eicosanoids, [14] and are thought to provide structural integrity to cell membranes [16–19].

In view of the importance of lipids, we have investigated the fatty acid composition of hepatic tissue from wild rockhopper (*Eudyptes crestatus*) and magellanic (*Spheniscus magellanicus*) penguins both pre-and post-moult.

MATERIALS AND METHODS

Penguins

Liver tissue samples were obtained at necropsy from adult healthy free-living rockhopper and magellanic penguins inhabiting the Falkland Islands during February 1987 after a post-breeding feeding period. This was undertaken as a result of

a penguin mortality investigation in the Falkland Islands in 1986 [20]. It was not possible to differentiate between adults and subadults even when taking into consideration the appearence of the gonads [11]. The period between the arrival on the moulting area and the beginning of the old feather loss was classified as pre-moult. Whereas, the penguins that had replaced their old feathers with new plumage were considered to be post-moult.

Lipid extraction

Total lipids were extracted from liver samples by the method of Folch *et al*, [21]. Tissues were homogenised in chloroform: methanol (2:1 v/v) containing 0.01% 2,6-di-t-butyl-4-methylphenol (BHT) as an antioxidant and left for 24 hr at 4°C. The homogenate-solvent mixture was filtered and transferred to a separatory funnel and left overnight at 4°C following the addition of 25% saline (0.85% NaCl) by volume. The lower organic phase was evaporated in a Rotavap-R (Buchi) under reduced pressure at 37°C. Samples were kept under nitrogen during and after the extraction

procedures and extracts stored at $-20°C$ until required.

Fatty acid separation and identification

Total lipids were transmethylated under nitrogen at 70°C for 3 hr with 5 ml of 5% sulphuric acid in methanol as an esterifying reagent. The fatty acid methyl ester derivatives were separated and identified as previously described [22] except that the chromatograph was a Varian model 3700, and the column a CP Sil88 (SP 2340).

Statistical analysis

Data are expressed as means and standard deviations with their maximum and minimum ranges. Interspecies mean differences and pre-and post-moult means were compared by Student's unpaired t-test.

RESULTS

The hepatic fatty acid composition of rockhopper and magellanic penguins pre-and post-moult

TABLE 1. Range and mean±SD percent liver fatty acids (16:0–22:6n-3) in wild pre-and post-moult rockhopper (*Eudyptes crestatus*) and magellanic (*Spheniscus magellanicus*) penguins.

Fatty acid	Rockhopper				Magellanic			
	Pre-moult (n=4)		Post-moult (n=4)		Pre-moult (n=3)		Post-moult (n=4)	
16:0	15.2–19.9	17.9 ±2.1	21.8–24.1	22.8 ±1.1	21.9–26.1	24.8 ±2.5	20.2–24.1	22.9 ±1.8
16:1	1.3–3.1	2.3 ±1.0	0.7–1.2	0.9 ±0.2	1.7–2.1	1.9 ±0.2	1.7–3.4	2.6 ±1.1
18:0	19.2–21.6	20.4 ±1.0	18.0–23.6	20.8 ±2.9	18.6–22.8	20.6 ±2.1	12.4–17.8	15.8 ±2.4
18:1n-9	18.6–25.3	21.4 ±2.8	16.5–19.1	18.1 ±1.1	18.0–22.4	20.3 ±2.2	18.2–24.5	20.9 ±3.0
18:2n-6	1.3–2.0	1.6 ±0.3	3.6–6.1	4.7 ±1.2	0.9–1.2	1.0 ±0.2	3.9–4.4	4.0 ±0.3
20:1n-9	1.0–2.7	1.7 ±0.9	0.9–1.9 (n=3)	1.4 ±0.5	0.5–1.0	0.7 ±0.3	2.9–5.1	4.1 ±1.0
20:4n-6	7.8–9.8	8.6 ±0.8	6.8–9.8	8.3 ±1.6	4.4–7.0	5.3 ±1.4	4.1–7.3	6.3 ±1.5
20:5n-3	8.6–10.6	9.3 ±1.0	5.0–7.7	6.4 ±1.5	8.5–8.9	8.8 ±0.2	2.9–3.7	3.4 ±0.3
22:5n-3	1.4–3.1	2.3 ±0.7	0.7–1.3	0.8 ±0.4	2.0–2.3	2.1 ±0.2	1.0–1.2	1.1 ±0.1
22:6n-3	7.5–14.7	11.2 ±3.0	8.2–14.5	11.4 ±2.6	9.2–12.5	11.0 ±1.7	11.8–14.3	12.9 ±1.1
22:1n-9	0.4–0.5 (n=3)	0.45 ±0.007	0.3–0.7 (n=3)	0.49 ±0.17	0.2–0.6	0.33 ±0.16	0.8–0.9	0.86 ±0.07

are shown in table 1. Linoleic (18:2n-6) and arachidonic (20:4n-6) acids were significantly (P <0.05 and P<0.025, respectively) higher and palmitic (16:0) acid significantly lower in the pre-moult rockhoppers (P<0.025) than the corresponding magellanics.

In both species of penguins moulting resulted in reduction in eicosapentaenoic (rockhoppers P< 0.025, magellanics P<0.001) and docosapentaenoic (22:5n-3) (rockhoppers P<0.01, magellanics P<0.001) and an increase in linoleic acid percent (rockhoppers P<0.005, magellanics P<0.001).

There was lower (P<0.05) stearic (18:0), and higher gadoleic (20:1n-9) (P<0.005) and erucic (22:1n-9) (P<0.05) acids in the post-moult magellanics compared to their pre-moult counterparts. The post-moult rockhoppers, however, had increased palmitic (P<0.01) and decreased palmitoleic (16:1) (P<0.05) acids compared to the corresponding pre-moult birds.

Post-moult magellanics had significantly higher palmitoleic (P<0.025), gadoleic (20:1n-9) (P< 0.01) and erucic (22:1n-9) (P<0.05) acids and significantly lower arachidonic (P<0.05), and eicosapentaenoic (20:5n-3) (P<0.025) acids compared to that of the post-moult rockhoppers.

DISCUSSION

In both the rockhopper and magellanic penguins the major hepatic fatty acids were palmitic (16:0), stearic (18:0), oleic (18:1), arachidonic (20:4n-6), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3). These findings are in general agreement with the reported fatty acid profiles for total body fat in wild Adelie (*Pygoscelis adeliae*) penguins [23] and dermal tissue in Emperor penguins (*Aptenodytes forsteri*) [24].

Pre-moult differences in hepatic fatty acids between rockhopper and magellanic penguins were likely to be due to differences in dietary habits. The rockhoppers including *Eudyptes crestatus* feed opportunistically on squid, crustaceans, euphausids, and small fish [11, 25, 26]. These foods would be rich in long chain n-3 fatty acids, with smaller amounts of the n-6 [27–30]. The magellanics' lower hepatic n-6 fatty acids imply a greater diet-

ary dependence on n-3 rich species. Diverse feeding ecologies have been reported for several penguin species [3, 31] and fatty acid compositions are known to be a reflection of both metabolism and diet [32–34]. Zar [24] suggested that the fatty acid differences between the adipose tissues of the Emperor (*Aptenodytes forsteri*) and of the Adelie (*Pygoscelis adeliae*) penguins were an effect of diet. In addition, Johnson and West [23] found that the proportions of fatty acids in Adelie penguin depot fat closely resembled the proportions of fatty acids in their normal diet of krill (*Euphausia sp*).

Both penguin species have relatively high proportions of liver arachidonic acid (n-6) despite living in a n-3 fatty acid rich environment. This may indicate a physiological requirement in penguins for arachidonic acid similar to that in mammalian species (33). Diet selection patterns or rates of desaturation and elongation could account for the relatively high arachidonic acid composition. Similarly we previously reported [35] significant proportions of n-6 fatty acids in the liver phosphoglycerides of wild dolphins feeding in n-3 fatty acid-rich environments.

The post-moult disparity in hepatic fatty acids between the rockhoppers and magellanics was likely to be the result of species differences in the metabolism of lipids. It could also be that the penguins were at different stages of moulting, utilising nutrients differently. Moulting is characterised by three distinct phases, I and II representing essentially lipid catabolism, with >90% of energy expenditure stemming from lipids in phase II; in phase III any remaining lipid reserves, are catabolised and proteins are also utilised [36, 37]. The rockhoppers were perhaps in the final phase of moulting, utilising more proteins; and the magellanics in the earlier phases, because of their greater fatty acid mobilisation. These findings are in agreement with our earlier observations [5]; that the post-moult rockhoppers had significantly lower plasma albumin and globulin compared to their magellanic counterparts indicating that the rockhoppers were utilising higher amounts of protein.

Stearic acid (18:0) is quantitatively a major fatty acid in animal tissues, and is not preferentially oxidised as fuel in mammals [38]. The post-moult

decrease of stearic and concomitant increase in gadoleic (20 : 1n-9) and erucic (22 : 1n-9) acid percent in the magellanics but not in the rockhoppers, is therefore interesting. Increased elongation and desaturation of stearic acid in the magellanics to compensate for the relative post-moult loss in unsaturation, may explain these findings. Physiological adaptation to low environmental temperatures result in increase of unsaturation in the tissues of many species [39–42]. The post-moult drop in unsaturation in the magellanics, may have induced this adaptive elongation and desaturation mechanism. Preferential mobilisation and utilisation of the specific long chain n-3 fatty acids eicosapentaenoic and docosapentaenoic (22 : 5n-3) during moulting was a consistent biochemical finding in both the rockhopper and magellanics. Because of the dietary abundance of n-3 fatty acids in marine ecosystems, it appears that these penguins have evolved metabolic mechanisms to preferentially utilise these fatty acids.

There was a significant increase in the proportion of hepatic linoleic acid (18 : 2n-6) after moulting both in the magellanics and rockhoppers, together with a reduciton in the long chain n-3 fatty acids, eicosapentaenoic (20 : 5n-3) and docosapentaenoic (22 : 5n-3). This n-6 and n-3 interaction is consistent with the findings of Gudbjarnason and Oskarsdottir [43] and Harbige *et al.* [22] in mammals. They found that increases in the proportion of long chain n-3 fatty acids was associated with a decrease in n-6 fatty acids, particularly linoleic acid. Linoleic acid is thought to have a role in the maintenance or formation of the epidermal water barrier [16]. It is conceivable that our observations of increased linoleic acid percent in the liver of both penguin species post-moult, may indicate specific mobilisation in relation to water barrier function during the vulnerable moulting period. Also there appears to be a differential sparing or conservation of the highly unsaturated docosahexaenoic acid (22 : 6n-3) and arachidonic acid in both species after moulting. Specific increases in membrane docosahexaenoic acid (22 : 6n-3) with cold adaptation have been reported [44, 45]; and may partly explain our findings as could the preferential retention of these fatty acids by hepatic cells. Conservation of docosahexaenoic acid, may also

have contributed to the post-moult decrease in the proportions of eicosapentaenoic and docosapentaenoic acid composition through chain elongation and or desaturation.

In conclusion we suggest that pre-moult hepatic fatty acid status of the rockhopper and magellanic penguins are mainly of dietary origin. Whereas, the post-moult values are a reflection of the stage of moult, and mobilisation and utilisation of lipids which appear to be both species dependent and species independent.

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