Wax-ester metabolism in the orange roughy \textit{Hoplostethus atlanticus} (Beryciformes: Trachichthyidae)

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Abstract. Freshly caught orange roughy (\textit{Hoplostethus atlanticus}) obtained at the edge of the Chatham Rise, east of the South Island of New Zealand, in June 1988, were used to investigate tissue-specific lipid synthesis and to obtain blood for plasma lipoprotein analysis. Tissue slices prepared from an intraneurocranial fat deposit, pieces of skull (neurocranium), swimbladder and liver were incubated with radioactive acetate, palmitate and oleate, and the labelled lipids extracted and analysed. All four tissues could incorporate acetate label into fatty acids which were themselves incorporated into triacylglycerols and phospholipids although not into wax esters, the most abundant lipid class in these tissues. Exogenous palmitate and oleate were also incorporated into more complex lipids, with the label from oleate (but not palmitate) being found in wax esters as well as triacylglycerols and phospholipids. The distribution of the label in the wax esters showed that some reduction of the fatty acid to fatty alcohol had occurred. The only exception to this pattern was the swimbladder, which incorporated small amounts of palmitate-label into both the fatty acid and fatty alcohol portions of the wax esters. Lipoproteins were isolated from serum by centrifugation. All lipoprotein classes contained phospholipid, cholesterol, triacylglycerol, cholesteryl esters and wax esters. A very low-density lipoprotein class also contained large amounts of unesterified fatty acids, which are possibly artefacts resulting from storage of the samples. Significant quantities of \(\omega-3\) fatty acids were also found in the serum lipids.

Materials and methods

\textit{Hoplostethus atlanticus} were caught at the edge of the Chatham Rise near the Chatham Islands (Fig. 1) during a cruise of the F.V. "Arrow" out of Nelson, New Zealand, during June 1988. The fish were caught in a trawl at 1 000 m depth and brought to the surface over a period of 20 min. Whole blood was collected from the tail and frozen at \(-20^\circ\text{C}\). Samples of intraneurocranial lipid, skull, swimbladder and liver, were dissected and used immediately for the lipogenesis experiments. For these, between 100 and 400 mg of tissue were minced and incubated in 5 ml of Tissue Medium B of D'Aoust (1967), containing the following salts (mmol l\(^{-1}\)): NaCl (164), KCl (5), Na\(_2\)HPO\(_4\) \(\cdot\)7H\(_2\)O (0.2), Na\(_2\)HPO\(_4\) (0.4), NaHCO\(_3\) (6.5), CaCl\(_2\) (1), MgSO\(_4\) (1). Glucose was included (30 mmol l\(^{-1}\)) along with trace amounts of \(1\)\(^{14}\)C-acetate, \(1\)\(^{14}\)C-palmitate or \(9,10\)-\(^3\)H-oleate, each at 20 \(\mu\)Ci. The two fatty acids were emulsified with 0.5% (w/v) bovine serum albumin. Incubations were carried out for 3 h at ambient temperature (\(14^\circ\text{C}\)) and reactions were stopped by removal of the medium and the addition of 2 vol of chloro-
form: methanol, 2:1 (v/v). Further analyses were carried out at the University of Otago.

Lipid extractions were completed by homogenising the samples using the small probe of an Ultra-Turrax blender, adding 1 vol of water, and centrifuging to separate the phases. The lipids in the chloroform layer were dried under a stream of nitrogen and weighed. They were then dissolved and stored at -20°C in a standard volume of chloroform. Aliquots of each were counted for radioactivity using a scintillation containing 4.5 g l⁻¹ diphenyloxazole and 0.2 g l⁻¹ phenyl-oxazolylphenyl-oxazolylphenyl in toluene: Triton X-100 (3:1 by vol).

Thin-layer chromatography

Samples of the total lipids were chromatographed in 2.5 cm lanes on 0.5 mm layers of silica-gel G in a solvent mixture containing hexane:diethyl ether:acetic acid (80:20:1 by vol). The position of the lipids was determined by staining with iodine, and areas corresponding to standards were scraped into scintillation vials and counted directly. The percentage radioactivity in each lipid class was determined and corrected to dis/min/mg total lipid using the data for total lipid radioactivity determined as above. In selected samples, the wax-ester fraction was isolated following thin-layer chromatography by scraping into a tube and eluting the waxes from the silica gel using chloroform. The waxes were purified by a second thin-layer chromatographic step in the same solvent system, and scraped and counted for radioactivity. In other samples, the source of label (acetate, palmitate and oleate) for 3 h. Distribution of the radioactivity amongst the phospholipids was determined colorimetrically (Stewart 1980) using human erythrocyte phospholipids as standard (Cynamon et al. 1984). Total protein was determined according to Lowry et al. (1951). Lipid was extracted from samples of each of the lipoprotein fractions according to Bligh and Dyer (1959), and analysed by thin-layer chromatography. Plates were developed in hexane:diethyl ether:acetic acid (80:20:1), sprayed with a solution of 5% v/v H₂SO₄ in ethanol, and charred at 200°C. The charred plates were scanned with a LKB Ultrascan laser densitometer.

The triacylglycerols, phospholipids and a combined wax ester/cholesteryl ester fraction were isolated from a sample of total serum lipids by preparative thin-layer chromatography, and fatty acid methyl esters were prepared as described above. These were analysed by gas-liquid chromatography on a 30 m × 0.32 mm-bonded, fused silica Superox column in a Varian 3700 chromatograph equipped with flame-ionisation detector. Helium was used as carrier gas at a pressure of 0.85 kg cm⁻². Split-injection (1:20 ratio) was used to introduce samples onto the column, and the column temperature was programmed at 2°C min⁻¹ from 100°C to 200°C. Methyl heptadecanoate was used as an internal standard.

Results

Although Hoplostethus atlanticus were caught at depths of 1000 m or more, the fish were still alive as they were brought to the surface. This enabled both the collection of blood for lipoprotein analysis and the preparation of samples for the lipogenesis experiments. Samples from one fish were tested with radioactive acetate to determine the time-dependency of the labelling. The results for the intraneurocranial fat and the liver showed that radioactivity was incorporated into lipid linearly with time up to 3 h, verifying that this time period was appropriate for subsequent experiments (data not presented).

Tissues from two fish were incubated with three sources of label (acetate, palmitate and oleate) for 3 h. The incorporation of radioactivity into the major identifiable lipid classes using thin-layer chromatography was determined as dis/min/mg total lipid (Table 1). The total lipid content of the tissues examined varied between 12 and 15% of the total tissue fresh weight for skull and liver to 32 and 67% for the intraneurocranial fat and swim-bladder, respectively (Table 1).

Lipid analysis by thin-layer chromatography was complicated by low specific activities of the acetate-labelled samples and the presence of large quantities of unreacted free fatty acid label in the palmitate and oleate-labelled samples. Thus, values have been listed for wax esters, triacylglycerols, free fatty acids and polar lipids (those remaining at the origin) for the acetate-labelled samples and only wax esters, triacylglycerols and polar lipids for the others. When acetate was used as the source of label, radioactivity could be detected in all lipid classes except the wax esters, even though these were the major lipids in the three non-hepatic tissues. A similar result was observed for the palmitate-labelled samples.

Fig. 1. The Chatham Rise, New Zealand. Contour lines show depth (m). Adapted from Henth (1981)