INCREASED CHAIN SHORTENING OF ERUCIC ACID IN PERFUSED HEART FROM RATS FED RAPESEED OIL

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1. Introduction

In feeding experiments rapeseed oil, rich in erucic acid (22 1, *n*-9*cis*), causes an accumulation of triacylglycerol in the heart and sceletal muscle of rats and other species [1]. The fat infiltration reaches a peak after 3 days of feeding. After prolonged feeding for one to three weeks the lipidosis gradually disappears. The metabolic reasons for this adaptation process are poorly understood. In vivo experiments with labelled erucic acid [2], and studies with isolated hepatocytes [3–5], have shown that erucic acid is rapidly shortened to eicosenoic acid (20.1, *n*-9), oleic acid (18 1, *n*-9) and palmitoleic acid (16:1, *n*-9) in the liver. The hepatic chain shortening system may explain why rapeseed oil feeding does not induce fatty liver. A relatively slow chain shortening of erucic acid in cultured heart myocytes has been described [6].

The aim of the present work was to study chain shortening of [^{14}C]erucic acid in the perfused rat heart, and to see if the activity of this system was altered after prolonged feeding with rapeseed oil.

2. Materials and methods

[14-^{14}C]Erucic acid was from Centre d'Etudes Nucleaires de Saclay, Gif sur Yvette and was purified by thin-layer chromatography using hexane/diethylether/acetic acid (80 20 1, v/v/v). The free fatty acid fraction was extracted with $CHCl_3/CH_3OH$ (2.1, v/v). Essentially fatty acid free bovine serum albumin and erucic acid were from Sigma Chemical Co., St Louis, MO.

Male Wistar rats 100 g were fed ad libitum semisynthetic diets containing 30% of the calories as either peanut oil or rapeseed oil for 3 weeks. Erucic acid accounted for 42.7% of the fatty acids in the rapeseed oil [7].

The rats were decapitated and their hearts perfused by a modified Langendorff preparation [8]. The perfusion apparatus was a recirculating system containing 50 ml Krebs-Henseleit buffer (pH 7.4), 11 mM glucose, 3% (w/v) bovine serum albumin and 0.5 mM [14-^{14}C]erucic acid. The hearts were preperfused with 10 ml buffer without fatty acid before perfusion in the recirculating system under constant 70 mm Hg for 30 min at 37°C. Coronary flow ranged from 6–9 ml/min, and the heart frequency was 200–240 beats/min. At the end of each perfusion the heart was quickly removed and rinsed 4 times in ice-cold NaCl (0.9%), and immediately homogenized in methanol.

The $^{14}CO_2$ leaving the perfusion chamber during the perfusion was collected in phenylethylamine/CH_3OH (1.2, v/v). Immediately after the perfusion a sample of the perfusate was acidified, the $^{14}CO_2$ released was collected in phenylethylamine/CH_3OH (1 1, v/v), and the radioactivity was measured.

The extraction of lipids and the separation of lipid classes were performed as in [9]. Total lipid extracts of phospholipids, free fatty acids and triacylglycerols were transmethylated [10], and analyzed by radio-gas chromatography using a Varian 2100 gas chromatograph connected to an ESI-Nuclear radioactivity detector with a 1 1 outlet splitter. Fatty acids methyl esters were separated at 190°C using 10% SP-2340 on Supelcoport 100/120 (Supelco Inc.,

Table 1
The chain-shortening of [14-^{14}C]erucic acid in perfused rat heart from rats fed a peanut oil diet or a rapeseed oil diet for 3 weeks

Diet	Lipid fraction	$C_{16:1}$	$C_{18:1}$	$C_{20:1}$	Sum of 16:1, 18:1 and 20:1	$C_{22:1}$
Peanut oil	Triacylglycerol	–	10.5 ± 4.6	11.0 ± 0.7	21.5 ± 5.3	635.4 ± 56.8
	Phospholipid	–	3.0 ± 0.6	3.1 ± 0.6	6.1 ± 1.2	97.8 ± 9.2
Rapeseed oil	Triacylglycerol	Trace	41.3 ± 4.5	13.8 ± 2.0	55.1 ± 6.5	695.5 ± 140.8
	Phospholipid	Trace	10.9 ± 2.7	4.6 ± 0.6	15.5 ± 3.3	68.8 ± 14.9

The experiments were performed as in section 2

The results are presented as nmol ^{14}C-labelled fatty acid/g heart wt (wet wt) (mean ± SD, n = 4)

Bellefonte, PA) The peaks were identified on the basis of the retention time compared with the standards The distribution of radioactivity between the peaks was calculated from counting data recorded on Printing Autoscaler 5680 (ESI-Nuclear).

3. Results and discussion

Table 1 shows that significant amounts of [^{14}C]oleic acid and [^{14}C]eicosenoic acid appear in the triacylglycerol and phospholipid fractions of rat hearts perfused with [^{14}C]erucic acid.

In the hearts from animals fed rapeseed oil (with a high content of erucic acid) the [^{14}C]oleic acid content was 3–4-times higher, and the [^{14}C]eicosenoic acid content was 1.2–1.5-times higher compared to hearts from animals fed peanut oil.

Trace amounts of [^{14}C]palmitoleic acid were detected in the hearts from animals fed rapeseed oil, but not in the hearts from the control group fed peanut oil.

The shortened fatty acids accounted for 12–15% of the ^{14}C-labelled fatty acids incorporated in the phospholipid fraction, and for 4–6% of the labelled fatty acids in the triacylglycerol fraction of the hearts from the animals fed rapeseed oil compared to 5–6% and 2–3%, respectively, in the control group fed peanut oil.

The total amount of labelled fatty acids incorporated in the triacylglycerol and phospholipid fractions did not differ significantly between the hearts from the two diet groups (table 2).

The labelled free fatty acid fraction in the hearts (table 2) did not contain detectable amounts of shortened fatty acids (not shown).

The oxidation to $^{14}CO_2$ was approximately the same in the hearts from the two diet groups (table 2). It is possible that a fraction of the [^{14}C]erucic acid oxidized is first shortened to [^{14}C]eicosenoic acid

Table 2
The metabolism of [14-^{14}C]erucic acid in perfused rat heart from rats fed a peanut oil diet or a rapeseed oil diet for 3 weeks

Diet	Oxidation products[a]	Triacyl-glycerol	Diacyl-glycerol	Free fatty acids	Phospho-lipids	Total uptake
Peanut oil	715.4 ± 54.4	656.9 ± 62.1	34.6 ± 5.7	252.0 ± 14.5	103.9 ± 10.4	1762.8 ± 147.1
Rapeseed oil	686.4 ± 93.5	750.6 ± 147.3	31.9 ± 1.1	245.5 ± 9.2	84.3 ± 18.2	1798.7 ± 269.3

[a] The oxidation products are the sum of the $^{14}CO_2$ released during perfusion and the $^{14}CO_2$ dissolved in the perfusion fluid after the perfusion. The erucic acid conc. was 0.5 mM in total vol. 50 ml, and the perfusion time was 30 min

The results are presented as nmol [14-^{14}C]erucic acid equiv./g heart wt (wet wt) (mean ± SD, n = 5)

and [^{14}C]oleic acid before ordinary mitochondrial β-oxidation. Experiments with liver suggest that [^{14}C]erucic acid can be shortened by extramitochondrial mechanisms, possible at the outer mitochondrial membrane [11], or in the peroxisomes [3–5].

It may be surprising that the increased chain shortening does not give an increased oxidation compared to the control group (table 2). This can possibly be explained by a dilution of [^{14}C]erucic acid by the presence of an endogenous pool of unlabelled erucic acid in the hearts from rats fed rapeseed oil.

The present finding of increased amounts of [^{14}C]oleic acid and [^{14}C]eicosenoic acid in perfused hearts from animals fed rapeseed oil for three weeks, suggest that rapeseed oil feeding induces an increased capacity for chain shortening of erucic acid in the heart. Experiments with perfused liver and isolated liver cells from rats fed rapeseed oil suggest an increased chain shortening of $C_{22\ 1}$ also in these systems. (R. Z. Christiansen, E. N. C., K. R. Norum and J. Bremer, unpublished). The combined effects of an increased chain shortening in the heart and the liver may explain why the initial heart lipidosis caused by rapeseed oil feeding, gradually decreases after 1–3 weeks of continued feeding.

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