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Changes in composition and enzyme activities of mitochondrial and post-mitochondrial fractions of tissues of rats given mustard oil diet with carnitine and/or fish oil

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Feeding vegetable oils at the level of 20 % to laboratory animals has no ill effects. But oils containing erucic acid such as rapeseed oil and mustard oil produce cardiac lipidosis (2, 4, 5, 16, 17) followed by fibrosis, if such feeding is prolonged (8, 15). Little work appears to have been carried out on the factors which could prevent such lipidosis. Renuka (16) demonstrated that fish meal in place of skim milk powder as a source of protein has a tendency to prevent lipidosis. Since fish meal contains carnitine and polyunsaturated fatty acids in addition to proteins, the effects of their addition to a mustard oil diet on the composition and activities of enzymes of lipid metabolism in subcellular fractions of heart, liver, and skeletal muscles of rats were studied.

Material and methods

Batches of 7 male rats were fed experimental diets supplying 20 % mustard oil, or 17.5 % mustard oil plus 2.5 % fish oil, with and without 0.1 % carnitine, over a period of 65 hours. The mustard oil contained 47.2 % erucic acid. After the experimental feeding, the animals were killed and the heart, liver and skeletal muscles were collected on ice, pooled, homogenized and fractionated into mitochondria and post-mitochondrial fractions (10). The homogenates from heart were also examined for lipase and phospholipase activities. The protein content (9), succinate dehydrogenase (3), phospholipase (13), lipase (13), cholesterol ester hydrolase and synthetase (14) were determined. The cholesterol and free fatty acids were also determined (19, 6), and the tissues were also examined for histopathological changes (12).

Results

Feeding carnitine and/or fish oil with a mustard oil diet to rats increased the activities of phospholipase and lipase (pH 6.0) in homogenates of heart and also increased the mitochondrial protein content of heart and liver, and fish oil with carnitine did so in skeletal muscles also. The carnitine and/or fish oil supplemented diets increased the activity of succinate dehydrogenase in the mitochondria from heart and skeletal muscles. The activities of phospholipase in mitochondria of heart and liver were decreased and the ratios of cholesterol to cholesterol esters were increased (table 1).

Table 1. Changes in the composition and enzyme activities of mitochondrial preparations from tissues of rats given a mustard oil diet with carnitine and/or fish oil.

Diet	Protein*	Lipase** (pH 6.0)	Phospho-lipase**	Succinate dehydrogenase***	Chol/Chol Est ratio
Heart					
MO	60	176 (18)	241 (6)	212	2.0
MO + FO	368	167 (36)	231 (46)	2512	2.1
MO + FO + C	184	152 (49)	183 (77)	1272	2.4
Liver					
MO	56	82 (20)	235 (7)	1193	1.0
MO + FO	99	83 (10)	149 (11)	799	1.5
MO + FO + C	82	116 (15)	144 (16)	1110	1.3
Skeletal muscles					
MO	180	–	–	212	2.0
MO + FO	106	–	–	550	2.1
MO + FO + C	315	–	–	583	1.9

* µg/mg tissue protein

** µg free fatty acids released/mg protein

*** pmoles succinate oxidized/mg protein/minute; MO, mustard oil; FO, fish oil; C, carnitine: Chol Est, cholesterol esters; figures in parentheses represent enzyme activities in tissue homogenates.

The mustard oil diet supplemented with carnitine plus fish oil increased the ratios of cholesterol ester hydrolase to cholesterol ester synthetase in the post-mitochondrial fractions of heart and liver. The activities of phospholipase in the post-mitochondrial fractions of heart were increased by supplementation with carnitine and/or fish oil. The protein content of the post-mitochondrial fraction was increased with the mustard oil diet supplemented with carnitine and fish oil (table 2).

On feeding the mustard oil diet, lipidosis was observed by Sudan IV and Haematoxylin-Eosin staining of sections of heart (fig. 1 a) and skeletal muscles (fig. 1 b). Inclusion of fish oil alone in a mustard oil diet had a little beneficial effect, whereas inclusion of fish oil plus carnitine in a mustard oil diet resulted in elimination of lipidosis in heart (fig. 2 a) and skeletal muscles (fig. 2 b).

Discussion

The increased activities of phospholipase and lipase (pH 6.0) in homogenates of heart indicated increased lipolysis when carnitine and/or fish oil were added (table 1). The diet with carnitine plus fish oil produced a greater increase in the activity of these enzymes. The higher ratios of cholesterol ester hydrolase to cholesterol ester synthetase in the post-mitochondrial fraction of the hearts of rats given the mustard oil diet with carnitine plus fish oil (table 2) also indicated increased lipolysis. This was also confirmed by Sudan IV and Haematoxylin-Eosin staining of the sections of heart and skeletal muscles from rats given mustard oil diet with carnitine and fish oil (figs. 1 and 2). The increase in mitochondrial protein content and succinate dehydrogenase activity after feeding the

Table 2. Changes in the protein and enzyme activities of postmitochondrial fractions from tissues of rats given mustard oil diet with carnitine and/or fish oil.

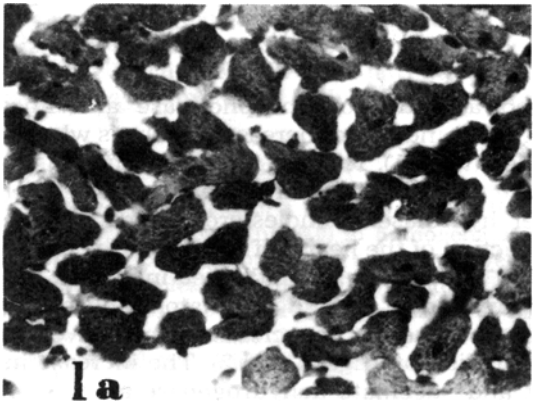
Diet	Protein*	Lipase** (pH 6.0)	Phospholipase**	CEH/CES ratio
Heart				
MO	202 (3.4)	141 (0.8)	24 (0.1)	2.3
MO + FO	150 (0.4)	90 (0.5)	124 (0.5)	0.5
MO + FO + C	381 (2.1)	73 (0.5)	138 (0.8)	3.8
Liver				
MO	247 (4.4)	111 (1.4)	56 (0.2)	0.6
MO + FO	222 (2.2)	26 (0.3)	50 (0.3)	0.2
MO + FO + C	310 (3.8)	34 (0.3)	31 (0.2)	1.0
Skeletal muscles				
MO	298 (1.7)	193 (4.6)	82 (5.1)	20.8
MO + FO	253 (2.4)	47 (5.2)	99 (1.2)	0.5
MO + FO + C	222 (0.7)	109 (2.3)	112 (1.6)	0.4

* µg/mg tissue protein

** µg free fatty acids released/mg protein; CEH and CES, cholesterol ester hydrolase and synthetase; MO, mustard oil; FO, fish oil; C, carnitine; figures in parentheses represent the ratio of activities of enzymes in the post-mitochondrial to mitochondrial fractions.

mustard oil diet containing carnitine plus fish oil (table 1) suggested that the integrity of the mitochondria was increased and consequently their oxidative capacity.

The carnitine plus fishoil-supplemented mustard oil diet increased the ratio of cholesterol to cholesterol esters in the heart (table 1) mitochondria, indicating increased incorporation of cholesterol into mitochondria. The insertion of cholesterol may modify the bilayer structure of membranes (1, 7, 18), and consequently the position of integral membrane proteins in the lipid bilayer may be altered (11).



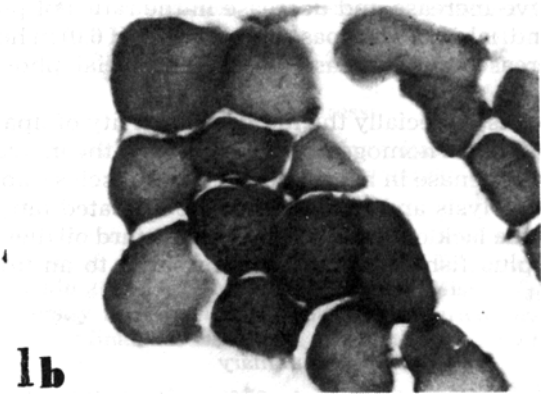


Fig. 1. Frozen sections of (a) heart and (b) skeletal muscles of rats given a mustard oil diet and showing fat deposition represented by dark areas stained with Sudan IV.

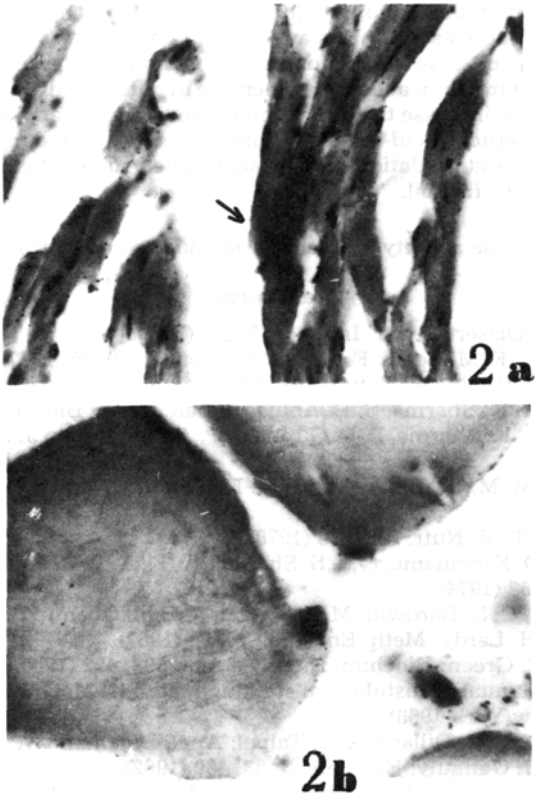


Fig. 2. Frozen sections of heart (a) and skeletal muscles (b) of rats given mustard oil diet supplemented with carnitine plus fish oil and showing lack of fat deposition after Sudan IV staining.

The respective increase and decrease in the ratios of post-mitochondrial to mitochondrial phospholipase and lipase (pH 6.0) in heart indicated a respective decrease and increase in mitochondrial phospholipase and lipase (table 2).

All these results, especially the increased activity of lipase (pH 6.0) and phospholipase in heart homogenates (table 1) and the increased activity of succinate dehydrogenase in heart and skeletal muscles (table 1), indicated that increased lipolysis and oxidation of the liberated fatty acids may be responsible for the lack of lipidosis when a mustard oil diet supplemented with carnitine plus fish oil is fed, as compared to an unsupplemented mustard oil diet.

Summary

The effects of short-term feeding of a 20 % mustard oil (containing 47 % erucic acid) diet with 0.1 % carnitine and/or fish oil on the activity of enzymes of lipid metabolism in mitochondrial and post-mitochondrial fractions of heart, liver, and skeletal muscles of rats were determined to study the reversal of erucic acid-induced lipidosis. Histopathological changes were also studied.

The inclusion of fish oil or fish oil plus carnitine in a mustard oil diet, especially the latter, eliminated the histopathologically detectable lipidosis in heart and skeletal muscles. The reason for this appears to be the increased activity of lipase and phospholipase in tissue homogenates and of cholesterol ester hydrolase in the post-mitochondrial fraction; and also an increase in mitochondrial succinate dehydrogenase which may increase the utilization of the liberated fatty acids. The results suggest that the erucic acid-induced lipidosis is prevented or eliminated by increased lipolysis and oxidation of fatty acids when such a diet is supplemented with carnitine and/or fish oil.

Key words: enzyme activity, mitochondria, mustard oil, carnitin, fish oil

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