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FAILURE OF DIETARY ERUCIC ACID TO IMPAIR OXIDATIVE CAPACITY OR ATP PRODUCTION OF RAT HEART MITOCHONDRIA ISOLATED UNDER CONTROLLED CONDITIONS*

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SUMMARY

1. Male, 8-week old rats were fed Purina Rat Chow or semi-synthetic diets containing 20 % by weight of rapeseed oil or corn oil for 3 days.

2. The hearts from the animals fed the three diets were analyzed for total lipid, phospholipid, free fatty acids, cholesterol esters, tri-, di- and monoacylglycerols. There was a seven-fold increase in the levels of triacylglycerols in the hearts of rats fed rapeseed oil diet compared to the levels in the hearts of animals fed the other two diets. Smaller increases in the content of other neutral lipid fractions were also observed.

3. Heart mitochondria from the three groups of animals were isolated under controlled conditions in the presence or absence of heparin. The rates of oxidation of different substrates and of ATP synthesis by these mitochondria were compared.

4. Mitochondria isolated in the absence of heparin from rapeseed oil-fed rats had much lower rates of oxidation and ATP synthesis than mitochondria isolated similarly from rats fed the other two diets.

5. With mitochondria freshly isolated in the presence of heparin, no significant differences in rates of oxidation or ATP synthesis were found among the three groups of animals.

6. It is concluded that, when properly isolated, mitochondria from rapeseed oil-fed rats are functionally intact with respect to oxidation and energy-coupling capacity.

INTRODUCTION

Feeding of rapeseed oils of high erucic acid content to rats results in marked changes in the lipid composition of the heart, liver and other organs [1–3]. In the first week of feeding, the most profound change in the heart is the accumulation of triacylglycerols containing long chain fatty acids. Houtsmüller et al. [4] claimed

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that ATP production from a variety of respiratory substrates is inhibited in isolated cardiac mitochondria from rapeseed oil-fed rats. They concluded that dietary erucic acid causes a considerable decrease in the capacity of heart mitochondria to oxidize substrates and a concomittant accumulation of heart triacylglycerols. However, in our earlier studies [3] no significant change was observed in the capacity of heart mitochondria to oxidize fatty acids when rats were fed rapeseed oil-containing diets for periods longer than two weeks. After three days of feeding such diets, the triacylglycerol accumulation in the heart begins to decline and reaches low levels after two weeks. Moreover, many claims in the literature, based on in vitro experiments, that dietary or hormonal changes induce in vivo changes in mitochondrial function have later been disputed. Thus, although earlier studies indicated impaired mitochondrial energy coupling in essential fatty acid deficiency [5], thyrotoxicosis [6] and diabetes [7], later improvements in the techniques for the isolation of mitochondria resulted in the demonstration of normal mitochondrial function in these conditions [8–10]. In view of these data, it was decided to re-investigate the effect of dietary erucic acid on the metabolism of heart mitochondria isolated and incubated under well-controlled conditions.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats maintained on Laboratory Purina Rat Chow (Chow) diet for 6–7 weeks and weighing 250–280 g were fed either the same diet or semi-synthetic diets containing 20 % by weight corn oil or rapeseed oil for the last 3 days before killing. The complete composition of the diets and the caging and care of the animals are described elsewhere [3]. The rapeseed oil used contained 23 % erucic acid [3].

Lipid analysis

Total lipids of the hearts were extracted twice according to the procedure of Bligh and Dyer [11]. A known portion was removed to determine total phosphorus [12]. The remaining lipids were fractionated by column chromatography on acid-treated Florisil according to Carroll [13]. The total neutral lipids (chloroform and 5 % methanol fraction) were separated by thin-layer chromatography on Silica Gel H plates (0.5 mm in thickness) using hexane/diethyl ether/acetic acid (85 : 15 : 1) as developing solvent. Bands were visualized under ultraviolet light after spraying the plates with rhodamine B. The separated lipid fractions were eluted and transesterified with 5 % (w/w) dry HCl gas in anhydrous methanol after addition of a known amount of internal standard (methyl heptadecanoate) and analyzed by gas-liquid chromatography as described previously [14].

Isolation of mitochondria

Heart mitochondria were isolated by methods already fully described [9, 10] and were suspended finally in a solution containing: mannitol, 0.21 M; sucrose, 0.07 M; EDTA, pH 7.4, 0.01 M; Tris · HCl buffer, pH 7.4, 0.01 M; heparin 330 units/ml medium (General Biochemicals). Heparin was employed in the isolation and suspension medium because it has been shown to preserve the functional integrity

of mitochondria [9, 10]. Mitochondria were also isolated in the absence of heparin for comparative purposes.

Measurement of oxygen uptake and ATP production

Oxidation rates and respiratory control indices were measured polarographically at 37 °C in the following media: potassium glutamate, 10 mM, with potassium malate, 10 mM, and potassium malonate, 10 mM, or potassium pyruvate, 10 mM with potassium malate, 10 mM, and potassium malonate, 10 mM, or potassium α-ketoglutarate, 10 mM, with potassium malonate, 10 mM, or potassium succinate, 20 mM, with rotenone, $5 \cdot 10^{-7}$ M; KCl to give 60 mM total potassium; potassium phosphate, pH 7.4, 10 mM; Tris · HCl, pH 7.4, 22 mM; $MgCl_2$, 5 mM; EDTA (sodium), pH 7.4, 7 mM; mannitol, to bring to isotonicity; bovine serum albumin, 0.2 %; NAD, 0.50 mM; Cytochrome *c*, 0.02 mM; mitochondrial protein, 0.20–0.25 mg; total volume 1.8 ml. A volume of 0.04 ml of 10 mM (standardized) ADP was added after the basal respiratory rate had been determined. The state 3 respiratory rate (in the presence of ADP), the state 4 respiratory rate (after exhaustion of ADP) and the respiratory rate in the presence of 5 μg/ml of oligomycin were determined. Because of increased oligomycin-sensitive ATPase at physiological temperature and a resultant reduced respiratory index, the basal respiratory activity was also measured in the presence of oligomycin. The uncoupling action of free fatty acids produced during incubation was prevented by the use of bovine serum albumin. The solubility of oxygen in isotonic salt medium was calculated to be 0.39 μg atoms of oxygen/ml at 37 °C, according to Chapell [15]. State 3 and state 4 respiratory rates, ADP : 0 ratios and respiratory indices were calculated as described by Chance and Williams [16].

The precise concentrations of the components of the isolation and incubation media are critical for reasons fully explained in previous publications [9, 10].

RESULTS

Table I shows that the feeding of rapeseed oil caused an increase in the total lipid content per heart from 2.9 % to 5.1 % of the wet tissue weight. While the phospholipid content remained constant, most of the neutral lipids increased: mono- and diacylglycerols, free fatty acids and methyl esters doubled in concentration, triacylglycerols increased 7-fold, and a slight increase occurred in cholesterol esters. By contrast, the feeding of 20 % corn oil diet caused only a slight increase in the triacylglycerol fraction compared to rats fed Purina chow. The level of incorporation of erucic (C22 : 1) and eicosenoic (C20 : 1) acids by rapeseed oil-fed rats was not the same in all neutral lipid classes: the methyl esters and free fatty acids contained only moderate amounts of these acids (about 2 % 22 : 1 and 1 % 20 : 1), the cholesterol esters and mono- and diacylglycerols contained significant amounts (about 7 % 22 : 1 and 3 % 20 : 1) and the triacylglycerols contained 23 % of 22 : 1, 13 % of 20 : 1 and 3 % of 24 : 1 (tetradocos-enoic acid).

Table II shows the oxidative capacity and ATP production observed for mitochondria obtained under conditions similar to those described by Houtsmüller et al. [4]. The results show that, in the absence of heparin in the isolation medium, there is indeed a 50–60 % reduction in the oxygen uptake of freshly-isolated mitochondria

TABLE I

EFFECT OF DIET ON THE WET WEIGHT, TOTAL LIPID, PHOSPHOLIPID AND NEUTRAL LIPID CONTENT OF RAT HEART

	Chow	Corn oil	Rape seed oil
Wet weight (mg)	800*	870	900
Total lipid (mg/heart; % wet weight)	23.0 (2.9 %)	25.0 (2.9 %)	45.9 (5.1 %)
Total lipid phosphorous (μg/heart)	408	453	436
Total neutral lipid (mg/heart; % total lipid)	5.00 (21.7 %)	5.89 (23.6 %)	22.11 (48.2 %)
Cholesterol esters (mg/heart, % neutral lipids)	0.75 (14.9 %)	0.73 (12.4 %)	0.97 (4.4 %)
Methyl esters** (mg/heart, % neutral lipids)	0.25 (5.0 %)	0.25 (4.2 %)	0.53 (2.4 %)
Triglycerides (mg/heart, % neutral lipids)	2.53 (50.6 %)	3.21 (54.5 %)	17.75 (80.3 %)
Free fatty acids (mg/heart, % neutral lipids)	1.20 (24.0 %)	1.43 (24.3 %)	2.32 (10.5 %)
Mono- and diglycerides (mg/heart, % neutral lipids)	0.28 (5.5 %)	0.27 (4.6 %)	0.53 (2.4 %)

* All values are means for 5 animals from each group.

** Methyl esters may be formed during the extraction procedure.

from rapeseed oil-fed rats and a proportionate reduction in respiratory control and ATP production (Table II). The results also show, in agreement with Houtsmüller et al. [4], that the ADP : O ratio was only slightly affected by the rapeseed oil diet. When mitochondria were isolated and re-suspended in the absence of heparin, those from rats fed the diet containing 20 % rapeseed oil deteriorated much faster than those from rats fed the other two diets, as indicated by a faster rate of decline of oxidative potential (Table II).

When heparin was used in order to preserve mitochondrial integrity during isolation and storage, the results obtained were quite different. Table III shows that the capacities for substrate oxidation, respiratory control and ATP production of freshly-isolated heart mitochondria from rats fed 20 % rapeseed oil were not significantly different from those for rats fed either 20 % corn oil or Purina Rat Chow.

It is apparent, on comparing the results presented in Tables II and III, that heparin in high concentration has a pronounced protective effect on respiratory activity and energy coupling in heart mitochondria isolated from rapeseed oil-fed rats.

TABLE II

OXIDATIVE AND ENERGY COUPLING ACTIVITIES OF HEART MITOCHONDRIA ISOLATED IN THE ABSENCE OF HEPARIN FROM RATS FED CHOW, CORN OIL OR RAPESEED OIL DIETS

Abbreviations used: GMM, glutamate+malate+malonate; PMM, pyruvate+malate+malonate; Succ+Rot, succinate+rotenone; αKg+malon, α-ketoglutarate+malonate.

Diet	Substrate	State 3 oxygen uptake (μg atom/mg protein/h)	Respiratory index		ADP : O	ATP production (μmol/mg protein/h)
			State 3/State 4	State 3/Oligo rate		
			1–2 h after isolation			
Chow	GMM	40.8±2.5	3.7±0.6	5.5±0.3	2.9±0.1	118.1±13.0
	PMM	37.7±1.1	4.3±0.4	5.1±0.1	3.3±0.2	125.2±11.5
	Succ+Rot	42.7±1.9	1.8±0.4	2.7±0.1	1.7±0.1	76.1±7.5
	αKg+Malon	18.7±2.1	4.5±0.8	10.6±2.3	3.6±0.2	67.8±8.4
Corn oil	GMM	44.2±4.6	3.2±0.8	7.9±0.8	2.8±0.1	123.2±16.7
	PMM	42.0±4.0	3.3±0.7	7.2±2.0	3.3±0.1	140.9±17.1
	Succ+Rot	45.6±6.0	1.6±0.2	2.7±0.3	1.7±0.1	74.3±12.4
	αKg+Malon	17.5±2.0	3.3±1.1	17.1±1.9	3.5±0.4	61.8±14.2
Rapeseed oil	GMM	20.8±7.0	2.7±1.2	5.6±1.3	2.7±0.4	59.2±28.6
	PMM	29.6±2.2	3.3±0.8	6.0±0.2	3.0±0.2	88.8±9.4
	Succ+Rot	31.9±2.5	1.5±0.3	2.3±0.4	1.6±0.1	51.0±8.6
	αKg+Malon	12.4±1.5	3.3±1.2	10.6±2.9	3.8±0.3	46.9±9.7
			5–6 h after isolation			
Chow	GMM	36.5	3.1	5.1	2.6	95.6
	PMM	21.8±0.5	3.2±0.3	4.1±0.3	3.1±0.1	97.7±4.8
Corn oil	GMM	34.6±2.5	2.2±0.6	4.4±0.6	2.4±0.3	83.4±15.9
	PMM	33.1±2.2	2.8±0.7	5.9±0.5	3.0±0.2	96.8±4.0
Rapeseed oil	GMM	14.6±0.9	1.5±0.3	2.6±0.5	2.0±0.1	29.0±3.5
	PMM	27.0±3.1	2.5±0.1	4.9±0.1	2.9±0.0	78.6±9.1

DISCUSSION

The rates of oxygen uptake and ATP synthesis obtained for heart mitochondria from control animals in the present study (Tables II and III) are about 10-fold higher than the rates reported by Houtsmüller et al. [4]. Thus, with glutamate as substrate, Houtsmüller et al. [4] reported an oxygen uptake of 4.2 μg atoms/mg protein/h and a rate of ATP synthesis of 11.5 μmol/mg protein/h. In the present work the corresponding values were in the range of 44–48 μg atoms of oxygen/mg protein/h and 123–145 μmol of ATP/mg protein/h. The high rates of oxygen uptake and ATP synthesis observed in the present study for heart mitochondria closely agree with the values reported earlier for carefully isolated skeletal muscle mitochondria [9, 10]. The lower rates obtained by Houtsmüller et al. [4] might be partially due to the omission of malate from their incubation medium. This would result in decreased oxidation of glutamate because glutamic dehydrogenase activity in muscle mitochondria is either absent or very low [17]. In addition the reactions were conducted at 25 °C instead of the 37 °C used in the present experiments.

TABLE III

OXIDATIVE AND ENERGY COUPLING ACTIVITIES OF HEART MITOCHONDRIA ISOLATED IN THE PRESENCE OF HEPARIN FROM RATS FED CHOW, CORN OIL OR RAPESEED OIL DIETS

Abbreviations for the substrates are explained under Table II.

Diet	Substrate	State 3 oxygen uptake (μg atom/mg protein/h)	Respiratory index		ADP : O	ATP production (μmol/mg protein/h)
			State 3/State 4	State 3/Oligo rate		
			1–2 h after isolation			
Chow	GMM	46.8±2.8	4.7±0.4	6.0±0.9	2.9±0.1	137.3±6.9
	PMM	45.4±1.5	5.3±0.4	6.8±0.6	3.4±0.1	150.5±2.1
	Succ+Rot	48.2±3.4	2.3±0.1	2.9±0.4	1.8±0.1	84.4±4.2
	αKg+Malon	19.2±1.4	8.4±1.7	14.1±1.3	4.0±0.1	75.0±5.5
Corn oil	GMM	48.2±1.0	3.7±0.1	6.6±1.2	3.0±0.0	145.1±5.2
	PMM	44.5±0.2	5.0±0.5	5.9±0.4	3.5±0.0	153.3±0.3
	Succ+Rot	48.8±1.4	2.3±0.2	2.9±0.4	1.9±0.1	93.0±5.8
	αKg+Malon	18.2±1.4	12.5±3.0	17.9±1.1	4.1±0.1	75.3±7.5
Rapeseed oil	GMM	43.1±2.9	4.5±0.3	6.3±0.3	3.1±0.0	134.3±8.0
	PMM	42.8±2.0	5.0±0.3	5.2±0.2	3.3±0.1	137.1±0.3
	Succ+Rot	44.9±1.5	2.3±0.3	2.8±0.3	1.9±0.1	85.7±1.0
	αKg+Malon	14.7±0.4	7.9±0.8	14.8±3.3	4.0±0.1	61.0±2.3
			5–6 h after isolation			
Chow	GMM	37.0±2.3	3.8±0.1	5.7±0.6	2.8±0.1	102.7±4.7
	PMM	33.2±0.5	3.8±0.1	5.1±0.4	3.2±0.3	105.2±9.3
Corn oil	GMM	37.7±0.0	3.1±0.7	4.1±0.8	2.7±0.3	101.3±10.4
	PMM	32.6±1.5	4.5±0.5	4.8±1.6	3.1±0.2	97.2±4.3
Rapeseed oil	GMM	28.2±1.0	2.6±0.2	3.4±0.3	2.5±0.1	72.0±4.0
	PMM	31.4±1.7	3.0±0.2	3.5±0.3	2.9±0.1	87.8±3.5

Freshly isolated mitochondria from the hearts of rapeseed oil-fed rats showed a reduced oxidative capacity compared with control animals (Table II). This effect was almost completely abolished if the mitochondria were isolated in the presence of heparin and tested within 2 h after isolation (Table III). The results in Table III, in disagreement with Houtsmüller et al. [4], show that such freshly-isolated mitochondria are functionally intact and show no significant reduction in their capacity to oxidize substrates or to synthesize ATP. Nor is any change observed in the respiratory indices or in the ADP : O ratios.

The presence of heparin in the isolation and incubation media has been shown to protect mitochondria from in vitro loss of oxidative function [9, 10]. Inhibition of lipolytic activity of mitochondria by heparin was suggested as a possible mechanism of this protection [9, 10]. Mitochondria from rapeseed oil-fed rats were more dependent on the presence of heparin for protection against in vitro loss of oxidative function than mitochondria from the other groups of animals (compare Tables II and III). The reason for the rapid in vitro loss of function by the mitochondria from rapeseed

oil-fed animals is not known. Rapeseed oil feeding is known to cause changes in the fatty acid composition of the phospholipids of cellular membranes [18]. Such fatty acid changes in membrane phospholipids could result in increased fragility of mitochondrial membranes in vitro due either to physical weakening of the membrane structure or to increased enzymatic breakdown of the phospholipids, either of which could cause loss of oxidative function in vitro.

Houtsmüller et al. [4] and Hornstra [19] suggested that the accumulation of triacylglycerols in the hearts of rapeseed oil-fed rats might be due to impaired mitochondrial oxidative capacity. In view of the present findings this does not appear to be so. Indeed, Gumpen and Norum [20] concluded that myocardial oxidation is not altered by rapeseed oil feeding. From in vitro studies with heart mitochondria Christophersen and Bremer [21] concluded that triacylglycerols accumulate in the heart as a consequence of an inhibitory effect of erucic acid or one of its metabolites on the β-oxidation of other fatty acids. On the other hand, Swarttouw [22] could not find inhibition of the oxidation of other fatty acids by erucic acid although she found, in agreement with Kramer et al. [3] that the oxidation of erucic acid itself is much lower, compared to that of other fatty acids. In conclusion, the accumulation of triacylglycerols in the hearts of rapeseed oil-fed rats is not due to a decreased capacity for ATP production but it may be due to a combination of increased uptake of fatty acids [20], a generally slower metabolism of erucic acid and erucic acid-containing acylglycerols [3, 22] and a possible impairment of ATP utilization due to changes in membrane fatty acid composition.

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