EFFECT OF DIETARY LONG CHAIN FATTY ACIDS ON ENERGY TRANSPORT IN CARDIAC MITOCHONDRIA

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1. Introduction

Studies to investigate effects of dietary long chain fatty acids on mitochondrial oxidative functions have shown that short term feeding of diets differing in fatty acid composition alters mitochondrial structural components [1–4] and metabolic functions [4–7]. Modification of mitochondrial membrane properties by dietary fat is also supported by recent ultrastructural studies of myocardial tissues [8–10]. These studies indicate morphological differences [11] in cardiac muscle and mitochondrial ultrastructure for rats and pigs fed diets of differing fatty acid composition. Thus, association of dietary fatty acid balance with mitochondrial structural–functional transitions is apparent. It has been proposed that similar interactions may affect the function of mitochondrial membrane-located control sites such as the adenine nucleotide translocase–creatine phosphokinase energy transport system [4,7], thus altering ATP utilization and substrate flux in cardiac mitochondria. Therefore, it is of interest to examine whether transitions in the fatty acid composition of cardiac mitochondrial membrane phospholipids are associated with corresponding changes in ATP translocation and utilization via mitochondrial creatine phosphate synthesis after long term feeding of diets differing in fatty acid composition.

Here, dietary fat-induced changes in the fatty acid composition of isolated cardiac mitochondrial phospholipids were associated with differences in mitochondrial capacity for synthesis and translocation of ATP utilized for mitochondrial creatine phosphate synthesis.

2. Materials and methods

Male weanling rats derived from the Sprague-Dawley strain were caged individually with water and experimental diets provided ad libitum. Three diets [7], containing 15% (w/w) of either soybean oil (SBO), low erucic acid rapeseed oil (LER) or high erucic acid rapeseed oil (HER) of edible quality were fed for 16 and 28 week periods. The fatty acid composition of the experimental oils has been described [7]. Hearts from 4 animals were pooled for each mitochondrial sample prepared. The number of separate samples analysed and statistical differences are indicated (tables 1–3).

Isolated cardiac mitochondria were prepared in the presence of heparin as in [7,12,13]. Respiratory studies were performed immediately on resuspended mitochondrial pellets. Protein was measured by a colorimetric method [14]. For analysis of membrane phospholipid fatty acids, isolated mitochondria were fractionated with digitonin to prepare the inner mitochondrial membrane matrix compartment [15] as in [4]. This inner membrane matrix compartment, free of membrane fragments and microsomal material was used as the source of membrane phospholipids for further study. Membrane phospholipids were extracted [2] and fatty acid methyl esters were prepared with boron trifluoride methanol reagent [16]. Fatty acid methyl esters were analysed by gas–liquid chromatography (Varian model 3700 gas chromatograph equipped with flame ionization detectors and a CDS 111 data system for automated peak area calculations). Glass columns (3 m × 2 mm i.d.) packed with Silar-5CP (10% w/w) coated on acid washed 80–100 mesh chromosorb were utilized for chromatographic separation and identification of fatty acid methyl esters Peak identities were verified by comparison of retention data with standards and by the method of equivalent chain length [17]

ATP translocation through the inner mitochondrial membrane was determined during steady state conditions of oxidative phosphorylation by coupling the rate of ATP appearance in the reaction medium to synthesis of glucose-6-P by hexokinase [18] The complete reaction medium contained 0 25 M sucrose, 10 mM Tris–HCl (pH 7 4), 5 mM K-pyruvate, 2 mM K-malate, 3.3 mM $MgCl_2$, 20 mM glucose, 0 25 mM ATP, 5 IU/ml hexokinase and mitochondria in 4 ml final vol Oxidative phosphorylation was initiated by addition of mitochondria (1 mg protein/ml) and the rate of oxygen uptake was monitored continuously [7] Glucose-6-P synthesized in the reaction mixture was measured at two different times by removing 1 ml samples out of the reaction cuvette The reaction in these samples was stopped by addition of 0 2 ml aliquots of perchloric acid (6%) and the glucose-6-P separated by a rapid filtration technique [18] Glucose-6-P was measured by a spectrophotometric method [19] The rate of ATP translocation from the mitochondrial matrix by adenine nucleotide translocase was also measured when ATP transport was coupled to mitochondrial creatine phosphokinase by replacing the glucose-hexokinase system with 20 mM creatine [18]

3 Results and discussion

Studies of the composition of mitochondrial membrane phospholipids have indicated marked transitions in fatty acid composition of membrane components after acute feeding of diets of differing fatty acid composition [2] Examination of membrane fatty acyl components after chronic periods of feeding (16 or 28 weeks) with similar diets should reflect the animals physiological capacity to counteract or regulate changes in the membranes structural lipids thereby eventually compensating for increased levels of unsaturated fatty acids known to characterize mitochondrial membranes after 7 or 28 days dietary treatment with long chain monoenoic fatty acids In the long term, metabolic adaptation to diets of high $n-9$ fatty acid content in combination with turnover of fatty acyl components of mitochondrial membrane phospholipids could result in mitochondrial membranes of similar composition and physical properties irrespective of the fatty acid composition of the diet fed Thus eventually minimizing the functional significance of acute changes in the fatty acid composition of mitochondrial membrane phospholipids In this regard, prolonged feeding of dietary treatments for 16 or 28 weeks resulted in a progression of both membrane total saturated fatty acid content and unsaturation index towards similar levels (table 1), indicating that some degree of continuous turnover of fatty acid components occurs in mitochondrial membrane lipids in the mature rat However, the level of $n-9$ monoenoic fatty acids present in membrane phospholipids after 16 and 28 weeks of dietary treatment remained higher for HER or LER treatments and at levels similar to those reported for short term feeding studies [2] The level of $C_{22\ 1}$ in membrane phospholipids declined from 16 to 28 weeks of feeding suggesting turnover of this fatty acid in the membrane lipids Levels of $n-6$ fatty acids in membrane phospholipids were also higher for long term treatments containing SBO, similar to levels reported for shorter feeding periods [2] and largely reflected the $C_{18\ 2}$ content of mitochondrial membrane phospholipids (table 1)

Diet-induced long term changes in the composition of mitochondrial membrane phospholipids for LER and HER fed rats were also associated with significant differences in mitochondrial respiratory capacity when steady state conditions of oxygen uptake and ATP synthesis were coupled to creatine phosphate synthesis by the mitochondrial membrane adenine nucleotide translocase–creatine phosphokinase energy transport system (table 2)

These rates of respiration, from 269–325 nmol oxygen uptake min^{-1} mg $protein^{-1}$, are consistent with in vitro rates reported in [7] Mitochondria isolated from rats fed LER or HER for prolonged periods (16 weeks) respire at significantly lower steady state rates relative to SBO fed rats, generating less ATP when mitochondrial energy metabolism is under the metabolic control of mitochondrial creatine phosphokinase or when mitochondrial energy utilization is coupled to creatine phosphate synthesis (table 2).

Assays of mitochondrial respiration requiring only

Table 1
Fatty acid composition of total phospholipids extracted from cardiac mitochondria of rats fed experimental fats for 16 or 28 weeks

Dietary treatment	SBO		LER		HER	
Weeks of feeding	16	28	16	28	16	28
Fatty acid (% w/w)(1)						
$C_{14:0}$	$4.2 \pm 1.0^{a(2)}$	4.8 ± 0.5^{a}	2.5 ± 0.3^{a}	2.6 ± 0.2^{a}	2.6 ± 0.5^{a}	3.0 ± 0.4^{a}
$C_{14:1}$	0.3 ± 0.2^{a}	0.3 ± 0.2^{a}	0.2 ± 0.1^{a}		0.2 ± 0.1^{a}	1.6 ± 0.6^{a}
$C_{16:0}$	12.2 ± 1.1^{a}	11.7 ± 0.9^{a}	11.5 ± 0.08^{a}	12.7 ± 0.8^{a}	8.7 ± 0.9^{a}	11.4 ± 1.0^{a}
$C_{16:1}$	1.0 ± 0.2^{a}	0.3 ± 0.1^{a}	0.9 ± 0.3^{a}		1.0 ± 0.3^{a}	1.5 ± 0.4^{a}
$C_{18:0}$	26.0 ± 2.1^{a}	25.4 ± 2.0^{a}	28.0 ± 2.3^{a}	26.0 ± 1.9^{a}	18.2 ± 1.9^{a}	21.5 ± 1.7^{a}
$C_{18:1}$	6.9 ± 0.7^{a}	6.3 ± 0.6^{a}	14.2 ± 1.7^{b}	20.0 ± 1.8^{b}	12.6 ± 1.3^{b}	12.0 ± 1.0^{c}
$C_{18:2}$	31.0 ± 2.7^{a}	35.6 ± 2.9^{a}	24.5 ± 2.0^{ab}	21.9 ± 2.0^{b}	22.8 ± 2.3^{b}	23.9 ± 2.1^{b}
$C_{18:3}$	TR^{a}	TR^{a}	0.2 ± 0.1^{a}	TR^{a}	0.5 ± 0.2^{a}	0.5 ± 0.1^{a}
$C_{20:1}$	0.8 ± 0.2^{a}		0.8 ± 0.3^{a}		2.0 ± 0.3^{a}	2.2 ± 0.3^{a}
$C_{20:4}$	14.8 ± 1.4^{a}	15.9 ± 1.1^{a}	16.7 ± 1.8^{a}	16.7 ± 1.9^{a}	14.1 ± 1.7^{a}	14.9 ± 1.8^{a}
$C_{22:1}$				0.2 ± 0.1^{a}	7.7 ± 1.0^{b}	2.1 ± 0.3^{b}
$C_{24:0}$					0.7 ± 0.3^{a}	1.0 ± 0.4^{a}
$C_{24:1}$					3.5 ± 1.0^{a}	4.0 ± 1.1^{a}
Others	3.6 ± 1.2		0.7 ± 4.0		5.4 ± 2.1	0.2 ± 0.1
% saturated	42.5 ± 1.1^{a}	41.9 ± 0.9^{a}	42.5 ± 1.0^{a}	41.3 ± 0.7^{a}	30.7 ± 0.9^{a}	39.9 ± 0.9^{a}
Total $n - 9$	7.7 ± 0.5^{a}	6.9 ± 0.2^{a}	15.0 ± 1.1^{b}	20.2 ± 0.5^{b}	25.8 ± 1.0^{c}	19.4 ± 0.7^{b}
Total $n - 6$	44.8 ± 2.2^{a}	51.5 ± 1.3^{a}	41.3 ± 1.8^{ab}	38.6 ± 1.6^{b}	37.3 ± 1.9^{b}	38.8 ± 1.1^{b}
Total $n - 3$	TR^{a}	TR^{a}	0.2 ± 0.1^{a}	TR^{a}	0.6 ± 0.2^{a}	0.5 ± 0.1^{a}
UI(3)	129.0 ± 4.1^{a}	142.0 ± 2.9^{a}	133.0 ± 4.2^{a}	131.0 ± 3.4^{a}	131.0 ± 4.4^{a}	133.0 ± 3.9^{a}

(1) Numbers before and after the colon represent the number of carbon atoms and double bands, respectively

(2) Mean ± standard error of mean. Four samples for each treatment were analysed by GLC. $TR \leqslant 0.1\%$. Different superscripts within a line indicate a significant difference ($p < 0.05$)

(3) *UI* = unsaturation index[2]

Table 2
Oxygen uptake when adenine nucleotide translocation is coupled to mitochondrial creatine phosphokinase and creatine phosphate synthesis(1)

Dietary treatment	Rate of oxygen uptake (nmol/min/mg mitochondrial protein)	ATP translocated to synthesize creatine phosphate
SBO	325^{a}	620^{a}
LER	264^{b}	536^{b}
HER	269^{b}	436^{c}
Mean	286	529
Pooled standard error ($n = 9$)	24.4	33

(1) Respiration was measured utilizing cardiac mitochondria isolated after 16 weeks of dietary oil treatment

Table 3
Rate of energy transport in cardiac mitochondria catalysed by adenine nucleotide translocase(1)

Dietary treatment	Oxygen uptake	ATP translocated	ATP translocated/ oxygen
	(nmol/min/mg mitochondrial protein)		
SBO	95^{a}	182^{a}	1 91^{a}
LER	97^{a}	199^{a}	2 03^{a}
HER	88^{a}	136^{b}	1 62^{b}
Mean	93	172	1 85
Pooled standard error ($n = 9$)	6 02	14 7	0 117

(1) Respiration was measured utilizing cardiac mitochondria isolated after 16 weeks of dietary oil treatment

ADP–ATP translocation independent of creatine phosphate synthesis (table 3) indicated similar respiratory rates for all dietary treatments suggesting that adenine nucleotide translocase does not limit respiration rate in mitochondria isolated from HER and LER fed rats relative to SBO treatments However, a lower rate of ATP translocation was observed for HER treatments confirming previous observations that the efficiency of oxidative phosphorylation is decreased in rats fed HER treatments

The mechanism linking these functional differences in energy utilization (table 2) to the kinetics of mitochondrial creatine phosphokinase remain to be investigated As ADP–ATP translocase, situated within the inner mitochondrial membrane, is known to transport ATP synthesized within the mitochondrial compartment to the active site of creatine phosphokinase [18], one might speculate that diet-induced alterations in membrane structural components, vis à vis the differing fatty acid composition of membrane phospholipids (table 1), may interact in the kinetic process of mitochondrial energy utilization under steady state conditions

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References

[1] Blomstrand, R and Svensson, L (1974) Lipids 9, 771–780
[2] Clandinin, M T (1976) FEBS Lett 68, 41–44
[3] Dewailly, P , Sezille, G , Nouvelot, A , Fruchant, J C and Jaillard, J (1977) Lipids 12, 301–306
[4] Renner, R , Innis, S M and Clandinin, M T (1979) J Nutr 109, 378–387
[5] Houtsmuller, U M T , Struijk, C B and Van der Beek, A (1970) Biochim Biophys Acta 218, 564–566
[6] Hsu, C M L and Kummerow, F A (1977) Lipids 12, 486–494
[7] Clandinin, M T (1978) J Nutr 108, 273–281
[8] Vodovar, N , Desnoyers, F , Cluzan, R and Levillain, R (1977) Biol Cell 29, 37–44
[9] Bhatnagar, M K and Yamashiro, S (1979) Res Vet Sci in press
[10] Yamashiro, S and Clandinin, M T (1979) submitted
[11] Clandinin, M T and Yamashiro, S (1979) submitted
[12] Dow, D S (1967) Biochemistry 6, 2915–2922
[13] Dow, D S (1967) Biochemistry 6, 3350–3355
[14] Lowry, O H , Rosebrough, N J , Farr, A L and Randall, R J (1951) J Biol Chem 193, 265–275
[15] Schnaitman, C and Greenawalt, J W (1968) J Cell Biol 38, 158–175
[16] Metcalfe, L D and Schmitz, A A (1961) Anal Chem 33, 363–364
[17] Miwa, T K , Mikokajaczak, K L , Earle, F R and Wolff, J A (1960) Anal Chem 32, 1739–1742
[18] Saks, V A , Lipina, N V , Smirnov, V N and Chazov, E I (1976) Arch Biochem Biophys 173, 34–41
[19] Lamprecht, W and Stein, P (1965) in Methods of Enzymatic Analysis (Bergmeyer, H U ed) pp 610–616, Academic Press, New York