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THE EFFECT OF DIETARY LIPIDS ON THE THERMOTROPIC BEHAVIOUR OF RAT LIVER AND HEART MITOCHONDRIAL MEMBRANE LIPIDS

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Diets supplemented with relatively high levels of either saturated fatty acids derived from sheep kidney fat (sheep kidney fat diet) or unsaturated fatty acids derived from sunflower seed oil (sunflower seed oil diet) were fed to rats for a period of 16 weeks and changes in the thermotropic behaviour of liver and heart mitochondrial lipids were determined by differential scanning calorimetry (DSC). The diets induced similar changes in the fatty acid composition in both liver and heart mitochondrial lipids, the major change being the ω6 to ω3 unsaturated fatty acid ratio, which was elevated in mitochondria from animals on the sunflower seed oil diet and lowered with the mitochondria from the sheep kidney fat dietary animals. When examined by DSC, aqueous buffer dispersions of liver and heart mitochondrial lipids exhibited two independent, reversible phase transitions and in some instances a third highly unstable transition. The dietary lipid treatments had their major effect of the temperature at which the lower phase transition occurred, there being an inverse relationship between the transition temperature and the ω6 to ω3 unsaturated fatty acid ratio. No significant effect was observed for the temperature of the higher phase transition. These results indicate that certain domains of mitochondrial lipids, probably containing some relatively higher melting-point lipids, independently undergo formation of the solidus or gel phase and this phenomenon is not greatly influenced by the lipid composition of the mitochondrial membranes. Conversely, other domains, representing the bulk of the membrane lipids and which probably contain the relatively lower melting point lipids, undergo solidus phase formation at temperatures which reflect changes in the membrane lipid composition which are in turn, a reflection of the nature of the dietary lipid intake. These lipid phase transitions do not appear to correlate directly with those events considered responsible for the altered Arrhenius kinetics of various mitochondrial membrane-associated enzymes.

Introduction

Phospholipids are known to exhibit both thermotropic and lyotropic mesomorphism which give rise to changes in the physical state of their acyl fatty acid chains [1–5]. The transition temperature and enthalpy for pure phospholipids are dependent on the nature of both the hydrocarbon chains and the polar headgroups, the amount of water [6] and the presence of components, including proteins, which may interact either with the hydrocarbon region of the bilayer and/or in the vicinity of the polar headgroups [2,3,5,7]. Phase transitions of aqueous lamellar dispersions of phospholipids are readily detectable by differential scanning calorimetry (DSC) [2–4]. Such transitions involve a highly co-operative reversible change in the fatty acids between a fluidus, liquid-crystalline phase

Abbreviations: Hepes 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mops, 4-morpholinepropanesulphonic acid.

and a solidus, crystalline or cogel phase [1,4–6].

Thermotropic phase transitions associated with the membranes and membrane lipids from a wide variety of organisms have been detected by thermal techniques [8–10]. These transitions are usually broad and occur at temperatures which are dependent on the membrane fatty acid composition [11]. Until recently, phase transitions in mammalian membranes were not apparent at temperatures above 0°C, due to the high content of unsaturated fatty acids in the membrane lipids [12]. However, recent reports indicate that the lipids of some membranes may undergo phase transitions at temperatures well above 0°C, although these transitions are of relatively low enthalpy. For example, rat liver microsomal and mitochondrial membranes exhibit phase transitions in the region 18–40°C, with the transition in the extracted lipids occurring at slightly lower temperatures [13,14]. Rat hepatocyte plasma membranes and extracted lipids exhibit reversible thermal phase transitions between 18 and 31°C [15], as do plasma membrane preparations from the small intestine of the rat [16]. Bovine heart submitochondrial particles also exhibit a reversible transition at about 20°C after thermal denaturation of the membrane proteins [17]. This relatively small transition is in addition to the main phase transition centred at about −10°C [12,17]. Phase transitions at about 20°C have been detected in the liver microsomal fraction of rats fed fat-free diets but not with rats fed normal diets, the differences being attributed to differences in the degree of saturation of the membrane lipids induced by the diet [18]. All the phase transitions described above occur at temperatures well above 0°C, and they may be responsible in part for the discontinuities which have been reported for the Arrhenius plots of numerous membrane-associated enzyme systems, at temperatures near 20°C [19–23].

In this study we have examined by differential scanning calorimetry, the thermal behaviour of liver and heart mitochondrial membrane lipids isolated from rats fed various lipid-supplemented diets. Such diets would be expected to alter the fatty acid composition and hence some of the physical properties of the membrane lipids which may affect certain characteristics associated with the thermal behaviour of the membrane lipids.

In addition to the above-mentioned phase transitions, various membrane phospholipids can undergo a phase transition between fluid lamellar (L_α) and reverse hexagonal (H_{II}) liquid-crystalline phases under certain conditions. These $L_\alpha \rightleftarrows H_{II}$ transitions may perform a functional role in biological membranes [24]. The majority of evidence for these transitions has relied on ^{31}P-NMR and freeze-fracture techniques[24,25]. However, in a study on aqueous dispersions of egg phosphatidylethanolamine, both gel $\rightleftarrows L_\alpha$ and $L_\alpha \rightleftarrows H_{II}$ transitions have been observed by differential thermal analysis, with the two types of phase transition being distinguished, in part, by their behaviour at different pH values [26].

Materials and Methods

Adult rats (Hooded-Wistar), weighing between 230 and 260 g at the commencement of the experiment were assigned to three different dietary regimes to achieve widely different levels of fat intake and unsaturation:saturation ratios. For the next 16 weeks, the animals were fed either a commercially available rat chow (Milling Industries Ltd., Australia) which contained 4% (w/w) total fat derived from a variety of sources (designated reference diet), or diets supplemented by the addition of either 12% (w/w) sunflower seed oil obtained from Nuttelex Pty. Ltd., or 12% (w/w) sheep kidney (perirenal) fat which was added to the reference chow at the time of pelleting.

After the 16-week feeding period, animals were killed by decapitation under light diethyl ether anaesthesia and liver and heart mitochondria were prepared. Liver mitochondria were prepared in isolating medium comprising 250 mM sucrose/2 mM Hepes/0.5 mM EGTA/0.05% (w/v) delipidated bovine serum albumin (pH 7.4) in the ratio of 1 g (wet weight) tissue to 10 ml isolating medium. After removal of the atria, subsarcolemmal heart mitochondria were prepared essentially according to the method of Tomec and Hoppel [27] in isolating medium comprising 100 mM KCl/50 mM Mops/2 mM EGTA/0.2% (w/v) delipidated bovine serum albumin (pH 7.2) with the tissue from one heart being homogenized in 20 ml of isolating medium. Liver or heart tissue was first chopped and rinsed in their respective ice-cold

isolation media, then homogenized using a Polytron tissue disintegrator (Kinematica, Switzerland) at setting 3.5 for two bursts of 6 s. The brei was filtered through cheesecloth and centrifuged at $500 \times g$ for 12 min. The pellets were resuspended in their respective isolating media to the original volume and recentrifuged at $500 \times g$ for 12 min. The combined supernatants were centrifuged at $6000 \times g$ for 15 min and the mitochondrial pellet was washed twice by recentrifugation at $6000 \times g$ for 15 min, with the liver mitochondria being washed in the original liver isolating medium and the heart mitochondria being washed in medium comprising the original heart isolating medium without the addition of bovine serum albumin. The final mitochondrial pellets were resuspended in their respective wash buffers to a concentration of 63 mg protein/ml (liver) and 23 mg protein/ml (heart) and used for the determination of mitochondrial respiratory activity and for the extraction of mitochondrial lipids.

Measurement of respiratory activity of liver and heart mitochondria by polarographic techniques was as previously described [21], except that the reaction medium was that of Tomec and Hoppel [27] comprising 80 mM KCl/50 mM Mops/5 mM potassium phosphate/1 mM EGTA/0.1% (w/v) delipidated bovine serum albumin (pH 7.0).

For lipid extraction, mitochondrial preparations were diluted in 50 vol. 20 mM Tris/2 mM EDTA (pH 7.2) and centrifuged at $250000 \times g$ for 60 min. The resulting membrane pellet was resuspended in glass-distilled water and the lipids extracted usisng the method of Bligh and Dyer [28]. The antioxidant, butylated hydroxytoluene was included in the lipid extract at the concentration of approx. 0.1% of the lipid dry weight. The fatty acid composition of the respective mitochondrial total lipid extracts was determined after methylation of an aliquot of the total lipids in 1% (v/v) H_2SO_4 in methanol heated at 70°C for 3 h. Fatty acid methyl esters were analysed by gas chromatography as described previously [29].

Differential scanning calorimetry (DSC) was performed on aqueous buffer dispersions of liver or heart mitochondrial total lipids. A concentrated solution of mitochondrial lipids in chloroform was added to 75-μl stainless-steel pans (Perkin-Elmer, Norwalk, CT, U.S.A.). For liver mitochondria, approx. 24 mg (dry weight) of lipid was added per pan with each pan containing the liver mitochondrial lipids isolated from one animal. For heart mitochondria in which the lipids from the hearts of eight animals per dietary group were included in each pan, the average sample dry weight was 18 mg. The lipid in the pan was dried of solvent by vacuum desiccation for 24 h and samples were then hydrated by adding at least 30 μl (i.e., over 100% hydration) of buffer comprising 50 mM Tris/2 mM EDTA/15% (v/v) ethylene glycol (pH 7.2) (liposome buffer). This concentration of ethylene glycol has been reported to have little effect on the phase transition behaviour of aqueous dispersions of distearoylphosphatidylcholine when investigated by DSC [30]. Pans were then hermetically sealed using a Perkin-Elmer sealing press and left to equilibrate (without sonication) at 4°C for at least 24 h.

Thermal scans were performed using a Perkin-Elmer Differential Scanning Calorimeter (Model DSC-2B) and analysed using a dedicated Perkin-Elmer Thermal Analysis Data Station. The instrument was calibrated with respect to both temperature and enthalpy measurements using water, indium (Perkin-Elmer) and aqueous dispersions of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) (Sigma, St. Louis, MO, USA). Scans were made at a rate of 5°C per min against an empty sealed reference pan. All samples were scanned three times in the cooling mode and then at least one heating scan was included which was firstly initiated below the ice $\rightleftarrows$ water phase transition to verify full hydration of the lipid samples and then initiated above the ice $\rightleftarrows$ water phase transition for observation of the thermal behaviour of the mitochondrial membrane lipids. The phase transition temperature of the lipid dispersions has been defined as that temperature at which a significant departure from the baseline is first evident in the cooling mode or when the scan reunites with the baseline in the heating mode. Cooling and heating scans of aqueous buffer dispersions of DMPC or DPPC in the presence of butylated hydroxytoluene (at a concentration of 0.1% of the dry weight of the phospholipid) had no effect on the reported transition temperature or enthalpy of these phospholipids.

Results

The fatty acid composition of the lipid-supplemented and reference diets is shown in Table 1. Both the sheep kidney fat and sunflower seed oil diets contained approx. 16% lipid by weight, whereas the reference diet contained 4% lipid. The sheep kidney fat diet was characterized by a relatively high proportion of palmitic (16 : 0), stearic (18 : 0) and oleic (18 : 1) acids and a low proportion of linoleic (18 : 2) acid in comparison to the other diets. The sunflower seed oil diet was characterized by a high proportion of 18 : 2 and a low proportion of 16 : 0 in comparison to the other two diets. All diets provided more than 1.3% of the total dietary energy as linoleic acid, which is reported to be the minimum requirement for this fatty acid in rats [31]. All diets contained at least 1% (of the total fatty acid content) as linolenic acid (18 : 3ω3). The level of lipid unsaturation ranged from about 41% for the sheep kidney fat diet to about 84% for the sunflower seed oil diet.

Rates of state 3 (ADP non-limiting) respiration and values of the respiratory control ratios for liver and heart mitochondria oxidizing glutamate or succinate are shown in Table II. The isolation procedures chosen were those which gave the optimal state 3 rates and respiratory control ratios. The dietary lipid treatment did not significantly affect the 'intactness' of either liver or heart mitochondria as determined by the constancy of the respiratory control ratio values for each particular substrate. The state 3 respiration rates were also not significantly affected due to the dietary lipid treatment, except for a slight reduction for glutamate oxidation in heart mitochondria isolated from the sheep kidney fat and sunflower seed oil dietary groups. Based on the above respiratory parameters, it would appear that the isolation of both liver and heart mitochondria from the differing dietary groups was not significantly affected by the particular dietary lipid treatments employed.

The fatty acid composition of rat liver mitochondrial lipids after 16 weeks dietary lipid supplementation is shown in Table III. The change in the overall level of both saturated and unsaturated fatty acids and the unsaturation index was not great, despite the fact that the diets differed considerably in their levels of lipid and degree of lipid unsaturation. There were, however, significant changes in the proportions of certain individual saturated and unsaturated fatty acids due to the dietary treatment. The most prominent changes in the saturated fatty acids were the decreased proportion of 16 : 0 and the increased proportion of 18 : 0, which occurred in both lipid-supplemented dietary groups relative to the reference group. For the unsaturated fatty acids, the proportion of linoleic acid (18 : 2ω6) was reduced in the sheep kidney fat dietary supplemented animals relative to the other two dietary groups. The proportion of arachidonic acid (20 : 4ω6) was elevated in the sunflower seed oil diet animals and slightly reduced in the sheep kidney fat diet animals, relative to the control. The opposite was the case for docosahexaenoic acid (22 : 6ω3). Changes in the proportions of oleic acid (18 : 1) were also evident as a result of the dietary lipid treatments. The changes in the proportion of the various unsaturated fatty acids can be viewed in terms of changes in the ω6/ω3 unsaturated fatty acid ratio.

TABLE I

FATTY ACID COMPOSITION OF LIPID-SUPPLEMENTED RAT DIETS

Fatty acids are designated by the number of carbon atoms followed by the number of double bonds together with the designated fatty acid series for particular unsaturated fatty acids. Data are presented as the mean relative percentage (w/w) determined from at least six different samples from each dietary group. Trace (tr.) present at less than 0.1%.

Fatty acid	Reference diet	Sunflower seed oil diet	Sheep kidney fat diet
14:0	1.6	2.6	0.3
16:0	20.8	22.3	9.2
16:1	3.2	1.6	0.6
17:0	0.9	1.5	tr.
18:0	7.2	29.4	5.6
18:1	22.2	29.8	22.8
18:2ω6	32.9	7.1	58.0
18:3ω3	2.6	1.1	1.0
20:0	0.1	0.6	0.3
20:1	3.1	0.6	0.7
22:1/20:5	3.6	0.5	0.7
22:6ω3	1.6	0.3	0.3
Saturated	30.8	56.4	16.2
Unsaturated	69.2	41.0	83.7

TABLE II

LIVER AND HEART MITOCHONDRIAL RESPIRATION FROM DIETARY LIPID-SUPPLEMENTED RATS

State 3 respiration rates are expressed as nmol O_2/min per mg protein at 30°C and are the mean ±S.E. for ($n = 4$) liver mitochondrial preparations from each dietary group and $n = 8$ heart mitochondrial preparations, with each preparation being prepared from the hearts of two animals. RCR, respiratory control ratio. Final concentrations of glutamate and succinate were 20 mM and 10 mM, respectively.

Respiratory substrate		Diet		
		Reference	Sheep kidney fat	Sunflower seed oil
Liver mitochondria				
Glutamate	state 3	63 ±12	67 ±12	64 ±10
	RCR	6.4± 0.8	6.4± 1.0	6.9± 0.7
Succinate	state 3	81 ±11	91 ± 7	93 ± 5
	RCR	4.1± 0.4	4.9± 0.5	5.0± 0.4
Heart mitochondria				
Glutamate	state 3	82 ± 4	66 ± 4	67 ± 3
	RCR	5.5± 0.5	4.5± 0.4	4.7± 0.4
Succinate	state 3	140 ± 8	126 ± 6	137 ± 5
	RCR	3.0± 0.2	2.9± 0.2	3.1± 0.2

TABLE III

RAT LIVER MITOCHONDRIAL FATTY ACID COMPOSITION AFTER 16 WEEKS DIETARY LIPID SUPPLEMENTATION

Data are presented as the mean relative percentate ±S.E. for mitochondrial preparations from the liver of $n = 4$ animals from each dietary group. The unsaturation index (U.I.) is $\Sigma[(a)(b)]$ where a is the percentage of each unsaturated fatty acid and b is the number of double bonds for that particular fatty acid. Trace (tr.) represents amounts present at less than 0.3%. Significance between sheep kidney fat and sunflower seed oil dietary groups was determined by Student's t-test with (–) designating not determined, n.s. not significant. All other data are as described in Table I.

Fatty acid	Diet			Significance
	Reference	Sheep kidney fat	Sunflower seed oil	Sheep kidney fat vs. Sunflower seed oil
16:0	18.8±0.3	14.0±0.2	12.5±0.2	$P < 0.01$
16:1	1.7±0.1	1.2±0	0.6±0	–
17:0	0.6±0	0.8±0	0.5±0	–
18:0	19.5±0.3	24.3±0.4	24.1±0.5	n.s.
18:1	9.1±0.2	11.5±0.2	7.5±0.2	$P < 0.001$
18:2ω6	14.1±0.2	11.2±0.3	13.8±0.5	$P < 0.01$
18:3ω6	0.3±0	0.3±0	0.4±0	–
18:3ω3	tr.	tr.	tr.	–
20:1	0.4±0	0.3±0	0.5±0	–
20:2/20:3	0.4±0	0.6±0	1.1±0.1	–
20:3ω6	1.1±0	1.5±0	0.5±0	–
20:4ω6	22.1±0.1	20.4±0.4	30.0±0.2	$P < 0.001$
20:5/22:1	1.7±0.1	1.6±0.1	tr.	–
22:4ω6	tr.	tr.	0.4±0.1	–
24:0	tr.	tr.	0.5±0.1	–
22:5ω3	1.0±0	0.7±0	0.3±0	–
22:6ω3	8.6±0.3	10.8±0.3	7.1±0.2	$P < 0.001$
Saturated	39.1	39.5	37.7	
Unsaturated	60.9	60.5	62.3	
U.I.	192	195	208	
ω6	37.7	33.5	45.1	
ω3	9.8	11.7	7.4	
ω6/ω3	3.85	2.86	6.09	

Values for this ratio ranged from 2.86 in the sheep kidney fat diet animals to 3.85 for the reference and 6.09 for the sunflower seed oil diet animals.

Although the diet-induced changes in the heart mitochondrial fatty acids were similar to those observed for the liver, particularly in terms of the altered $\omega 6/\omega 3$ unsaturated fatty acid ratio, changes in the proportion of some of the major unsaturated fatty acids were quite different from those observed in liver (Table IV). Whereas in liver the major change in the $\omega 6$ unsaturated fatty acids was due to an alteration in the proportion of 20 : 4, in heart both 20 : 4 and 18 : 2 were elevated in the sunflower seed oil dietary animals relative to those on the sheep kidney fat diet. However, relative to the reference group, both the sheep kidney fat and sunflower seed oil dietary groups exhibited a decrease proportion of 18 : 2 and an increased proportion of 20 : 4. The pattern of change between the proportions of 22 : 6 in heart mitochondrial lipids due to the dietary lipid treatment was also similar to that observed with liver mitochondrial lipids. Although the absolute values for the $\omega 6/\omega 3$ unsaturated fatty acid ratio for each dietary lipid group in heart mitochondria differed from that observed in liver mitochondria, the extent by which the ratio was either increased or decreased by the sunflower seed oil or sheep kidney fat dietary treatments respectively, was similar.

The total level of the dimethyl acetal derivatives

TABLE IV

RAT HEART MITOCHONDRIAL FATTY ACID COMPOSITION AFTER 16 WEEKS DIETRARY LIPID SUPPLEMENTATION

Data are presented as the mean relative percentage ± S.E. for single heart mitochondrial preparations from $n = 8$ animals from each dietary group. DMA, dimethyl acetal derivative. All other details are as described in Table III.

Fatty acid	Diet			Significance sheep kidney fat vs. Sunflower seed oil
	Reference	Sheep kidney fat	Sunflower seed oil	
DMA 16:0	2.1±0	1.4±0	1.6±	–
16:0	11.1±0.1	9.1±0.2	7.8±0.1	$P < 0.001$
16:1	1.0±0.1	0.7±0	tr.	–
17:0	0.5±0	0.5±0	tr.	–
DMA 18:0	0.9±0.1	1.9±0	1.6±0	–
18:0	20.6±0.3	23.0±0.4	25.1±0.4	$P < 0.01$
18:1	9.0±0.3	9.7±0.1	6.3±0.1	$P < 0.001$
18:2ω6	21.2±0.8	13.7±0.5	18.4±0.3	$P < 0.001$
18:3ω6	tr.	tr.	tr.	–
18:3ω3	tr.	tr.	tr.	–
20:1	0.5±0	0.4±0.1	0.4±0.1	–
20:1/20:3	tr.	0.3±0	0.4±0	–
20:3ω6	0.4±0	0.6±0	0.4±0	–
20:4ω6	14.2±0.3	16.6±0.2	19.6±0.2	$P < 0.001$
20:5/22:1	0.7±0.1	1.4±0.3	0.9±0.2	–
22:4ω6	tr.	0.3±0.1	1.0±0	–
24:0	tr.	0.3±0.1	1.1±0.1	–
22:5ω3	1.7±0.1	1.9±0.1	1.0±0.1	$P < 0.001$
22:6ω3	14.2±0.5	16.7±0.3	13.2±0.3	$P < 0.001$
Saturated	35.4	36.2	37.7	
Unsaturated	62.6	62.4	61.8	
U.I.	205	202	212	
ω6	36.0	31.2	39.4	
ω3	15.9	18.6	14.2	
ω6/ω3	2.26	1.68	2.77	

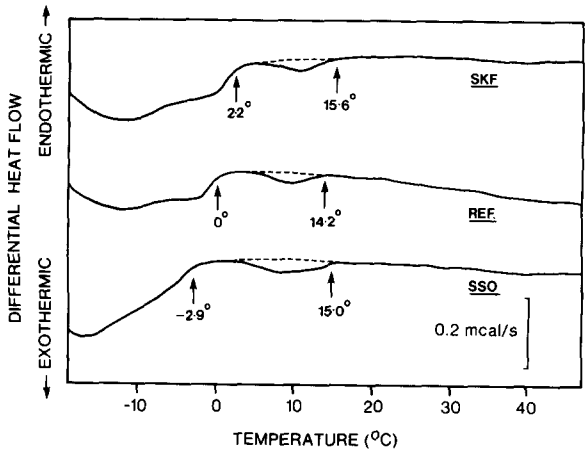

Fig. 1. Representative differential scanning calorimetric scans of aqueous buffer (pH 7.2) dispersions of liver mitochondrial lipids isolated from rats fed either the sheep kidney fat (SKF) diet, the reference (REF.) diet, or the sunflower seed oil (SSO), as described in the Materials and Methods. Scans were made in the cooling mode at a rate of 5 C deg./min. Arrows indicate the temperature at which a significant departure from the baseline was observed. The scans shown are those obtained in the second or subsequent cooling scans.

of 16:0 and 18:0 were 3.0, 3.3 and 3.2% of the total heart mitochondrial fatty acids for the reference, sheep kidney fat and sunflower seed oil dietary animals, respectively. These derivatives

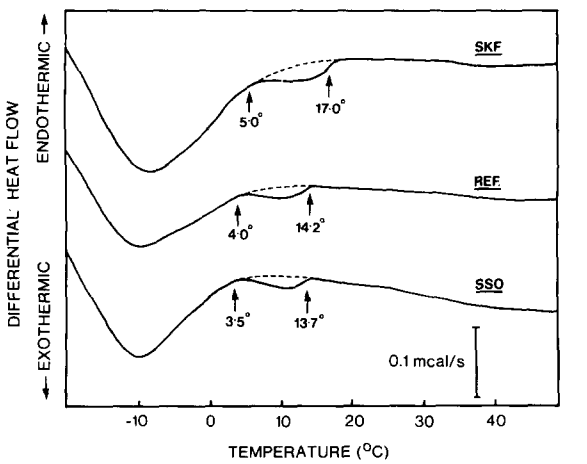

Fig. 2. Representative differential scanning calorimetric scans of aqueous buffer (pH 7.2) dispersions of heart mitochondrial lipids isolated from rats fed either the sheep kidney fat (SKF) diet, the reference (REF.) diet, or the sunflower seed oil (SSO) diet. Scans were performed as described for Fig. 1.

would presumably occur during the methylation of plasmalogens which are present in the membrane lipids of heart mitochondria, but are not present in liver mitochondrial lipids [32].

Representative DSC thermograms obtained in the cooling mode for aqueous buffer dispersions of liver and heart mitochondrial lipids from the various dietary lipid supplemented animals are shown in Figs. 1 and 2, respectively. The results for both the transition temperatures and the enthalpy values for all experiments are described in Tables V and VI. For all mitochondrial lipid samples at least two, but sometimes three, exothermic transitions were observed (for convenience, these transitions have been labelled as T_1, T_2 and T_3). For all samples, the enthalpy value (ΔH) for the T_1 transition was far greater than that of the other two transitions occurring at the higher temperatures. The transitions T_1 and T_2 were observed at the temperatures indicated in Tables V and VI when repeated scans were made in the cooling mode. They were also observed in the heating mode after repeated scans but the transition temperatures (T_1 and T_2) were of slightly different value than those reported for the cooling scans, due presumably to the change in the scanning mode. The transitions T_1 and T_2 were also repeatedly observed when control (reference) liver mitochondrial lipids were suspended in aqueous buffers at pH 10.0 and 4.0, although slight differences were observed in the respective transition temperatures in comparison to the values obtained for the same samples in the pH 7.2 liposome buffer (results not shown). In those samples where T_3 was observed, it was usually only observed on the first cooling scan and was then lost on the second and subsequent cooling scans. Furthermore, in contrast to the T_1 and T_2 transitions, the temperature at which the T_3 transition was evident was highly variable, although the enthalpy was of similar magnitude to the T_2 exothermic transition in the various dietary groups of animals investigated.

The major effect of the dietary lipid supplementation was on the transition temperature of T_1. For the liver mitochondrial lipids, this transition was 2.9 C deg. lower in the sunflower seed oil diet animals and 2.2 C deg. higher in the sheep kidney fat diet animals in comparison to the animals on the reference diet. No significant effect of the

TABLE V

TRANSITION TEMPERATURES AND ENTHALPY VALUES FOR LIVER MITOCHONDRIAL LIPIDS FROM RATS FED VARIOUS LIPID-SUPPLEMENTED DIETS FOR 16 WEEKS

Exothermic phase transitions were determined for the lipids on $n = 6$ separate rat liver mitochondrial preparations. Data are presented as the mean ± S.E. The enthalpy (ΔH) value is expressed as cal/g dry weight of lipid, and for ΔH_1 these values have been determined by extrapolation of the cooling scan to the baseline. The number of times the transition T_3 was observed in the six samples tested for each dietary treatment is indicated in brackets. Differences in the transition temperature of T_1 were significant at $P < 0.001$ when comparing reference vs. sheep kidney fat; reference vs. sunflower seed oil and sheep kidney fat vs. sunflower seed oil groups. Similar comparisons for ΔH_1, T_2 and ΔH_2 were not significantly different.

Exotherm		Diet		
		Reference	Sheep kidney fat	Sunflower seed oil
T_1	°C	0 ± 0.3	2.2 ±0.3	−2.9 ±0.6
	ΔH_1	−1.74±0.18	−1.95±0.13	−1.87±0.09
T_2	°C	14.2 ±0.3	15.6 ±1.1	15.0 ±1.0
	ΔH_2	−0.08±0.01	−0.08±0.02	−0.10±0.01
T_3	°C	34.1 ±4.2(4)	38.2(2)	38.2 ±4.2(6)
	ΔH_3	−0.08±0.01	−0.06	−0.10±0.02

dietary lipid treatments on the transition T_2 or on the enthalpy values, ΔH_1 and ΔH_2, was observed. In addition, there did not appear to be any dietary lipid effect on the T_3 exothermic transition (Table V). The effects of the dietary lipid treatment on the thermotropic properties of heart mitochondrial lipids were slightly different from those on liver mitochondria with regard to the following. Firstly, the sunflower seed oil dietary lipid treatment did not reduce the T_1 transition temperature in com-

TABLE VI

TRANSITION TEMPERATURES AND ENTHALPY VALUES FOR HEART MITOCHONDRIAL LIPIDS FROM RATS FED VARIOUS LIPID-SUPPLEMENTED DIETS FOR 16 WEEKS

Exothermic phase transitions were determined for the lipids on $n = 4$ samples, with each sample comprising mitochondria isolated from eight rat hearts from each dietary group. Data are as described in Table V. The number of times the transition T_3 was observed in the four samples tested for each dietary treatment is indicated in brackets. Differences in the transition temperature of T_1 were significant for the following comparisons, reference, vs. sheep kidney fat ($P < 0.01$), sheep kidney fat vs. sunflower seed oil ($P < 0.02$). Differences in T_1 between reference and sunflower seed oil were not significant. Differences in the enthalpy value, ΔH_1, were significant at $P < 0.01$ for comparisons of reference vs. sheep kidney fat and sheep kidney fat vs. sunflower seed oil. Differences in ΔH_1 between reference and sunflower seed oil groups were not significant. Similar comparisons for T_2 and ΔH_2 were not significantly different. n.o., not observed.

Exotherm		Diet		
		Reference	Sheep kidney fat	Sunflower seed oil
T_1	°C	3.9 ±0.09	4.7 ±0.15	3.1 ±0.43
	ΔH_1	−1.52±0.06	−1.97±0.06	−1.48±0.06
T_2	°C	14.1 ±0.4	15.9 ±1.33	13.9 ±1.23
	ΔH_1	−0.06±0.01	−0.06±0.01	−0.05±0.01
T_3	°C	n.o.	35.5 ±4.0(3)	41.8 ±3.1(3)
	ΔH_1	n.o.	−0.15±0.05	−0.16±0.01

parison to the reference group. Secondly, the ΔH_1 for the sheep kidney fat group was significantly higher than for the other two dietary groups. Thirdly, the transition temperature of T_2 did appear to be higher than for the other two dietary groups, although this was not statistically significant in the number of samples tested. Fourthly, the frequency for the occurrence of the T_3 exothermic transition did appear to be related to the nature of the dietary lipid treatment in that the transition T_3 was not observed in heart mitochondrial lipids from the reference group of animals under the experimental conditions employed.

Discussion

The various dietary lipid supplements used in this study resulted in significant changes in the fatty acid composition of both liver and heart mitochondria, and these changes in composition influenced the physical properties of the lipids as determined by DSC. These changes in the mitochondrial lipids would be reflecting events occurring in the mitochondrial membrane phospholipids, as it has been reported that more than 95% of the fatty acids found in isolated mitochondrial membranes are contained within the phospholipid fraction [33]. In addition, we have observed that the fatty acid profiles of both rat liver and heart mitochondrial phospholipids are virtually identical to those of the mitochondrial total lipids after similar dietary lipid treatments (unpublished results).

The thermotropic behaviour of the mitochondrial lipids indicates that certain lipids can undergo a phase transition independently of other lipids. As this phenomenon is observed in the isolated lipids of the mitochondria, it eleminates the possibility that the transitions arise from denatured proteins or as some consequence of lipid-protein interactions. The transitions labelled T_1 and T_2 were observed repeatedly at their respective temperatures in both cooling and heating modes for all samples of liver or heart mitochondrial lipids examined, and they were observed in the absence of any added divalent cations. For liver mitochondrial lipids, at least, they were observed at pH values for the liposome buffer system which ranged from 4.0 to 10.0. They therefore most likely represent various gel $\rightleftarrows$ liquid-crystalline phase transitions rather than any form of $L_\alpha \rightleftarrows H_{II}$ transition.

On the basis of the enthalpy values, the transition T_2 comprises a much smaller proportion of the lipid than transition T_1. For liver mitochondria, the enthalpy value is about 18-times greater for the transition T_1 in comparison to transition T_2, whilst for heart mitochondrial lipids, the enthalpy of the transition T_1 is about 28-times greater than that of transition T_2. The phase transition T_2 may represent the formation of the solidus phase of some higher melting-point lipids. On further cooling, the bulk of the lipids which were not already part of the solidus phase lipid domain formed from the transition T_2, would then undergo solidus formation at lower temperatures. In this respect, the phase transitions designated T_2, which begin at temperatures between 14 and 16°C, are similar to those phase transitions which have been recently observed in membrane lipids prepared from rat liver mitochondria [14] and bovine heart submitochondrial particles [17]. The T_1 phase transitions occurring in the mitochondrial lipids resemble those previously reported for other mammalian mitochondrial membranes [12,34,35], where it is considered that they represent at the molecular level a reversible order-disorder change in the acyl fatty acids within the membrane [3–5]. In contrast, the T_3 transition appears to be quite different in its thermal behaviour to the T_1 and T_2 phase transitions. The T_3 transition was not observed in all samples, nor was it observed with repeated scans. We have recently shown that its thermal behaviour is markedly dependent on the pH of the liposome buffer, a property not shared by the other two transitions but shared by the $L_\alpha \rightleftarrows H_{II}$ phase transition of egg-yolk phosphatidylethanolamine [26]. On this basis, it is possible that the T_3 transition may represent an $L_\alpha \rightleftarrows H_{II}$ lipid phase transition as reported by others [24–26], but its identification in these experiments remains uncertain.

The temperature at which the phase transitions occur in both rat liver and heart mitochondrial lipids is influenced by the nature of the dietary lipid intake. Although the dietary-induced changes in the fatty acid profile involved changes in the level of certain saturated and unsaturated fatty

acids, there was, however, little change in the overall percentage level of saturated and unsaturated fatty acids. This observation, particularly for the saturated fatty acids, may indicate the need to maintain relatively constant levels of saturated fatty acids in the membrane despite large variations in the dietary intake of saturated fatty acids. The most prominent change in the membrane fatty acid composition was in relation to the $\omega 6$ to $\omega 3$ unsaturated fatty acid ratio. For both liver and heart mitochondria, the sunflower seed oil diet increased the value of this ratio, whilst the opposite was the case for the sheep kidney fat diet. Changes in this ratio would reflect changes in the flux of the various unsaturated fatty acids through their particular conversion pathways. The observation that an elevation of some of the unsaturated fatty acids of one particular series was accompanied by a reduction in some of the fatty acids of the alternate unsaturated fatty acid series, would suggest that regulation of the various desaturase activities was responsible in part for the change in the types of unsaturated fatty acid present, as has previously been suggested [36,37]. For both tissues, changes in the levels of the $\omega 3$ unsaturated fatty acids between the two lipid-supplemented diets were brought about principally by changes in the proportion of docosahexaenoic acid. The changes in the levels of the various $\omega 6$ unsaturated fatty acids as a result of the two lipid-supplemented diets were far more tissue-specific, as has been discussed before [38]. Thus, the major effect of the two dietary lipid treatments was on the type, rather than on the overall amount, of unsaturated fatty acids present in the mitochondrial membrane. These results are similar to the diet-induced modifications recently observed in the fatty acid composition of rat liver and heart mitochondrial membranes by Tahin et al. [37].

In terms of the thermotropic behaviour of the mitochondrial lipids, the major effect of the diet-induced lipid changes was on the transition temperature of transition T_1, which for liver was inversely proportional to the value of the $\omega 6/\omega 3$ unsaturated fatty acid ratio. The transition temperature of T_2 was not significantly altered, despite variation in both the temperature of T_1 and the $\omega 6/\omega 3$ unsaturated fatty acid ratio. For heart mitochondria the effect of altered lipid composition on the transition temperature of T_1 was not as clear as it was for liver mitochondria. In general however, the altered transition temperature of T_1 accompanying the changed $\omega 6/\omega 3$ fatty acid ratio could indicate a possible change in the fluidity of the mitochondrial lipids, with the sunflower seed oil diet increasing and the sheep kidney fat diet decreasing the fluidity, respectively. As liver mitochondria do not contain plasmalogens [32] and the content of the dimethyl acetal derivatives produced during methylation of heart mitochondrial lipids was not significantly affected in these or other similar dietary lipid experiments [39], it is unlikely that the shifts which were observed in the transition temperatures were the result of changes in the thermal behaviour of certain plasmalogens.

Of major significance in this study is the possible relationship of these lipid phase transitions (i.e., T_1 and T_2) to the thermal behaviour of various mitochondrial-membrane-associated enzymes. This is particularly so with regard to the effect of changes in membrane lipid composition on the temperature-activity profiles of such enzymes as a result of dietary lipid treatment. Changes in the slope ('breaks') in Arrhenius plots have been observed for a variety of membrane-associated enzymes [5] and in particular for mammalian mitochondrial membrane enzymes such as succinate oxidase [19–21]. In addition, the motional characteristics of infused fatty acid spin labels also show marked temperature dependence, which has been considered the result of some form of lipid phase transition in the host membrane lipids [19,20,40]. Although the presence of a small domain of higher melting point lipids in the mitochondrial membrane may be responsible for changes in the thermotropic behaviour of certain mitochondrial membrane-associated enzymes via the influence of the phase transition T_2, at this stage the correlation is uncertain. We have observed significant changes in the Arrhenius profile and critical temperature of rat liver [23] and heart [41] mitochondrial succinate cytochrome *c* reductase after identical dietary lipid treatment, yet, in this parallel study on isolated liver mitochondrial lipids, no significant change in the phase transition temperature of T_2 was observed by DSC, as a result of the dietary lipid treatment. Furthermore,

the DSC-determined phase transition temperatures were not in agreement with the critical temperatures at which changes in the Arrhenius plot of succinate cytochrome *c* reductase were observed, although lipid extracts, rather than native membranes, were the subject of this study. In view of these findings, it is possible that the dietary-induced alterations in the Arrhenius critical temperature, reported for rat liver mitochondrial succinate oxidase [21] and succinate cytochrome *c* reductase [23], may be reflecting a greater sensitivity of these particular enzyme systems to critical changes in membrane lipid viscosity, as suggested by Aloia [42], rather than to the presence of lipid phase transitions of the type reported in this present study.

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