

Dynamic modulation of mitochondrial membrane physical properties and ATPase activity by diet lipid

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A longitudinal cross-over feeding design was used to investigate the relationship of dietary lipid composition to the membrane lipid environment and activity of mitochondrial ATPase *in vivo*. Rats were fed a polyunsaturated fatty-acid-rich oil (soya-bean oil) for 12 days, crossed-over to a monounsaturated fatty-acid-rich oil (rapeseed oil) for the next 11 days, then returned to soya-bean oil for 11 more days. Additional rats were fed either soya-bean oil or rapeseed oil throughout. Rats fed rapeseed oil had lower rates of ATPase-catalysed ATP/[³²P]P_i exchange than rats fed soya-bean oil. Arrhenius plots showed higher transition temperature (T_t) and activation energy (E_a) for rats fed rapeseed oil. Switching from soya-bean oil to rapeseed oil was dynamically followed by changes in the thermotropic and kinetic properties of the mitochondrial ATPase exchange reaction. Returning to soya-bean oil reversed these changes. The rapid and reversible modulation of T_t caused by a change of the type of fat ingested suggests that membrane physicochemical properties are not under rigid intrinsic control but are continually modified by the profile of exogenously derived fatty acids. The studies suggest that *in vivo* the activity of mitochondrial ATPase is in part determined by dietary lipid via its influence on the microenvironment of the enzyme. The rapidity and ready reversibility of changes observed for this subcellular-membrane-bound enzyme suggest that dietary fatty-acid balance may be an important determinant of other membrane functions in the body.

Studies of the interaction between protein and lipid components of biological membranes have indicated that membrane-associated proteins may be arranged peripherally, anchored at the membrane surface, or integral, penetrating into or through the structural matrix of the lipid bilayer (Singer & Nicolson, 1972). It is logical to suppose that activity of membrane-associated enzymes may be dependent on association with specific polar head groups at the membrane surface, may be modulated by the surrounding bilayer matrix determined by fatty-acyl content and/or phospholipid distribution, or, more specifically, may be dependent on interaction with specific fatty acyl chains at the protein-lipid interface.

Specific phospholipids are required before membrane-bound enzymes can express full activity (Sanderman, 1978). In addition, the transversal asymmetric distribution of phospholipids (Crain & Marinetti, 1979; Krebs *et al.*, 1979; Op den Kamp, 1979) further implies a specific purpose for phospholipid orientation. Demonstration of the import-

ance of the fatty acid milieu surrounding integral membrane proteins has been achieved through correlation of change in enzyme activity with change in membrane fatty-acid composition (Bloj *et al.*, 1973; Haeffner & Privett, 1975; Gidwitz *et al.*, 1980) and through differences reported in membrane physical properties defined by temperature of lipid phase transition (T_t) together with differences in Arrhenius activation energy (E_a) of enzymes from membranes with differing fatty-acid distributions (Cronan & Gelman, 1975; Solomonson *et al.*, 1976; Im *et al.*, 1979; McMurchie & Raison, 1979). Differences exist in the T_t reported for different enzymes associated with a given membrane (Fourcans & Jain, 1974; Im *et al.*, 1979; Nohl, 1979), thus implying dissimilarity among the annular lipid microenvironment of enzymes within one membrane. Together the foregoing facts suggest a specificity not only for phospholipid arrangement, but also for fatty-acyl-chain placement within the proximity of membrane proteins.

In artificial membranes T_t decreases as the degree

of membrane unsaturation increases (Cronan & Gelman, 1975; Lee, 1977), indicating a fluidizing effect of unsaturated fatty-acyl chains. Phase changes in membrane lipid are accompanied by marked changes in the E_a of lipid-dependent membrane-bound enzymes (Raison *et al.*, 1971; Raison & McMurchie, 1974; Solomonson *et al.*, 1976; McMurchie & Raison, 1979; Parenti-Castelli *et al.*, 1979). Above the T_t value, lipids are considered to be in a fluid, liquid-crystalline phase and E_a is decreased. Conversely, below the T_t value, lipids are in a more solid, or gel-like phase and E_a is increased. These findings have been interpreted (McMurchie & Raison, 1979; Silvius & McElhaney, 1980) to imply that more fluid environments permit greater ease of mobility to the enzyme for attainment of optimal conformation for catalytic activity, thus producing, and reflected in, a lower reaction enthalpy. In contrast, a more saturated or gel-state lipid would hinder transient movement and thus increase E_a .

We have previously demonstrated (Innis & Clandinin, 1981) that the composition of mitochondrial inner-membrane lipids of rat myocardium can be dynamically controlled by dietary fat. Phospholipid fatty-acyl components were rapidly altered by switching from a diet rich in polyunsaturated fatty acids to a monounsaturated-rich diet and in a dynamic state, continually susceptible to modulation by diet fat. The present report describes studies to determine whether diet-induced changes in membrane lipid composition are paralleled by altered membrane physical properties, defined as T_t from Arrhenius plots, and changes in kinetic activity and E_a of mitochondrial ATPase (EC 3.6.1.3). Mitochondrial ATPase is the terminal transphosphorylating enzyme of oxidative phosphorylation (Senior, 1979). Conformational change in this protein has been proposed as an essential part of its catalytic activity (Senior, 1979). Thus diet-induced changes in the lipid microenvironment might be expected to result in modulation of the enzyme configurational mobility and hence alter kinetic behaviour. In the present paper the isotopic exchange reaction of ATP/[^{32}P]P_i, which is dependent on phospholipids and is thought to be a partial reaction of ATP synthesis (Senior, 1979), was studied. The results clearly establish *in vivo* that dietary lipid composition can dynamically affect alterations in membrane physical properties and in the kinetic activity of membrane-bound mitochondrial ATPase in a rapid and reversible manner that parallels diet-induced change in membrane lipid composition.

Materials and methods

Animals and diets

Two replicates of male Sprague-Dawley rats (Biobreeding Laboratories, Ottawa, Que., Canada)

weighing 55–60 g were fed diets containing 20% (w/w) soya-bean oil or rapeseed oil (Innis & Clandinin, 1980) in a longitudinal design previously described (Innis & Clandinin, 1981). The fatty-acid composition of the oils has also been described (Innis & Clandinin, 1981). To ensure adequate sample size each group contained eight rats on day 0, four rats on days 2, 5, 8, 12, 23 and 34, and three rats on days 17, 28 and 61. In addition, two replicates of rats were fed the diet containing soya-bean oil for 12 days, switched to the diet containing rapeseed oil for the next 11 days, then returned to the diet containing soya-bean oil for 11 more days. Of the latter, two groups of rats were killed by decapitation on days 14, 17, 20, 23 (rapeseed oil cross-over period) and on days 25, 28, 31, 34 and 61 (soya-bean oil cross-over period) from the start of dietary treatment.

Preparation of mitochondria

Rat hearts were quickly excised, and mitochondria were prepared by the established procedures previously described (Clandinin, 1978), except that heparin was omitted at all stages. Protein was measured by a colorimetric method (Lowry *et al.*, 1951).

ATP/[^{32}P]P_i exchange assays

The mitochondrial ATPase ATP/[^{32}P]P_i exchange reaction was measured as described by Pullman (1967), except that the reaction mixture contained 20 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH 7.4), 10 mM-MgSO₄, 0.2 M-sucrose, 0.4% (w/v) bovine serum albumin and 20 mM-KH₂PO₄ (pH 7.4) containing 10⁶ c.p.m. of [^{32}P]P_i (New England Nuclear, Lachine, Que., Canada) and 0.5 mg of mitochondrial protein in a final volume of 1.0 ml. The reaction was initiated with ATP (Sigma Chemical Co., St. Louis, MO, U.S.A.) provided at concentrations as indicated in the appropriate Figures and conducted at the temperatures indicated in the Results section. For temperatures below ambient, reactions were carried out in a Haake E1 refrigerated water bath and circulator (Fisher Scientific, Toronto, Ont., Canada). The reaction was stopped at the end of 5 min by the addition of 0.1 ml of 35% HClO₄ and the mixture was centrifuged in a clinical centrifuge at 3000 rev./min for 10 min. Deproteinized supernatant (0.2 ml) was extracted exhaustively as described by Pullman (1967) to remove [^{32}P]P_i and 2 ml of the remaining organic phase counted for incorporation of [^{32}P]P_i into adenine nucleotides. Samples were counted in plastic vials by Čerenkov counting in a liquid-scintillation spectrometer (Unilux II; Nuclear Chicago). Paper chromatography of 0.1 ml of deproteinized reaction mixture using a solvent

system of isobutyric acid/conc. NH_3/water (66:1:33, by vol.) was routinely performed to establish exchange of label from ATP to ADP and AMP.

Results

Oligomycin is known to inhibit membrane-bound mitochondrial ATPase through binding to the membrane sector of the enzyme (Senior, 1979). Consequently, this antibiotic at concentrations of $14 \mu\text{g}$ of oligomycin/mg of protein was used to establish that more than 93% of the $\text{ATP}/[^{32}\text{P}]\text{P}_i$ exchange reaction measured in the present study represented mitochondrial ATPase activity. Rates of

exchange in the absence of exogenous ATP were less than 5% of those in the presence of ATP and were similar among different diet treatments. $\text{ATP}/[^{32}\text{P}]\text{P}_i$ exchange rates found here are similar to those previously reported for rat liver mitochondria (Sandoval *et al.*, 1970).

The Arrhenius plots of $\text{ATP}/[^{32}\text{P}]\text{P}_i$ exchange reaction at ATP concentrations of 10 mM (Fig. 1) show a biphasic plot of two intersecting straight lines over the temperature range 3–30°C. These plots are analogous to those of other studies of thermotropic properties of membrane-bound mitochondrial ATPase activity (Lenaz *et al.*, 1978; Parenti-Castelli *et al.*, 1979; Nohl, 1979; Rottenberg *et al.*, 1980)

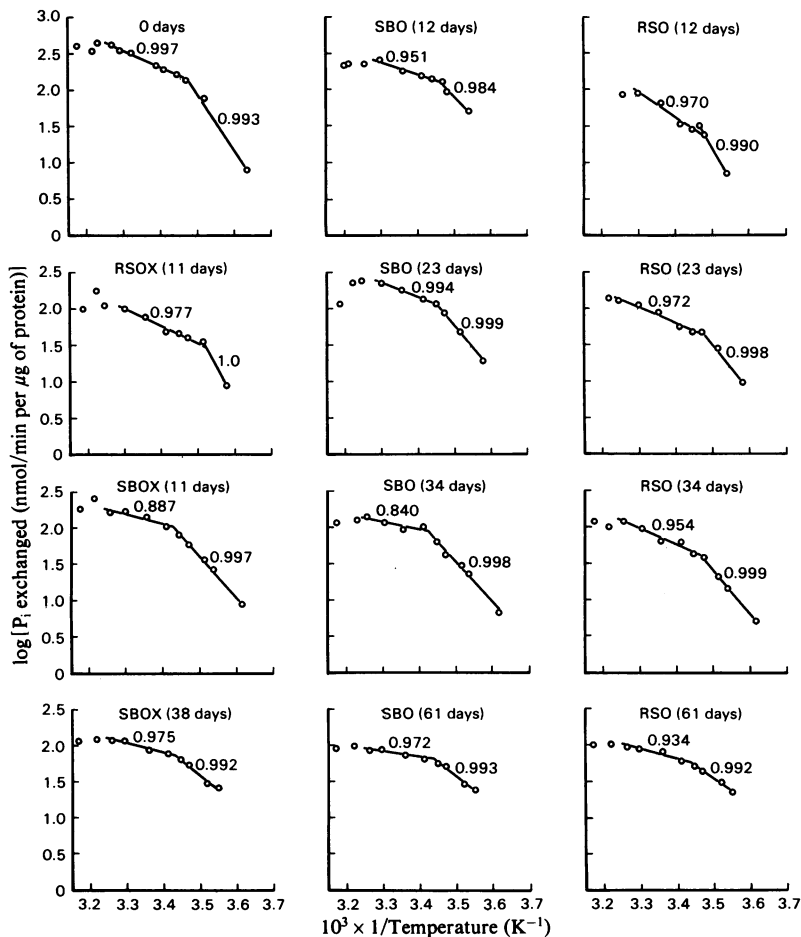


Fig. 1. Arrhenius plots showing dynamic changes in thermotropic behaviour of $\text{ATP}/[^{32}\text{P}]\text{P}_i$ exchange activity induced by diet

Mitochondria were prepared from hearts of rats fed SBO (soya-bean oil), RSO (rapeseed oil), RSOX (crossed to RSO days 12–23 after 12 days SBO) or SBOX (crossed to SBO after 11 days RSOX). The concentration of ATP in the reaction mixture was 10 mM. Each point represents the mean of two groups of rats per diet treatment. Two separate assays were performed on each group. The data were analysed by the method of least squares, and straight lines were fitted. Regression coefficients are given on the line. Transition temperatures and energies of activation calculated from these slopes are given in Table 1.

Table 1. *Energies of activation and transition temperatures for ATP/[³²P]P_i exchange activity of mitochondria from rats given different dietary fat treatments*

Values given are calculated from Arrhenius plots shown in Fig. 1. Values given are as follows: E_{a1} , energy of activation below transition temperature; E_{a2} , energy of activation above transition temperature; T_t , transition temperature. The dietary treatment is indicated as follows: SBO, rats fed diet containing soya-bean oil; RSO, rats fed diet containing rapeseed oil; RSOX, rats switched to RSO after 12 days SBO; SBOX, rats switched to SBO after 11 days RSOX.

Diet treatment	Duration (days)	E_{a1} (J/mol per °C)	E_{a2} (J/mol per °C)	T_t (°C)
O	0	146.4	43.5	14.8
SBO	12	99.2	30.5	16.1
SBO	23	113.8	21.8	16.8
SBO	34	105.9	19.2	19.0
SBO	61	72.8	16.7	17.8
RSO	12	169.5	64.4	14.3
RSO	23	121.3	51.9	14.8
RSO	34	113.8	42.7	15.4
RSO	61	65.3	27.6	18.1
RSOX	11	168.6	45.6	11.3
SBOX	11	107.1	34.7	18.6
SBOX	38	77.8	26.4	17.9

and other enzymes dependent on phospholipid association for full activity (Raison & McMurchie, 1974; Solomonson *et al.*, 1976; Im *et al.*, 1979; McMurchie & Raison, 1979). The presence of a second discontinuity approx. 15°C above the first is also evident in all Arrhenius plots (Fig. 1). The occurrence of a second break point in thermotropic analyses of mitochondrial ATPase activity has been reported (Rottenberg *et al.*, 1980). In the present study insufficient determinations were conducted at higher temperatures to enable accurate calculation of the higher break point. As established for mitochondrial ATPase (Lenaz *et al.*, 1978; Parenti-Castelli *et al.*, 1979; Nohl, 1979; Rottenberg *et al.*, 1980) and other membrane-bound enzymes (Raison & McMurchie, 1974; Solomonson *et al.*, 1976; Im *et al.*, 1979; McMurchie & Raison, 1979; Nohl, 1979) showing characteristic breaks in Arrhenius plots, E_a decreased as the temperature increased above the value T_t value (Fig. 1, Table 1).

Diet effects on thermotropic activity of ATP/[³²P]P_i exchange

The nature of the dietary fat fed influenced both E_a and T_t values (Fig. 1, Table 1). For rats fed soya-bean oil the abrupt change in E_a causing the Arrhenius plot break occurred at a higher temperature than for rats fed rapeseed oil for 12, 23 or 34 days. At the same time E_a , particularly above the T_t value, was lower in rats fed soya-bean oil. Since T_t is dependent on the lipid environment of the enzyme (Cronan & Gelman, 1975) these results clearly establish that the fatty-acid composition of the dietary lipid can influence membrane physico-chemical properties *in vivo*. The thermotropic

activity of the ATP/[³²P]P_i exchange reaction was dynamically manipulated by altering the diet fat. Switching from the polyunsaturated fatty-acid-rich soya-bean oil diet to the monounsaturated fatty-acid-rich rapeseed oil diet resulted in a decrease in T_t and an increase in E_a both below and above the T_t value (Fig. 1, Table 1). By returning to soya-bean oil the T_t value increased and the E_a fell. Thus changes in dietary lipid can rapidly and reversibly influence the thermotropic properties of ATPase bound to the mitochondrial inner membrane, presumably via the modulation of membrane lipid composition, which accompany shifts in dietary fat (Innis & Clandinin, 1981). At the end of the experimental period (61 days) little difference in T_t or E_a values occurred among rats given the three dietary treatments (Fig. 2).

Developmental changes in T_t and E_a values for the rate of ATP/[³²P]P_i exchange reaction is evident in rats fed soya-bean oil or rapeseed oil. From weaning (day 0) to 61 days post-weaning the T_t value increased and E_a , above and below T_t , fell in both rats fed soya-bean oil and rats fed rapeseed oil (Table 1). We have previously reported that definite changes in the fatty-acyl composition of mitochondrial inner-membrane phospholipids occur with maturation (Innis & Clandinin, 1981). For example, the percentage C_{18:2ω6} content of cardiolipin and the percentage C_{22:6ω3} content of phosphatidylethanolamine rises over this time period irrespective of whether a soya-bean oil or rapeseed oil diet is fed. Similarly, distinct changes in the phospholipid polar head group profile of rat heart and liver mitochondria (Hallman & Kankare, 1979) and rabbit heart mitochondria (Nagatomo *et al.*, 1980) occur with increase in age. It is apparent that growth-related

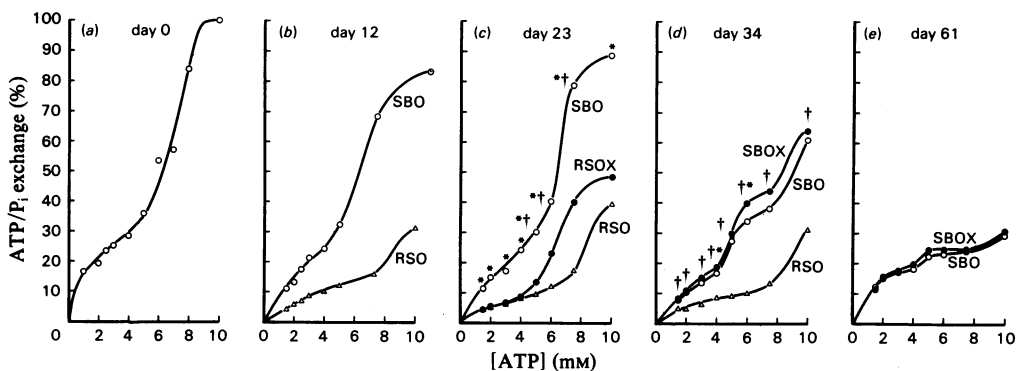


Fig. 2. Dynamic alteration in ATP/[^{32}P]P_i exchange activity induced by diet

Reactions were performed at 30°C. Heart mitochondria were prepared for: 0 days, rats killed before dietary treatment; SBO, rats fed soya-bean oil; RSO, rats fed rapeseed oil. RSOX, rats crossed to RSO after 12 days SBO; SBOX, rats crossed to SBO after 11 days RSOX. Each point represents the mean for two groups of rats per diet treatment (two separate assays were performed on each group). *, RSOX or SBOX statistically significantly different from SBO; †, RSOX or SBOX significantly different from RSO ($P < 0.05$). Reaction velocity is expressed as a percentage of the maximal velocity observed before dietary treatment was initiated (day 0) and is plotted against substrate concentration. The day 0 velocity was $324.8 \pm 58.3 \mu\text{mol}$ of P_i/mg of protein. All SBO and RSO means were significantly different from each other ($P < 0.05$). The s.e.m. of all values after day 0 was less than $\pm 13.5\%$ of the mean.

changes in thermotropic properties of mitochondrial ATPase observed here are at least partly a consequence of developmentally controlled changes in the lipid environment of the enzyme.

Diet effects on ATP/[^{32}P]P_i exchange activity

Assay of the ATP/[^{32}P]P_i exchange reaction clearly indicated that the type of oil fed influenced the rate of ATP/[^{32}P]P_i exchange by ATPase of heart mitochondria (Fig. 2). After 12, 23 or 34 days of diet treatment, for all substrate concentrations used, the rate of ATP/[^{32}P]P_i exchange by rats fed rapeseed oil was significantly lower than rats fed soya-bean oil. By switching rats from soya-bean oil to rapeseed oil (Fig. 2c) rates of ATP/[^{32}P]P_i exchange were significantly decreased from that of rats maintained on soya-bean oil to a value similar, at five of eight substrate concentrations used, to that of rats maintained on rapeseed oil. By returning these rats from rapeseed oil to soya-bean oil (Fig. 2d) ATP/[^{32}P]P_i exchange was significantly increased to a rate equal to or higher than that of rats fed soya-bean oil throughout. After continuing diet treatment for an additional month the exchange rate curves for the latter two groups were identical (Fig. 2d). Incomplete data were obtained for rats fed rapeseed oil for 61 days, but since the rate of ATP/[^{32}P]P_i exchange for these animals with a substrate concentration of 10 mM-ATP (Fig. 3b) and the Arrhenius plot behaviour (Fig. 1, Table 1) was similar to the other two treatment groups, it is apparent that no difference in exchange activity of

mitochondrial ATPase existed among the three diet treatments after 61 days of feeding. The similarity between rats fed rapeseed oil and rats fed soya-bean oil after 61 days of dietary treatment may be the result of the induced peroxisomal metabolism of long-chain monoenoic fatty acids that is known to occur in animals fed rapeseed oil (Christiansen *et al.*, 1979; Norseth *et al.*, 1979). This induction of compensatory extramitochondrial β -oxidation of dietary long-chain monoenoic fatty acids would alter the substrates available for membrane incorporation.

Rates of ATP/[^{32}P]P_i exchange declined with increasing age (Fig. 2). The maximum exchange rate achieved at 2 months post-weaning was only 30% of the rate observed in weanling animals.

The rate of ATP/[^{32}P]P_i exchange by heart mitochondria when the ATP concentration in the reaction mixture was either well below V_{max} (5) or close to V_{max} (10) clearly indicated the influence of dietary lipid treatment on the catalytic activity of mitochondrial ATPase (Fig. 3). Rats fed rapeseed oil exhibited lower rates of ATP/[^{32}P]P_i exchange than rats fed soya-bean oil for both substrate concentrations illustrated. Substituting one diet lipid source for another was dynamically followed by changes in this enzyme function. Thus, at 5 days after switching rats from soya-bean oil to rapeseed oil rates of exchange were similar to those of rats fed rapeseed oil throughout. By returning to soya-bean oil, rates of ATP/[^{32}P]P_i exchange had increased by day 8 to become similar to that of rats fed soya-bean oil from day 0. The return from rapeseed oil to

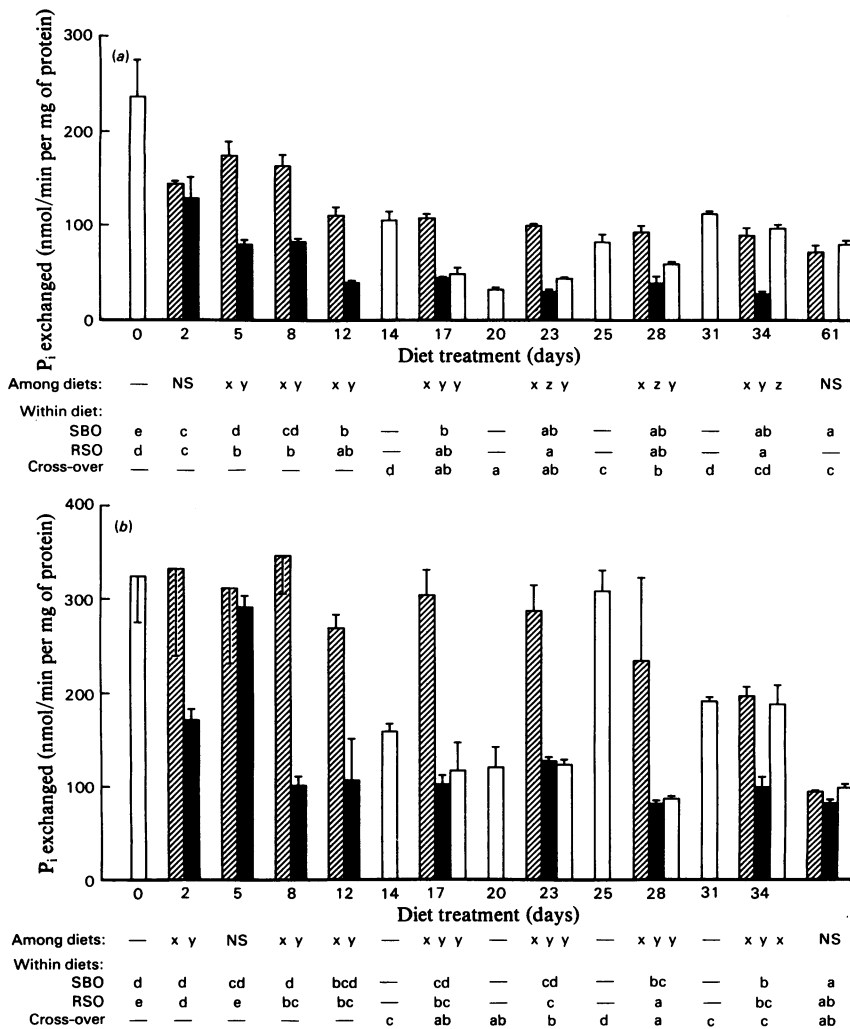


Fig. 3. *Dynamic effect of dietary fat treatment on rate of mitochondrial ATP/[³²P]P_i exchange*
 Values given are mean (\pm S.E.M.) rates of ATP/[³²P]P_i exchange (μ mol of P_i/mg of protein per min) with ATP concentration equal to (a) 5 mM and (b) 10 mM for rats fed soya-bean oil (SBO; hatched bars), rapeseed oil (RSO; solid bars) or cross-over (fed SBO days 0–12, RSO days 13–23, SBO days 24–61, open bars). Analysis of variance was performed for difference in exchange rate due to diet treatment x, y or z, for a given day, or due to length of feeding a, b, c, d or e for a given diet. Different indicators of significance indicate significance within days or within diets at the *P* < 0.05 level. Further abbreviation used: NS, not significant.

soya-bean oil resulted in a transient elevation in ATP/[³²P]P_i exchange activity after 2 days (day 25) at both 5 mM-ATP (Fig. 3a) and 10 mM-ATP (Fig. 3b) substrate concentration. The reason for this observation is not apparent but it is possible that increased activity of this enzyme is related to the higher food consumption observed when rats are switched from diets containing rapeseed oil to diets containing soya-bean oil. Overall, the data show a general decline in the rate of ATP/[³²P]P_i exchange activity of mitochondria from hearts of rats fed

either soya-bean oil or rapeseed oil with increase in age from 0 to 61 days post-weaning (Fig. 3).

Discussion

The present paper clearly establishes that the type of dietary fat fed has a significant controlling influence on the activity of oligomycin-sensitive ATPase of rat heart mitochondria. Rats fed the polyunsaturated fatty-acid-rich soya-bean oil exhibited higher rates of ATP/[³²P]P_i exchange, higher

T_t and lower E_a values in Arrhenius plots than rats fed the monounsaturated fatty-acid-rich rapeseed oil. By manipulating the dietary fat consumed, activity and thermotropic properties of the enzyme were altered. The effect of changing from one diet lipid source to another (soya-bean oil to rapeseed oil to soya-bean oil) was rapid and reversible.

In a previous report (Innis & Clandinin, 1981) we have demonstrated that the fatty-acyl compositions of phosphatidylcholine, phosphatidylethanolamine and cardiolipin isolated from the inner membrane of rat heart mitochondria rapidly and reversibly respond to flux in dietary fat composition. Employing an identical experimental design to that described herein, the dynamic nature and short half-lives of membrane fatty-acyl chains were demonstrated. Parallel changes in activity (Figs. 2 and 3) and thermotropic property (Fig. 1, Table 1) of mitochondrial ATPase, imply a cause-and-effect relationship among diet fat composition, membrane lipid composition and ATPase function. Therefore, it is logical to conclude that the physicochemical properties of the lipid microenvironment of mitochondrial ATPase was altered by dietary lipid. The reversible manipulation of the T_t value and reaction rate by changing the diet fat from soya-bean oil to rapeseed oil strengthens this conclusion. Consequently, we propose that the catalytic activity of mitochondrial ATPase is in part dependent on the diet fat consumed, defined as an effect produced via modulation of the lipid microenvironment of the enzyme. The present study supports the concept that intrinsic control over membrane lipid composition and/or physical property *in vivo* is not absolute.

Studies with artificial membranes have demonstrated that the T_t value of lipid-associated enzymes decreased as the degree of lipid unsaturation increased (Cronan & Gelman, 1975). The decrease in T_t value would imply a decrease in molecular ordering of fatty acids within the bilayer and thus an increase in fluidity. Based on the foregoing facts, the T_t value for rate of ATP/[32 P]P_i exchange by mitochondrial ATPase from rats fed the polyunsaturated-fatty-acid rich soya-bean oil might be expected to be lower than that for rats fed rapeseed oil. On the contrary, the results (Fig. 1, Table 1), consistently showed that feeding rapeseed oil produced a lowering of the T_t value. There are several possible explanations for these results. Rapeseed-oil feeding causes depletion of saturated fatty acids from mitochondrial phospholipids (Innis & Clandinin, 1981), which would depress T_t . This, however, cannot be the simple explanation because the effect of decreased membrane saturation would be counteracted by the increased monounsaturated-fatty-acid content concomitant to lowered polyenoic, particularly long chain ($\omega-3$ and $\omega-6$) fatty acids in mitochondrial phospholipids of rats fed

rapeseed oil. Further, mitochondrial inner-membrane phospholipids of rats killed before dietary treatment (day 0) contained a much higher proportion of saturated fatty acids, especially C₁₄ and C₁₆, than rats fed either soya-bean oil or rapeseed oil, whereas T_t for mitochondrial ATPase activity at day 0 was very low (Fig. 1, Table 1). T_t of membrane-bound enzymes is not solely dependent on the proportion of unsaturated to saturated bonds; the position of a C-C double bond also influences T_t (Lee, 1977). Mitochondrial membrane phospholipids from rats fed rapeseed oil are higher in ($\omega-9$)-series fatty acids and lower in ($\omega-6$) fatty acids than mitochondrial membrane phospholipids from rats fed soya-bean oil respectively (Innis & Clandinin, 1981).

In agreement with the increase in T_t caused by feeding a polyunsaturated-fatty-acid-rich diet observed in these studies, Solomonson *et al.* (1976) have reported that the T_t for (Na⁺ + K⁺)-dependent ATPase (EC 3.6.1.3) of plasma membranes from Ehrlich ascites tumour cells grown in mice fed diets containing 16% sunflower seed oil was 8°C lower than for mice fed diets containing 4% tristearoylglycerol as the only lipid source. These studies and others unequivocally establish that diet fat influences the activity of membrane-associated enzymes (Bloj *et al.*, 1973; McMurchie & Raison, 1979). It is also possible that analysis of total membrane lipid composition does not adequately reflect the composition of lipid associated with a particular protein. Thus the ability to correlate T_t to membrane fatty-acyl composition in some instances may be fortuitous. Indeed, the lipid-protein association required for maintenance or induction of appropriate protein conformation may demand specificity of fatty-acyl chain length and configuration around the irregular surfaces of the protein rather than a more general requirement for phospholipid and/or fatty acid combination giving appropriate fluidity to the protein milieu. In fact at homeothermic body temperature most membrane lipid is above T_t and in a liquid-crystalline phase.

The temperature at which membrane lipids undergo a change in phase is also influenced by the presence of cholesterol (Chapman *et al.*, 1979). No difference was present in the mitochondrial inner-membrane non-esterified cholesterol content relative to protein between rats fed soya-bean oil and rapeseed oil (Innis & Clandinin, 1980). Further, it has been suggested that cholesterol is excluded from the immediate environment of membrane proteins (Warren *et al.*, 1975; Hesketh *et al.*, 1976). Polar-head-group distribution is a further important determinant of T_t . The T_t value of phosphatidylethanolamine is 20°C higher than that of phosphatidylcholine with the same fatty acid composition (Lee, 1977). Thus in agreement with model

systems, rapeseed oil, which increased the phosphatidylcholine/phosphatidylethanolamine ratio (Innis & Clandinin, 1980), decreased T_t (Fig. 1, Table 1). An age-related increase occurred in T_t from Arrhenius plots of mitochondrial ATPase activity of rats fed soya-bean oil or rapeseed oil. Similar results have been reported by McMurchie & Raison (1979) for liver mitochondrial succinate oxidase activity. The mitochondrial pool of phosphatidylcholine in comparison with phosphatidylethanolamine is higher in newborn rat heart than 20-day-old rat heart (Hallman & Kankare, 1979), and in newborn versus adult rabbit heart (Nagotomo *et al.*, 1980). Thus, phospholipid polar-head-group distribution may be an important determinant of T_t *in vivo* that may change during development. The relationship of polar head groups to T_t is complex depending not only on the polar head group itself but also on the resultant surface charge and ionic interaction of the membrane (Lee, 1977). Changes in phospholipid species intimately associated with membrane enzymes and transport activities could have important regulatory implications. Potential questions arise as to whether or not the activity of membrane enzymes can be purposefully manipulated *in vivo* via the intimately bound phospholipids, by extrinsic dietary manipulation.

The appearance of a break in Arrhenius plots of mitochondrial ATP/[32 P]P_i exchange activity indicates that membrane lipid physical state influences E_a for this enzyme. Above the T_t value, when membrane lipids are in a liquid-crystalline state, E_a of mitochondrial ATPase is decreased (Table 1; Lenaz *et al.*, 1978; Parenti-Castelli *et al.*, 1979; Nohl, 1979; Rottenberg *et al.*, 1980). The tertiary structure or mobility of ATPase is, therefore, dependent on membrane lipid-phase condition. It is probable, therefore, that below T_t , where lipids are in a more gel-like, rigid phase, conformational mobility of the enzyme is restricted, thus producing an elevation in E_a .

E_a above and below the value T_t was lower in rats fed soya-bean oil than in rats fed rapeseed oil (Table 1). The mediation of these differences by diet lipid is unequivocally confirmed through the longitudinal cross-over manipulation. Since the composition of mitochondrial inner-membrane lipids of rats fed soya-bean oil is different from that of rats fed rapeseed oil and is dynamically modulated between soya-bean oil and rapeseed oil by diet change (Innis & Clandinin, 1981), it can be concluded that either the maintenance of appropriate conformation or the ease of mobility in catalytic activity is different between ATPase in the inner membrane of rats fed soya-bean oil and rats fed rapeseed oil. This conclusion is confirmed by the lower rates of ATP/[32 P]P_i isotopic exchange of rats fed rapeseed oil in comparison with soya-bean oil (Figs. 2 and 3).

Rates of ATP/[32 P]P_i exchange paralleled changes in E_a and T_t (Table 1, Figs. 1, 2 and 3) and membrane lipid compositional change (Innis & Clandinin, 1981) evoked by switching from soya-bean oil to rapeseed oil to soya-bean oil diets. The control of membrane lipid on the thermotropic behaviour and kinetic properties of mitochondrial ATPase *in vivo* appears complex, underlining potential limitations in extrapolation from readily interpreted results of more homogeneous model membrane systems to the situation *in vivo* where many intrinsic and extrinsic forces are interplayed.

The essentiality of phospholipid in ATP synthesis and ATP/[32 P]P_i exchange reactions by mitochondrial ATPase is not understood. The studies presented here suggest that specific lipid microenvironment rather than general fluidity may be more important to the function of this enzyme complex. The architecture of mitochondrial ATPase with a membrane sector linked to the globular F1 protein, which extends from the matrix side of the inner membrane, demand intimate association with not only the fatty-acyl components of the membrane interior but also with surface polar head groups. F1 protein undergoes conformational change as a result of changes in magnitude of hydrophobic bonding within the enzyme. Thus it has been proposed (Gomez-Puyou *et al.*, 1978) that any agent or condition that alters the magnitude of this bonding will also change the catalytic properties of the enzyme. Change could, therefore, result from diet-induced modification of either membrane fatty-acyl composition or phospholipid composition alone, or in combination, or might stem from changes in the net surface charge of the membrane due to altered polar-head-group distribution.

These studies have used a novel longitudinal cross-over feeding design to demonstrate *in vivo* that the influence of diet fat extends to the lipid microenvironment of oligomycin-sensitive ATPase, resulting in altered catalytic activity. The rapidity of diet effect and ease of reversal suggests that this may be a phenomena that is unique neither to this enzyme nor this subcellular membrane system. Conceivably many processes involving biological membranes are modified by diet-lipid conditioning of membrane lipid composition. It is evident that in any study of membrane-related processes attention must be paid to diet lipid preconditioning and developmental changes in membrane lipid.

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