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The Calcium Uptake of the Rat Heart Sarcoplasmic Reticulum is Altered by Dietary Lipid

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Summary. Small amounts of dietary n-3 fatty acids can have dramatic physiological effects, including the reduction of plasma triglycerides and an elevation of cellular eicosapentanoic (EPA) and docosahexanoic acids (DHA) at the expense of arachidonic acid (AA). We investigated the effects of alterations in the fatty acid compositions of cardiac sarcoplasmic reticulum (CSR) produced by dietary manipulation on the calcium pump protein that is required for energy dependent calcium transport. CSR was isolated from rats fed menhaden oil, which is rich in n-3 fatty acids, and from control animals that were given corn oil. Relative to control membranes, those isolated from rats fed menhaden oil, had a lower content of saturated phospholipids, an increased DHA/AA ratio, and an increased ratio of n-3 to n-6 fatty acids. These changes were associated with a 30% decrease in oxalate-facilitated, ATP-dependent calcium uptake and concomitant decreased Ca-ATPase activity in the membranes from the animals fed menhaden oil. In contrast, there was no alteration in active pump sites as measured by phosphoenzyme formation. Thus, the CSR Ca-ATPase function can be altered by dietary interventions that change the composition, and possibly structure, of the phospholipid membranes thereby affecting enzyme turnover.

Key Words sarcoplasmic reticulum · diet fatty acids · phospholipids · calcium adenosine triphosphatase · turnover

Introduction

The cardiac sarcoplasmic reticulum (CSR) is an important store of calcium for the activation of myocardial contraction. Relaxation occurs primarily by the energy-dependent reuptake of calcium by the CSR. The CSR Ca-ATPase (ATP phosphohydrolase 3.6.1.3) pumps calcium across a steep ion gradient energized by the hydrolysis of adenosine triphosphate (ATP) (Tada, Yamamoto & Tonomura, 1978). This protein, like other integral membrane transport proteins, may have altered activity when the membrane phospholipid milieu that surrounds it is changed. For example, the skeletal muscle Ca-ATPase activity is dependent upon membrane phospholipids. Specifically, alterations in phospholipid head groups (Martonosi, 1964) in phospholipid acyl chains (Fiehn & Hasselbach, 1970; Hidalgo, Petrucci & Vergara, 1982) and in the ratio of phospholipid to protein (Meissner & Fleischer, 1972; Hidalgo, de la Fuente & Gonzalez, 1986), alter calcium uptake or Ca-ATPase activity in vitro. In vitro experiments utilizing the purified cardiac pump protein, similar to those performed with the skeletal muscle pump protein, have not been reported. However, dietary manipulation of the CSR phospholipid has been accomplished, and alterations in CSR function have been associated with these phospholipid changes (Croset et al., 1989; Croset & Kinsella, 1989). Here we attempt to correlate these fatty acyl chains with CSR activity changes. In this paper, dietary manipulation was utilized to alter type and content of the acyl chains of rat CSR phospholipids and to alter the CSR activity. An examination of the mechanisms responsible for the increased calcium accumulation following corn oil supplementation and the decrease in CSR function produced by fish oil supplementation was made. The results strongly support (i) the concept that n-3 fatty acids are readily accumulated in CSR phospholipids, (ii) the lack of alteration in membrane permeability produced by such manipulation, and (iii) the importance of the interaction of the pump protein with surrounding phospholipid in governing enzyme turnover and, ultimately, enzyme activity.

Materials and Methods

Animals

Female Sprague-Dawley rats (200 g) were fed either 20% corn oil (CORN) or 17% menhaden oil (FISH) + 3% Corn Oil. The supplemented diets were purchased from ICN Biochemicals (Ir-

vine, CA) and kept at −20°C until used. Individual bags of FISH diet were sealed under nitrogen and changed every other day. FISH diet also included recommended antioxidants (Fritsche & Johnston, 1988).

Isolation of CSR

Rats were anesthesized with an 0.7 ml/kg intramuscular injection of a combination rodent anesthetic (acepromazine 1.4 mg/ml, ketamine 42.8 mg/ml, xylazine 8.6 mg/ml). The hearts were removed, cut open and rinsed in ice-cold buffer (25 mM imidazole, pH 7.0). All subsequent procedures were performed at 4°C. The hearts were rapidly trimmed of atria, right ventricles and connective tissue, then blotted and weighted. The whole left ventricle of the rat was finely minced with scissors in 15 volumes of ice-cold 25 mM imidazole, 200 μM phenylmethylsulfonyl fluoride (PMSF), 10 mM dithiothreitol (DTT; made fresh daily), pH 7.0 (Medium A). The tissue was homogenized with a motorized Teflon pestle (Thomas type BB) using 10 full passes at 600 rpm, taking care to homogenize along the sides of the tube, not on the bottom (Pagani & Solaro, 1984). After a one-minute period on ice, ten more passes were made. The homogenate was centrifuged at 500 × *g* for 10 min, then the supernatant was poured through one layer of cheesecloth into another chilled centrifuge tube and centrifuged at 15,000 × *g* for 10 min. The supernatant was decanted and centrifuged at 15,000 × *g* for 10 min. The resultant supernatant was removed carefully and centrifuged at 44,000 × *g* for 30 min. The supernatant was discarded, and the pellet was washed with a small amount of ice-cold Medium B (25 mM imidazole, 0.6 M KCl, 10 mM DTT, pH 7.0) which was then pipetted off. The pellet was resuspended in Medium B, using half the original volume of Medium A, via 10 passes of a glass Teflon homogenizer and centrifuged at 44,000 × *g* for 30 min. The supernatant was discarded and the pellet was washed with a small volume of Medium C (25 mM imidazole, 100 mM KCl, 10 mM DTT, pH 7.0) and then resuspended in 250 μl of the same buffer utilizing a Thomas-type homogenizer size O. All solutions were made fresh daily and kept ice cold. Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. All measures of activity were determined using freshly isolated vesicles.

Standard Assay Procedures

Sarcolemmal contamination was assayed by cholesterol content following thin layer chromatography using a commercially available kit [Boehringer Diagnostics] with a cholesterol standard purchased from Boehringer. Mitochondrial contamination was determined by cardiolipin content and was found to be less than 10% in the CSR preparations. Cholesterol content of the CSR preparations was <0.01 μmol/mg protein. Furthermore, there was a 30% reduction in cholesterol content, when CSR was compared to the initial heart homogenate.

ATPase Activity

ATPase activity was measured by continuously monitoring the oxidation of NADH at 340 nm with a linked enzyme system as previously described (Van Winkle et al., 1981). The reaction medium included 40 mM Tris maleate (pH 7.4), 100 mM KCl, 1 mM $MgCl_2$, 2 mM phosphoenolpyruvate, 400 μM NADH, 8.75 and 12.5 units/ml of pyruvate kinase and lactate dehydrogenase respectively, and 10 μg of SR protein/ml in a final volume of 2 ml. Sodium azide (10 mM) was included to inhibit any contaminating mitochondria. The reactions were started with 50 μM ATP (unless noted otherwise) after a 5-min equilibration period at 30°C. The difference between the rate measured in the presence of 40 μM $CaCl_2$ and the rate in the presence of 1 mM Tris-EGTA (Mg^{2+} dependent) was taken as the calcium-dependent activity.

Phosphoenzyme Content

Phosphoenzyme estimation at 0°C was carried out using (γ-^{32}P)ATP with the identical reaction mixture as that for the ATPase assay (Shigekawa, Finegan & Katz, 1976). Briefly, 25 μg CSR protein in 0.5 ml total reaction volume was incubated on ice. The reactions were started with 50 μM (γ-^{32}P)ATP (specific activity of 400 cpm/pmol) and quenched 10 sec later with 1 ml of ice-cold 10% trichloroacetic acid (TCA). Blanks contained no protein. After 15 min on ice, the quenched reaction mixture was filtered through a Whatman GF-F glass filter which had been soaked in distilled water. The test tube was washed with 3 × 8 ml of wash solution (ice-cold 0.114 M perchloric acid/0.06 M phosphoic acid). The glass filter was then washed with 100 ml of wash solution. The filter was placed in a vial, to which 10 ml of Ecolum was added as scintillant, and counted in an LKB Rackbeta.

Calcium Uptake Assay

Oxalate facilitated ATP-dependent calcium uptake was determined by the Millipore filtration method as previously described (Tate et al., 1985). The reaction mixture included 40 mM Tris maleate (pH 6.8), 100 mM KCl, 10 mM $MgCl_2$, 10 mM NaN_3, 5 mM potassium oxalate, in a total volume of 1 ml at 30°C. An ATP regenerating system (2 mM phosphoenolpyruvate, 4 units/ml pyruvate kinase) was included. Calcium (specific activity of 5,000–10,000 cpm/nmol) and EGTA were added to produce a free calcium concentration of approximately 4 μM. The reaction was initiated with 5 mM ATP and was terminated by filtration through 0.45 μm Millipore filters which were then washed with 5 ml of cold 40 mM Tris maleate (pH 6.8), 100 mM KCl. Nonspecific counts were determined by filtering aliquots removed immediately before the addition of ATP. The filters were placed in scintillation vials to which 10 ml of Ecolum was added and counted as above. Rates were derived over the linear portion of uptake (usually the first two minutes).

Phospholipid Determinations

Freshly isolated CSR (500 μg, approximately 100–200 μl) was placed into a screw capped tube, and 5 ml of chloroform/methanol, 2 : 1, was added. This mixture was vortexed and shaken for 5 min. The top, aqueous layer was removed to a separate tube and the organic layer was filtered through glass wool. This organic layer was taken to dryness under a stream of nitrogen. The aqueous layer and the glass wool were extracted with 5 ml of isopropanol/heptane, 1 : 1, made slightly acid by the addition of two drops of 1N HCl. Mixing was performed as above; the aqueous layer was discarded and the organic extract added to the chloroform/methanol residue. This mixture was taken to dryness under nitrogen.

The resulting residue was dissolved in 100 μl of 2 : 1 chloro-

form/methanol and 25 μl aliquots were spotted on 20 × 20 cm silica gel Type 60 plates (Merck) and separated using a solvent system of chloroform/methanol/NH_4OH/H_2O (70 : 30 : 4 : 1). The lipids were visualized in iodine vapor, scraped into acid-washed tubes and quantitated for phosphate (Rouser, Siakotos & Fleischer, 1966).

For plasmalogen determinations, the procedure was as above, except the plates were developed in two directions with exposure to HCl vapor for 15 min between dimensions (Horrocks, 1968). Plasmalogen content was taken as the amount of lysophosphatide appearing from the phospholipid following acid treatment.

Fatty Acyl Chain Determinations

Aliquots (25 μl) from the organic extracts as prepared under Phospholipid Determination, were mixed with 1 ml of methanolic base (Supelco, PA) and boiled for 3 min. After cooling, 1 ml of boron trifluoride (Supelco, PA) was added to each sample, and they were boiled for an additional 3 min. The samples were allowed to cool, then 10 ml of petroleum ether was added and the tubes were shaken vigorously for 2 min. The top, organic layers were removed and taken to dryness under a stream of nitrogen. The residues were dissolved in 50 μl of carbon disulfide and 5 μl portions were injected into an Antek 3000 GLC fitted with a 30 m Supelcowax 10 column (ID = 0.32 mM). The temperature was set at 180° and increased to 245° with a 2.5 degree/minute ramp.

Statistical Analysis

All comparisons were made using unpaired *t*-test. The level of statistical significance was chosen at 0.05.

Abbreviations

CSR, cardiac sarcoplasmic reticulum; Ca-ATPase, cardiac calcium + magnesium dependent adenosine triphosphatase; ATP, adenosine triphosphate; DTT, Dithiothreitol; TCA, trichloroacetic acid; NADH, nicotinamide adenine dinucleotide, reduced form; EP, phosphorylated enzyme; AA, arachidonic acid; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; DPA, docosapentanoic acid; Tris, Tris(hydroxymethyl)aminomethane; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; EGTA, ethyleneglycol bis tetraacetic acid.

Results

Animals

All animals were female Sprague-Dawley rats weighing 200 g at the time of random assignment to the two diets. There were no differences in the weight at time of sacrifice, 21 days later. There were no differences in heart weight from these groups of young animals; menhaden oil (FISH, N = 12) 0.71 ± .02 gm, corn oil (CORN, N = 11) 0.78 ± 0.06 gm.

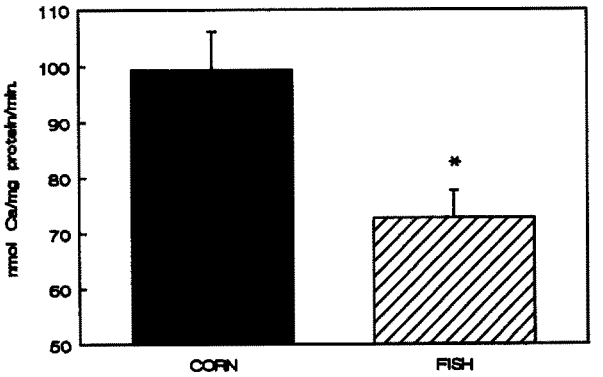

Figure. Dietary effect on calcium uptake. Oxalate-facilitate ATP-dependent calcium uptake was determined as described in Materials and Methods. Rates were measured over the linear portion of the uptake curve, usually the first two minutes. Vertical bars represent the mean ± SEM of 12 determinations for FISH and 11 for CORN. *$P < 0.05$, CORN *vs.* FISH.

Relative Contamination

The yield of CSR per gram wet weight of heart was not significantly different between the two diet groups; FISH 1.72 ± 0.19 mg/gm, CORN 1.86 ± 0.17 mg/gm. As noted previously, there were no significant differences in the mitochondrial (as measured by cardiolipin) and sarcolemmal (as measured by cholesterol) content of these preparations.

Oxalate-Facilitated ATP-Dependent Calcium Accumulation

The dietary manipulation produced significant effects on calcium uptake of CSR. This is shown in the Figure. For comparison, CSR isolated from animals fed a standard Purina 5001 diet, have a calcium uptake of 81 ± 3 nmol/mg protein/min.

Calcium-Dependent ATPase Activity

The total and calcium dependent ATPase activities at 50 μM ATP are seen in Table 1. In the absence of ionophore, the Ca-ATPase activity from CORN CSR was 30% higher than that of the FISH CSR ($P < 0.05$). With the addition of ionomycin the mean Ca-ATPase for CORN was also 30% higher than the FISH ($p < 0.05$). The same pattern was seen at 1 mM ATP (Table 1) where the Ca-ATPase activity of CORN was greater than FISH ($P < 0.05$). The percentage of total ATPase activity that was calcium

Table 1. Summary of ATPase activity

	50 μM ATP		1 mM ATP	
	Total	Calcium	Total	Calcium
Fish (n = 11)	0.15 ± .01	0.08 ± .01	—	—
Corn (n = 11)	0.20 ± .02*	0.11 ± 0.1*	—	—
		+800 mM ionomycin		
Fish (n = 11)	0.19 ± .01	0.16 ± .01	0.64 ± .03	0.37 ± .02
Corn (n = 11)	0.26 ± .02*	0.22 ± .01*	0.84 ± .05*	0.48 ± .03*

All values are in μmol Pi/mg protein/min. Total ATPase is in the presence of 40 μM calcium at 30°C. Mg-ATPase rate (in the presence of EGTA) was subtracted to determine the calcium-dependent rate. Values are means ± SEM (n = 11 for CORN and n = 12 for FISH) in mol%. Ratio of 1 mM ATP/50 μM ATP values; FISH, 2.31; CORN, 2.18. *$P < 0.05$, CORN *vs.* FISH.

Table 2. Diet-related measures of cardiac SR function

	Fish	Corn
Ca Accumulation by isolated SR	72.8 ± 4.9	99.2 ± 7.0*
Ionomycin stimulation (% control)	208 ± 18	201 ± 19
EP Total (pmol/mg protein)	404 ± 22	398 ± 50
EP; Ca dependent (%)	79 ± 9	84 ± 6
Turnover (CaATPase/TotEP;min^{-1})*	927 ± 53	1216 ± 67*
Turnover (CaATPase/CalEP;min^{-1})*	1223 ± 37	1476 ± 64*
Coupling (ATP/Ca uptake)	5.4 ± 0.4	5.0 ± 0.3

All values are mean ± SEM. Calcium accumulation is expressed in nmol/mg/min., *$P < 0.05$, different from corresponding value. n = 11 for CORN, n = 12 for FISH except AcylPhosphate (EP) where n = 4 for both groups.

dependent was similar for the two groups at all conditions (ionophore, increased ATP) consistent with similar cationic regulation of the Ca-ATPase.

Effect of Ionophore

Ionomycin renders the SR membrane permeable to calcium and releases any inhibitory effects of accumulated calcium. If a significant increase in membrane permeability were present as a result of the membrane lipid alteration, then ionophore would be expected to have a blunted stimulatory effect on Ca-ATPase activity. This was not found; ionomycin produced a doubling of Ca-ATPase activity in both groups consistent with the absence of diet associated alterations in leakiness of the CSR membranes (Table 2).

Relative Affinity for ATP

A decreased affinity for ATP could result in lower Ca-ATPase activity and lower calcium transport. Complete ATP dose response curves were not performed, however a comparison of Ca-ATPase activities with 50 μM ATP to that at 1 mM ATP was made (Table 1). For both groups approximately 40 percent of the activity present at 1 mM was measured at 50 μM, implying that no significant alteration in affinity of ATP is present within this concentration range.

Phosphoenzyme Content

If dietary manipulation resulted in significant changes in the number of active pump sites, this would affect function. Using [γ-^{32}P]-ATP, the phosphoprotein content was determined and there were no differences between the two groups (Table 2). Furthermore, there were no differences in the cationic regulation of the acyl-phosphate formation, in that approximately 80% of the total phosphoprotein was calcium dependent for both groups.

Turnover Number

Turnover was calculated at 50 μM ATP by dividing the calcium dependent ATPase activity by the two estimates of active pump sites (Total EP and Cal-

cium-dependent EP). As shown in Table 2, using Total EP as the estimate for active Ca-ATPase, there was a statistically higher turnover for the CORN CSR Ca-ATPase than for the CSR isolated from the FISH group. A similar pattern is seen when the Ca-dependent EP values are used to estimate active pump sites. The percent increase in turnover number for CORN was equal to the increase in calcium uptake *vs.* the FISH values.

Coupling Ratio

Although the ATPase activities and oxalate-facilitated calcium uptakes were determined under different experimental conditions, a crude measure of coupling of hydrolysis of ATP to calcium transported was made. Although there was substantial variation in ATP/Ca ratios within the same diet group, there were no differences between the two diet groups (Table 2). This is consistent with a lack of diet-regulated change in the efficiency of ion transport.

Calcium-Independent, Magnesium-Dependent ATPase Activity

Though there was no statistical difference in the Mg-ATPase activity at 50 μM ATP and there was a small decrease in Mg-ATPase activities for both groups with ionophore, there was an increased Mg-ATPase activity at 1 mM ATP (Table 1). This resulted in an increased Mg-ATPase activity for the CORN compared to the FISH group ($P < 0.05$), paralleling the changes in Ca-ATPase activity. Again, the percentage of total ATPase activity that was calcium independent was the same in both groups.

Fatty Acyl Chains

The fatty acyl chains from isolated CSR are shown in Table 3. The fish oil diet caused a decrease in the total amount of shorter chain fatty acids ($\leq$18 : 2) contained in the CSR membranes, the percentage from CORN being 68% and from FISH 47%. Most of this decrease in the FISH samples is due to the 55% decrease in linoleic acid (18 : 2). Although on a single chain basis, many of the lipid changes appear modest, the summary ratios of lipid groups reveal substantial changes produced by the dietary manipulation. The ratio of Polyunsaturated (PUFA) to Saturated (SFA) was; FISH 1.82 and 1.13 for CORN, and this correlated with CSR function in that the calcium uptake was greater with the lower PUFA/SFA ratio. The ratio of arachidonic (AA) to docosohexanoic acid (DHA) (20 : 4/22 : 6) has been correlated with function in total rat heart lipid extractions (Gudbjarnason, 1990). This ratio also appeared to correlate with calcium accumulation in our diet groups. The $(n - 6)/(n - 3)$ ratio clearly is altered by the FISH diet, compared to the CORN diet which contains 59% $n - 6$ fatty acids. Amounts of measured unsaturated fatty acids greater than C18, i.e., arachidonic, eicosapentanoic, docosapentanoic and docosahexanoic acids, were also significantly different between the two groups.

Table 3. Fatty acyl chain content of CSR membranes following dietary manipulation

C#	Fish	Corn
14 : 0	2.7 ± 0.4	0.6 ± 0.2*
16 : 0	12.0 ± 1.1	14.7 ± 2.8
18 : 0	21.7 ± 2.6	29.8 ± 2.3
18 : 1	2.6 ± 0.4	5.2 ± 0.7*
18 : 2	7.7 ± 1.1	17.7 ± 1.4*
18 : 3	trace	trace
20 : 4	13.0 ± 1.2	18.4 ± 2.1*
20 : 5	9.9 ± 0.8	1.3 ± 0.2*
22 : 5	17.9 ± 3.2	8.2 ± 0.7*
22 : 6	17.6 ± 1.7	5.3 ± 0.8*
P/S	1.82	1.13
AA/DHA	0.74	3.47
(n-6)/(n-3)	0.46	2.44

Values are mean ± SEM. *$P < 0.05$.

Table 4. Fatty acid content of test diets (%)

C#	Fish	Corn
14 : 0	12.3	—
16 : 0	24.1	12.0
16 : 1	15.0	0.2
18 : 0	3.5	2.3
18 : 1	13.3	26.1
18 : 2	13.1	59.3
18 : 3	1.3	—
20 : 4	1.0	—
20 : 5	16.5	—

Pellets of diet were extracted as described under Materials and Methods for SR. Values are in mol %.

CSR Phospholipids

Phospholipid headgroup amounts in the isolated CSR from the diet groups showed only small, non significant differences (*data not shown*). The amount of phospholipid/mg protein was CORN 383 ± 17 μg, FISH 407 ± 44 μg, also not significantly different.

Discussion

The sarcoplasmic reticulum, in both the heart and skeletal muscle, actively transport calcium via their Ca-ATPases (Tada et al., 1976). The structures of these pump proteins are postulated to consist of membrane spanning domains which undergo energy dependent conformational changes that can be affected by the phospholipid milieu (Brandl et al., 1984; Navarro, Toívio-Kinnucan, Racker, 1984; Hidalgo et al., 1986; Blasie et al., 1990). The skeletal muscle SR Ca-ATPase function can be altered by manipulation of its phospholipid milieu (Cheng et al., 1986) and it was previously shown that some specificity was apparent as to the fatty acyl chains residing in the terminal cisternae and longitudinal portions of the SR (Van Winkle et al., 1982). The protein has a number of intimately associated phospholipids (Bick et al., 1991); however, alterations in the bulk lipid result in changes in activity of the skeletal muscle SR Ca-ATPase (Moore, Lentz, & Meissner, 1978), the phospholipid alterations being in acyl chains, headgroups or both. Johansson et al. (1981) found that the ATPase activity increased 50% by reconstitution in di-(20 : 1)PC compared to the native vesicles. Martonosi noted that reconstitution of skeletal muscle SR Ca-ATPase with dipalmitoyl PC decreased the Km for ATP from 25 μM to 6.6 μM (Martonosi, Donley & Halpin, 1968). East and Lee (1982) reported increased activity of Ca-ATPase reconstituted with di-acyl PC. Di-acyl PE and di-acyl PS decreased activity. Thus, many aspects of the skeletal pump protein can be altered by membrane lipid manipulation.

Dietary manipulation of membrane lipids to alter skeletal muscle SR Ca-ATPase activity has been less conclusive, consistent with a decreased magnitude of lipid alteration by diet supplementation when compared to in vitro techniques. A corn oil diet produced a "minimal" (17%) increase in Ca-ATPase activity with rabbit skeletal SR (Gould et al., 1987). Our results with skeletal muscle SR also showed minimal alterations (*data not shown*) and small changes in membrane lipids.

The Ca-ATPase from CSR is similar to the skeletal muscle enzyme in many respects. However, there, there are important differences. One difference is that the stability of the cardiac enzyme during purification and lipid alteration effects using reconstitution have proved to be more difficult to produce in vitro. In vivo, calcium uptake by CSR is increased 300% in mice fed corn oil supplemented diet compared to controls (Swanson et al., 1989), with ATPase activity increased to a similar degree. Fish oil supplemented diet produced little change in CSR function (Croset et al., 1989). PC acyl chains were very responsive to variation in diet, with corn oil increasing linoleic acid 80% over control, while fish oil decreased linoleic 60%. DHA decreased 40% with corn oil and increased 75% with fish oil diet. Though the authors offered no kinetic explanation for their results, they did correlate n-6/n-3 ratios and ATPase activity, but not with calcium uptake (Swanson et al., 1989). An inverse relationship existed between uptake and DHA; i.e., more DHA, lower uptake. This relationship has been seen in other studies (Croset & Kinsella, 1989) and is supported by our data.

In vivo, increased DHA is accompanied by a decrease in AA, so it is unclear which, if any, fatty acid is more important. The reciprocal relationship is reflected in the AA/DHA ratio which correlated strongly with calcium uptake (Table 3). In summary, significant alterations in phospholipid acyl chains are associated with large changes in enzyme activity and function.

The CORN calcium uptake was 30% greater than FISH, this increase not being due to the number of active pump sites, unlike increased uptake following thyroid hormone treatment (Dillmann, 1990), as measured by total and calcium-dependent acyl phosphate content.

Though Ca-ATPase activity and uptake measurements were made under somewhat different conditions, there were no differences in the coupling ratios indicating no alterations in pump efficiency resulting from the diets. Also, no apparent alterations in the affinity of the pump for ATP were evident.

Coupling of beta adrenergic receptors to stimulatory G proteins is dependent on phospholipids in certain systems (Bakardjieva, Galla & Helmreich, 1979) and beta-stimulation of the CSR is primarily via the phosphorylation of phospholamban, which produces an increase in affinity of the cardiac pump for calcium but does not change the V_{max} (Tada et al., 1980). Because our experiments were performed at high free calcium concentrations (V_{max} conditions), alterations in phospholamban-Ca-ATPase coupling are not implicated in these differences in calcium uptake.

Increases in skeletal muscle SR Ca-ATPase activity have been noted after adding phospholipid to the native membranes and altering the protein/lipid ratio. The lipid to protein ratios were similar in both groups in this study, so, increased lipid does not explain the rapid turnover in the CORN group (Table 2).

Isolation of the CSR was performed with 10 mM DTT, inhibiting oxidation of fatty acids during the isolation procedure and possible membrane damage. Stimulation of ATPase activity by ionophore was similar between groups, implying no differences in

leakiness. Using oxalate in the calcium uptake assay means only a small calcium gradient exists across the SR membrane, because calcium oxalate precipitates, so minimizing the role of any membrane permeability.

The skeletal muscle calcium pump undergoes a conformational change that includes displacement of membrane lipids as the "plunger" contracts (Blasie et al., 1990) and it is assumed the cardiac pump has similar properties. Resistance to this displacement could be modified by the lipids present in the SR and we hypothesize it is by this mechanism that the acyl chain changes alter the Ca-ATPase turnover, with the relevant lipids being those intimately associated with the Ca-ATPase, with some extension into the bulk lipid.

Reconstitution of skeletal Ca-ATPase into phospholipid membranes of varying thickness results in a large variation of activity (Johansson et al., 1981), and membranes too thin or too thick inhibit the Ca-ATPase. The faster enzyme turnover occurred in the CORN membranes which included a high proportion of saturated fatty acyl chains and smaller number of n-3 fatty acids. Since the saturated and n-6 fatty acids are the least fluid (Holmes et al., 1983), the traditional view of fluidity may not be important here. However, none of the phospholipids in Table 3 would be expected to be in the crystalline phase at 30°C. Membrane thickness, which is dependent on fatty acyl chain length and degree of saturation, may be a more significant factor.

Although n-3 fatty acids comprise less than 20% of the menhaden oil in the diet, more than 60% of the phospholipids contain DHA or EPA, if we assume that these two fatty acids are located only at the sn-2 position. This might occur via two mechanisms (i) the synthetic pathways more readily incorporate n-3 fatty acids into the sn-2 position of phospholipids, or (ii) phospholipids having n-3 fatty acids at the sn-2 position may be degraded or hydrolyzed more slowly. These results support the concept that membrane phospholipids may not be tightly regulated to optimize protein function.

Isolated CSR may not be free of eicosanoid and phospholipase activities and, consequently, differences due to activation (protein kinase C or otherwise) of phospholamban or the calcium release channel by phosphorylation may exist, but our choice of conditions (saturating free calcium, presence of oxalate) has minimized with possibility. Nevertheless, these possibilities will necessitate further experimentation.

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