Effect of reversal of diet-induced changes in acyl group composition of cardiac membrane lipids on adenylate cyclase activity in rats

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The purpose of the present investigation was to determine if the diet-induced changes in cardiac adenylate cyclase activity and membrane lipids can be reversed. Three groups of male weanling Sprague-Dawley rats were fed purified diets containing different fats: 9% butter (Btr) + 1% corn oil (CO) (group I), 10% CO (group II), and 9% ethyl ester concentrate of n-3 fatty acids (EEC) + 1% CO (group III). After 5 weeks of feeding, rats from each group were killed. Cardiac membranes were prepared and assayed for adenylate cyclase activity. The fatty acid composition of membrane total phospholipids was also determined. The remaining rats in group I were divided into four subgroups and fed the following diets for the reversal study: 9% Btr + 1% CO (group Ia), 9% EEC + 1% CO (group Ib), 10% CO (group Ic), and 7% Btr + 2% EEC + 1% CO (group Id). Rats in groups II and III were maintained on their original diets. Rats were killed 5 weeks after changing the dietary regimes, and membranes were prepared from heart and analyzed for their fatty acid composition of total phospholipids. Adenylate cyclase activity (basal, fluoride- and forskolin-stimulated) was also measured. The enzyme activity was lower in membranes of rats in group Ia than those in groups II or III. Whereas the diet-induced changes in fatty acid composition were essentially reversed by dietary modification (groups Ib and Ic), the changes in adenylate cyclase activity were only partially reversed. (J. Nutr. Biochem. 5:106–112, 1994.)

Keywords: adenylate cyclase; heart; phospholipids; fatty acids; reversal of diet-induced changes

Introduction

There is substantial evidence that dietary lipids can influence the lipid composition of cardiac membranes.[1–10] The diet-induced structural changes in cardiac membrane lipids are associated with changes in the activities of a number of membrane-associated enzymes such as F_1-ATPase in mitochondria,[11,12] phosphodiesterase, p-nitrophenylphosphatase and 5′-nucleotidase in sarcolemma,[13] and adenylate cyclase.[14–18] Adenylate cyclase plays a critical role in the generation of cellular cyclic AMP in heart, which is important in regulating contractility.[19] In our previous studies, we reported that essential fatty acid deficiency,[20] the feeding of diets rich in *trans*-fatty acids[21] and those containing high levels of polyunsaturated fatty acids,[22] can alter the cardiac membrane fatty acid composition and adenylate cyclase activity. In the latter study,[22] the specific activity of adenylate cyclase was lower in cardiac membranes of rats fed diets containing saturated fats than those fed high levels of polyunsaturated fatty acids. The purpose of the present investigation was to determine if the decrease in adenylate cyclase activity induced by feeding a saturated fat can be restored by subsequent feeding of a diet rich in n-6 or n-3 polyunsaturated fatty acids and, if so, is it related to the diet-induced changes in the acyl group composition of cardiac membrane phospholipids?

Methods and materials

Male weanling Sprague-Dawley rats (Holtzman Co., Madison, WI USA) were fed regular rat ration for 3 days to acclimate

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them to the laboratory conditions. Rats were weighed and randomly divided into three groups with the same average body weight per group. Thirty-six rats were assigned to group I and 13 rats each to groups II and III. Rats were fed semipurified diets containing 9% butter + 1% corn oil (group I, rich in saturated fatty acids), 10% corn oil (CO) (group II, rich in n-6 fatty acids), or 9% ethyl ester concentrate (EEC) of n-3 fatty acids + 1 CO (group III, rich in n-3 fatty acids). The basal diet was essentially an AIN-76 diet[23,24] except that it contained 10% fat instead of 5%. Diets were prepared every week and stored at 4° C. Dietary ingredients including corn oil were purchased from Teklad (Madison, WI USA), unsalted butter was purchased locally. The ethyl ester concentrate, which was obtained under the Fish Oil Test Material Program (National Institute of Health [NIH], Bethesda, MD USA) contained supplements of antioxidants to prevent autoxidation. Therefore, antioxidants (α-tocopherol, γ-tocopherol and t-butylhydroxyquinone) were added to corn oil to match their concentration in EEC of n-3 fatty acids. This was based on the recommendation of the supplier of EEC of n-3 fatty acids (Southeast Fisheries Science Center, Charleston Laboratory, Charleston, SC USA). No antioxidants were added to butter because it contains very low levels of the easily oxidizable polyunsaturated fatty acids. The fatty acid composition of the dietary lipids is given in *Table 1*. Rats were housed individually in suspended stainless-steel cages in a temperature and light-controlled room (22 to 23° C, 12-hr light-dark cycle) and were given diets and tap water ad libitum. Rats were weighed twice weekly and their food intake was also measured 3 to 4 times during the study. After 5 weeks on their respective diets, 6 to 8 rats from each of the three groups were sacrificed by decapitation; hearts were dissected out, rinsed with cold physiological saline, blotted with a filter paper, and weighed. Hearts from rats within each group were chopped into small pieces and homogenized in a medium containing 0.25 M sucrose, 0.05 M Tris buffer, pH 7.7, and 10 mM $MgCl_2$. Crude membranes were prepared by differential centrifugation using a slight modification of the method of Foster et al.[25] In brief, the homogenate was filtered over two layers of cheese cloth and the filtrate was centrifuged at 14,000*g* for 10 min at 4° C. The supernatant was collected and centrifuged at 37,000*g* for 30 min. The pellet was resuspended in 10 mL of the homogenizing buffer and centrifuged again for 30 min at the same speed. After this spin, the supernatant was discarded and the pellet was resuspended in the homogenization buffer (without sucrose), divided into several aliquots of 1 mL each, and stored under liquid nitrogen.

To test the hypothesis that the saturated fatty acid-induced changes in adenylate cyclase activity can be reversed, the remaining rats in group I were divided into four subgroups of seven rats each and were fed for an additional 5 weeks period the following diets: 9% butter + 1% CO, Group Ia; 9% EEC + 1% CO, Group Ib; 10% CO, group Ic; and 2% EEC + 7% butter + 1% CO, group Id. Rats in groups II and III were maintained on their respective diets. After 10 weeks, all the rats were killed, hearts were dissected out, and membranes were prepared as described above.

Cardiac membranes from each group were assayed for adenylate cyclase activity by measuring the conversion of α-[^{32}P]-ATP to [^{32}P]-cAMP, which was isolated by two-step column chromatography as described by Salomon et al.[26] Radioisotopes, α-[^{32}P]-ATP and [^{3}H]-cAMP were purchased from Amersham (Arlington Heights, IL USA). Details of the adenylate cyclase assay have been previously reported.[27] The activity of adenylate cyclase was measured without any exogenous stimulation (basal activity) and in the presence of 15 mM NaF or 0.1 mM forskolin (stimulated activity). Fluoride is known to activate adenylate cyclase via G-proteins,[28–31] whereas forskolin activates the enzyme directly.[32–34] The incubation medium also contained 100 μM dithiothreitol, 10 mM $MnCl_2$ and 5 μg alamethicin (all final concentrations). Dithiothreitol is known to protect the sulfhydryl groups, Mn^{2+} increases the intrinsic activity of the catalytic unit of the enzyme,[35] and alamethicin unmasks the latent adenylate cyclase activity.[36]

Total lipids were extracted from aliquots of cardiac membranes using chloroform-methanol.[37] Phospholipids were separated by column chromatography on silicic acid columns. The fatty acid composition of total phospholipids was determined after transesterification with 14% BF_3/methanol.[38] Fatty acid methyl esters (FAME) were purified by thin layer chromatography (TLC) on 0.25 mm-thick silica gel G plates using hexane-diethylether (9:1) as solvents. The FAME spots were ex-

Table 1 Fatty acid composition of the dietary lipids

Fatty acid	10% Corn oil	9% Butter + 1% corn oil	9% EEC + 1% corn oil	7% Butter + 2% EEC + 1% corn oil
6:0		1.4		1.1
8:0		1.2		0.9
10:0		3.0		2.3
12:0		3.6		2.8
14:0		11.2		8.7
16:0	11.6	29.9	1.2	23.5
16:1 n-7		1.4		1.1
16:3 n-4			3.2	0.7
16:4 n-3			2.4	0.5
18:0	1.7	10.2	0.2	7.9
18:1	23.1	19.7	2.3	15.8
18:2 n-6	60.0	8.5	6.0	8.0
18:3 n-3	1.0			
18:4 n-3			6.3	1.4
20:4 n-6			2.3	0.5
20:5 n-3			41.7	9.3
22:5 n-3			1.6	0.4
22:6 n-3			21.6	4.8

Values are wt. %, average of 2 determinations.
EEC, ethyl ester concentrate of n-3 fatty acids.

tracted with 3 mL of chloroform-methanol (2:1). The recovery of FAME from the TLC plates was greater than 95%. The fatty acid composition was determined by gas chromatography using a megabore column (15 m × 0.53 mm FSOT, DB-Wax, 1.0 μm, J & W Scientific, Folsom, CA USA). Column temperature was programmed from 70° C (initial temperature for 1 minute) to 215° C at 8° C/min. The detector temperature was 250° C, and the injection port temperature was 225° C. The identification was based on relative retention data using standards. The FAME standards were obtained from Alltech Associates (Deerfield, IL USA) and the dimethylacetals for 16:0 and 18:0 were obtained from Deva Biotech (Hartboro, PA USA).

Results

There was no significant difference ($P > 0.05$) among the three groups in terms of final body weights or heart weights of rats fed diets containing different fats for 5 weeks *(Table 2)*. The data pertaining to the reversal study are shown in *Table 3*. Similar to the data at 5 weeks, there was no significant difference among the various groups in body weights or heart weights.

Adenylate cyclase activities are shown in *Table 4*. Thespecific enzyme activities (basal, fluoride-, and forskolin-stimulated) were significantly higher in cardiac membranes of rats fed diets containing corn oil (group II) or EEC of n-3 fatty acids (group III) than those fed the butter diet (group I). The fatty acid composition of total phospholipids of cardiac membranes of rats fed the three diets for 5 weeks is shown in *Table 5*. Changes characteristic of feeding the specific dietary lipid were observed. This included an increased incorporation of n-6 fatty acids, mainly 18:2, 22:4, and 22:5n-6, in membrane phospholipids of rats fed 10% corn oil diet and an increase in n-3 fatty acids in the group fed EEC of n-3 fatty acids relative to those fed butter.

The fatty acid composition of cardiac membranes after the reversal study (an additional 5 weeks of feeding) is shown in *Table 6*. Changes in fatty acid composition of membrane phospholipids as a result of feeding the 9% butter + 1% corn oil diet for 5 weeks were essentially reversed when butter was replaced by EEC of n-3 fatty acids (compare group Ib with group III) or corn oil (compare group Ic with group II). However, the decrease in adenylate cyclase activity in membranes of rats fed the 9% butter + 1% corn oil diet was only partially restored *(Table 7)*. The results show that adenylate cyclase activity (forskolin-stimulated) was significantly higher in membranes of rats fed the diet containing corn oil (group Ic) or EEC of n-3 fatty acids (group Ib) than those fed the diet containing butter (group Ia). Similar results were obtained when 2% of the butter was replaced by an equivalent amount of EEC of n-3 fatty acids (compare group Id versus group Ia). However, the enzyme activities were still significantly lower in cardiac membranes of rats pertaining to groups Ia, Ib, Ic, and Id (the reversal study) than those in groups II and III, which were fed the corn oil or EEC diet throughout the entire duration of 10 weeks. The basal or fluoride-stimulated enzyme activity was not significantly in-

Table 2 Body weights and heart weights of rats fed different diets for 5 weeks

Dietary fat	Final body weight (g)	Heart weight (g)	Heart weight (mg/100 g body wt)
9% butter + 1% corn oil	414 ± 12	1.40 ± 0.05	339 ± 6
10% corn oil	408 ± 6	1.46 ± 0.04	357 ± 9
9% EEC + 1% corn oil	387 ± 4	1.37 ± 0.07	354 ± 17

Values are mean ± SEM of 6 to 8 rats per group. The initial body weights were 88 ± 2 g. None of the values among the three groups are significantly different from each other ($P > 0.05$) using analysis of variance, Newman-Keul's test.
EEC, ethyl ester concentrate of n-3 fatty acids.

Table 3 Body weights and heart weights of rats in the reversal study

Group no.	Dietary fat and feeding duration	Final body wt. (g)	Heart wt. (g)	Heart wt. (mg/100 g body wt.)
Ia	9% butter + 1% corn oil (10 wks)	585 ± 18	1.87 ± 0.07	322 ± 15
Ib	9% butter + 1% corn oil (5 wks) → 9% EEC + 1% corn oil (5 wks)	531 ± 16	1.67 ± 0.05	315 ± 8
Ic	9% butter + 1% corn oil (5 wks) → 10% corn oil (5 wks)	563 ± 31	1.68 ± 0.08	300 ± 11
Id	9% butter + 1% corn oil (5 wks) → 7% butter + 2% EEC + 1% corn oil (5 wks)	575 ± 20	1.70 ± 0.04	297 ± 10
II	10% corn oil (10 wks)	579 ± 12	1.75 ± 0.04	302 ± 10
III	9% EEC + 1% corn oil (10 wks)	532 ± 17	1.76 ± 0.04	332 ± 9

Values are mean ± SEM of seven rats per group. None of the values among the various groups are significantly different from each other ($P > 0.05$) using analysis of variance, Newman-Keul's test.
EEC, ethyl ester concentrate of n-3 fatty acids.

Table 4 Adenylate cyclase activity in cardiac membranes of rats fed diets containing different lipids for 5 weeks

Group no.	Dietary fat	Basal	+ Fluoride	+ Forskolin
I	9% butter + 1% corn oil	26.9 ± 3.2[a]	118.6 ± 7.3[a]	266.5 ± 24.6[a]
II	10% corn oil	50.7 ± 3.1[b]	174.4 ± 7.1[b]	419.8 ± 26.1[b]
III	9% EEC n-3 + 1% corn oil	45.0 ± 4.8[b]	169.6 ± 18.1[b]	453.1 ± 38.8[b]

Values are mean ± SEM of 4 to 6 assays per group, each done in triplicate. Values with different superscripts in the same column are significantly different from each other ($P < 0.05$) using analysis of variance, Newman-Keul's test.
Enzyme activity is shown in pmol cAMP/mg protein/min. The final concentration of fluoride was 15 mM and forskolin 0.1 mM.
EEC n-3, ethyl ester concentrate of n-3 fatty acids.

Table 5 Fatty acid composition of total phospholipids of cardiac membranes of rats fed diets with different lipids for 5 weeks

Fatty acid	I 9% Butter + 1% Corn oil	II 10% Corn oil	III 9% EEC + 1% Corn oil
16:0 DMA	2.4 ± 0.2[a]	2.1 ± 0.2[b]	0.4 ± 0.1[c]
16:0	10.4 ± 0.4[a]	10.3 ± 0.8[a]	10.3 ± 0.8[a]
18:0 DMA	1.6	1.1 ± 0.2	0.2
18:0	19.1 ± 0.3[a]	19.8 ± 0.6[a]	22.7 ± 0.6[b]
18:1	8.5 ± 0[a]	6.3 ± 0.8[b]	3.9 ± 0.1[c]
18:2 n-6	11.6 ± 0.6[a]	16.1 ± 0.6[b]	6.8 ± 0.1[c]
20:3	0.9 ± 0.1[a]	0.6 ± 0.1[b]	0.4 ± 0[c]
20:4 n-6	23.5 ± 0.5[a]	21.2 ± 0.6[b]	11.3 ± 0.3[c]
20:5 n-6	3.2 ± 0.1[a]	3.0 ± 0.2[a]	3.2 ± 0.1[a]
20:5 n-3	tr[a]	tr[a]	5.0 ± 0.2[b]
22:4 n-6	1.2 ± 0.4[a]	3.0 ± 0.6[b]	tr[c]
22:5 n-6	4.4 ± 0.2[a]	7.0 ± 0.2[b]	1.5 ± 0.4[c]
22:5 n-3	1.1 ± 0.2[a]	1.0 ± 0.2[a]	3.2 ± 0.2[b]
22:6 n-3	5.5 ± 0.2[a]	4.5 ± 0.2[b]	27.5 ± 0.9[c]

Values are wt. % (mean ± SD of 3 to 4 determinations per group). Values with different superscripts in the same row are significantly different from each other ($P < 0.05$) using analysis of variance, Newman-Keul's test.
DMA, dimethylacetal; EEC, ethyl ester concentrate of n-3 fatty acids; tr, trace (< 0.1%).
Values with no SD are average of two determinations.

Table 6 Fatty acid composition of phospholipids of cardiac membranes of rats from the reversal study

Fatty acid	Ia 9% Btr + 1% CO	Ib 9% Btr + 1% CO→ 9% EEC + 1% CO	Ic 9% Btr + 1% CO→ 10% CO	Id 9% Btr + 1% CO→ 7% Btr + 2% EEC + 1% CO	II 10% CO	III 9% EEC + 1% CO
14:0	0.2 ± 0.1[a]	0.2 ± 0.1[a]	0.1 ± 0[a]	0.1 ± 0[a]	0.2 ± 0[a]	0.2 ± 0.1[a]
16:0 DMA	2.4 ± 0[b]	2.2 ± 0.1[a]	2.0 ± 0[c]	2.1 ± 0.1[a]	2.5 ± 0[d]	1.8 ± 0.1[e]
16:0	11.8 ± 0.2[a,b]	12.6 ± 0.3[b]	10.9 ± 0.4[a]	12.8 ± 0.2[b]	10.9 ± 0.2[a]	11.5 ± 0.2[a,b]
18:0 DMA	1.1 ± 0.1[a]	1.0 ± 0.1[a]	0.9 ± 0.2[a]	1.1 ± 0[a]	1.2 ± 0.1[a]	1.2 ± 0.1[a]
18:0	20.7 ± 0.3[a]	22.0 ± 0.5[b]	20.2 ± 0.3[a]	19.5 ± 0.3[c]	20.4 ± 0.1[a]	22.0 ± 0.4[b]
18:1	9.0 ± 0[a]	6.2 ± 0.1[b]	6.6 ± 0.1[c]	7.7 ± 0[d]	6.1 ± 0.1[b]	4.9 ± 0.1[e]
18:2 n-6	8.5 ± 0.2[a]	6.9 ± 0.1[b]	17.5 ± 0.4[c]	11.3 ± 0.3[d]	15.7 ± 0.4[e]	6.2 ± 0.1[f]
20:2	0.6 ± 0[a]	0.4 ± 0.2[a]	0.5 ± 0.1[a]	0.3 ± 0[a]	0.7 ± 0.1[a]	0.3 ± 0.3[a]
20:3	0.5 ± 0.1[a]	0.3 ± 0.1[a]	0.4 ± 0[a]	0.5 ± 0[a]	0.4 ± 0[a]	0.5 ± 0.3[a]
20:4 n-6	24.6 ± 0.6[a]	11.8 ± 0.3[b]	22.0 ± 0.4[c]	13.0 ± 0.3[d]	21.8 ± 0.3[c]	11.8 ± 0.1[b]
20:5 n-6	2.3 ± 0.1[a]	2.2 ± 0.1[a]	1.4 ± 0.6[b]	1.9 ± 0.1[a]	1.9 ± 0.1[a]	3.0 ± 0.1[c]
20:5 n-3	0.1 ± 0.1[a]	4.4 ± 0.1[b]	0.2 ± 0.1[a]	3.2 ± 0[c]	0.2 ± 0.1[a]	4.4 ± 0.2[b]
22:4 n-6	1.2 ± 0.1[d]	0.1 ± 0.1[b]	2.5 ± 0.1[c]	0.2 ± 0[b]	2.8 ± 0.1[d]	0.2 ± 0[b]
22:5 n-6	4.1 ± 0.2[a]	0.7 ± 0.1[c]	5.7 ± 0.2[b]	0.4 ± 0[c]	6.9 ± 0.1[d]	0.5 ± 0.1[c]
22:5 n-3	1.1 ± 0.1[a]	3.0 ± 0.1[b]	1.0 ± 0[a]	3.6 ± 0.1[c]	0.9 ± 0.1[a]	2.9 ± 0.2[b]
22:6 n-3	6.2 ± 0.3[a]	22.0 ± 0.5[b]	4.1 ± 0[c]	17.9 ± 0.3[d]	3.7 ± 0.2[c]	24.1 ± 0.7[e]

Values are wt. % (mean ± SD of three determinations per group). Values with different superscripts in the same row are significantly different from each other ($P < 0.05$) using analysis of variance, Newman-Keul's test.
Btr, butter, CO, corn oil, EEC, ethyl ester concentrate of n-3 fatty acids, DMA, dimethylacetal.

Table 7 Effect of reversal of diet-induced changes in fatty acid composition of cardiac membranes on their adenylate cyclase activity

Group no.	Dietary fat and duration of feeding	Basal	+ Fluoride	+ Forskolin
Ia	9% butter + 1% corn oil (10 wks)	6.5 ± 0.9[a]	43.8 ± 4.4[a]	34.7 ± 1.4[a]
Ib	9% butter + 1% corn oil (5 wks) → 9% EEC + 1% corn oil (5 wks)	7.7 ± 0.9[a]	56.0 ± 6.9[a]	60.2 ± 7.9[b]
Ic	9% butter + 1% corn oil (5 wks) → 10% corn oil (5 wks)	10.9 ± 1.2[a]	51.3 ± 1.8[a]	70.3 ± 3.5[b]
Id	9% butter + 1% corn oil (5 wks) → 7% butter + 2% EEC + 1% corn oil (5 wks)	6.9 ± 0.8[a]	44.0 ± 2.8[a]	58.6 ± 1.8[b]
II	10% corn oil (10 wks)	21.0 ± 2.7[b]	84.1 ± 5.0[b]	141.2 ± 8.9[c]
III	9% EEC + 1% corn oil (10 wks)	19.8 ± 2.0[b]	87.0 ± 11.8[b]	120.3 ± 9.2[a]

Values are mean ± SEM of 3 to 5 assays per group, each done in triplicate. Values with different superscripts in the same column are significantly different from each other ($P < 0.05$) using analysis of variance, Newman-Keul's test. Enzyme activity is shown in pmol cAMP/mg protein/min. The final concentration of fluoride was 15 mM and forskolin 0.1 mM. EEC n-3, ethyl ester concentrate of n-3 fatty acids.

creased in cardiac membranes of rats that had been previously fed the diet rich in saturated fatty acids and then switched over to the diets rich in n-6 or n-3 PUFA (compare groups Ib, Ic, and Id versus Ia).

Discussion

The results of the present study are consistent with our previous finding that adenylate cyclase activity was higher in cardiac membranes of rats fed diets rich in polyunsaturated fatty acids (PUFA) than those fed saturated fatty acids.[22] Coupled with the evidence that phosphodiesterase activity is decreased in cardiac membranes of rats fed unsaturated fats compared with the saturated fats,[39] one would expect that the intracellular cAMP levels will be higher in heart of rats fed diets rich in unsaturated fatty acids. The present results extend our previous observations and show that although the diet-induced changes in the fatty acid composition of cardiac membrane phospholipids in rats fed a saturated fat could be readily reversed by feeding diets rich in unsaturated fatty acids, the decrease in adenylate cyclase activity was only partially restored. This observation is consistent with the findings of Charnock et al.,[4] who showed that in contrast to compositional changes that can occur in several weeks, dietary lipid effects on the functional changes such as cardiac contractility and arrhythmia develop over a much longer time period. They also reported that the changes in rat myocardial phospholipids by feeding a saturated fat for 9 months were reversed by feeding the unsaturated fat for 9 months,[7] and that the adverse effects of dietary saturated animal fatty acids on vulnerability to cardiac arrhythmias in rats could be modified by crossover to diets rich in PUFA.[10] However, the adenylate cyclase activity was not measured in their study.

Although the specific activity of adenylate cyclase in membranes of rats pertaining to the reversal study *(Table 7)* was several fold lower than that at 5 weeks *(Table 4)*, similar differences due to the feeding of different dietary fats were observed. Thus, at each time point, the enzyme activity was 2 to 4 fold higher in membranes of rats fed a diet rich in unsaturated fatty acids (group II and III) than in membranes of rats fed the saturated fatty acids (group I or Ia). We don't know why the specific activities of adenylate cyclase were several fold lower in all the dietary groups from the reversal study than that of the 5 weeks-feeding study. The same enzyme assay was used at each of the two time points. This may possibly be related to the fact that by the end of the reversal study, the rats were 5 weeks older than in the beginning of the reversal study.

Typical changes in fatty acid composition of total phospholipids of cardiac membranes as a result of feeding a diet containing 10% corn oil were observed. Surprisingly, the levels of arachidonic acid (20:4n-6) in this group were lower relative to the group fed diet containing 9% butter + 1% corn oil *(Tables 5 and 6)*. It is conceivable that the high levels of linoleic acid (18:2n-6) in membrane phospholipids of rats fed 10% corn oil diet may be inhibiting its further desaturation and chain-elongation to form arachidonic acid (20:4n-6) by Δ^6 or Δ^5-desaturase. Apparently, further chain elongation and desaturation of 20:4n-6 to 22:4n-6 and 22:5n-6 was not inhibited in this group, as shown by higher levels of n-6 docosanoic fatty acids in membranes of rats fed 10% corn oil diet. The competitive inhibition of Δ^6-desaturase by high levels of n-3 fatty acids most likely resulted in the lowest arachidonic acid levels in membrane phospholipids of rats fed diets containing 9% EEC of n-3 fatty acids + 1% corn oil. Evidence exists for competition between the fatty acids of n-3 and n-6 families at the level of desaturation and chain elongation.[40]

Docosahexaenoic acid (22:6n-3) constituted 22 to 27.5% of the total fatty acids in membrane phospholipids of rats fed a diet rich in n-3 fatty acids, reflecting the dietary intake of this fatty acid. Similarly, high levels of 22:6n-3 in cardiac lipids from feeding diets containing fish oils have been reported in other studies.[5,7,13,41] The levels of 22:6n-3 were 4.5 to 5.5% in total phospholipids of membranes from rats fed no EEC of n-3 fatty acids. This observation suggests high capability of heart tissue to synthesize 22:6 n-3 by chain elongation and desaturation from its precursor, 18:3n-3, the levels of which were lower than 0.1% in these two groups. Similar observations have also been made by McMurchie et al.[16]

The mechanism as to how the diet-induced changes in fatty acid composition of cardiac membrane phospholipids can alter adenylate cyclase activity is not completely known. Possible explanations include changes in membrane fluidity that could influence interaction among the various components of the adenylate cyclase system such as the β-adrenergic receptor, G-proteins, and adenylate cyclase. However, in our previous two studies, one with an essential fatty acid deficiency model[20] and another with a somewhat similar dietary regime as in the present study,[22] we did not find significant differences in fluorescence polarization of diphenylhexatriene in cardiac membranes of rats fed the various diets, suggesting no significant changes in membrane fluidity in spite of the large differences in fatty acid composition of membrane phospholipids. However, the membrane bulk fluidity may be less important than the fluidity in the immediate vicinity of the enzyme. To our knowledge, no suitable method is currently available to examine the nature of the lipid microenvironment for the enzyme.

That the bulk membrane fluidity may not be the primary factor is also suggested by our adenylate cyclase activity data. If membrane fluidity were the main factor important in regulating adenylate cyclase activity, one would expect a greater difference among the dietary groups when NaF was used to stimulate adenylate cyclase rather than forskolin. However, the proportional increase in enzyme activity was identical in all groups despite the lower basal activity in cardiac membranes of rats fed diet containing 9% butter + 1% corn oil.

Because both fluoride- and forskolin-stimulated adenylate cyclase activity was increased in cardiac membranes of rats fed high PUFA diets (10% corn oil or 9% EEC n-3 + 1% corn oil), it appears that the levels/activity of G-proteins or those of adenylate cyclase or their interaction may be altered. Indeed, in a previous study,[22] we found that the concentrations of [^{3}H]-forskolin-binding sites (B_{max}) were significantly higher in cardiac membranes of rats fed a diet containing 10% menhaden oil than those fed 8% coconut oil + 2% corn oil or 10% corn oil. There was no significant difference in the affinity of the forskolin binding sites among the three dietary groups. The discrepancy between the results of the present study in which we observed no significant difference in cardiac adenylate cyclase activity between the group fed diets enriched with n-3 versus n-6 fatty acids and those of a previous study,[22] which showed higher adenylate cyclase activity and forskolin binding sites in the former group, may possibly be related to the source of n-3 fatty acids. Whereas we used 10% menhaden oil in the previous study,[22] 9% ethyl ester concentrate was used in the present study. Diet-induced changes in the levels of G-proteins or their coupling with adenylate cyclase is another possibility that remains to be explored. In a previous study,[42] which was conducted with salivary glands, we observed an increase in the ADP-ribosylation of membranes in the presence of cholera toxin, a suggestive evidence of increased activity of G_S, in rats fed diets containing 10% menhaden oil compared with rats fed diets containing 10% corn oil.

When one compares the adenylate cyclase activity data *(Table 4)* on the basis of fold-stimulation by NaF or forskolin, there was no difference among the three groups. Irrespective of the nature of the dietary fat, fluoride-stimulated activity was 3.4 to 4.4 fold the basal activity and the forskolin-stimulated activity was 8.3 to 10.1 fold the basal activity. Thus, the primary effect of the dietary fat was on the basal enzyme activity, suggesting that the dietary effects were most likely mediated at the level of adenylate cyclase. This argument is further substantiated by the adenylate cyclase activity data pertaining to the reversal study *(Table 7)*. For the reversal groups (Ib, Ic, and Id), the basal or fluoride-stimulated activity was not significantly different from that of the group fed 9% butter + 1% corn oil (Ia). However, the forskolin-stimulated activity was significantly higher in these three groups than that of group Ia.

Our observation that the effects of a partial substitution of butter with EEC of n-3 fatty acids during the reversal study (group Id, *Table 7*) were similar to those of the group in which all the butter was substituted for by EEC of n-3 fatty acids (group Ib, *Table 7*) may be important from a nutritional standpoint. Notwithstanding, the fact that it is often inappropriate to draw conclusions that are applicable to humans based on the animal data, our observation suggests some mechanism(s) worth further exploration to explain the beneficial effects of partial substitution of saturated fats with n-3 fatty acids.

In conclusion, the results of this study show that adenylate cyclase activity was only partially restored, whereas the changes in fatty acid composition were reversed when diets rich in PUFA were fed to rats that had been previously fed saturated fat. At this time, we cannot offer an explanation for this observation. We have made similar observations with cardiac adenylate cyclase in essential fatty acid (EFA) deficiency. Whereas the fatty acid composition of the membrane phospholipids was restored to normal, the changes in adenylate cyclase activity were only partially restored when the EFA-deficient rats were fed an EFA-supplemented control diet for 6 weeks.[20] It is conceivable that a specific pool of membrane phospholipids adjacent to adenylate cyclase is critical for its activity, and that the changes once induced in this lipid pool are not readily reversible. In a recent study on the lipid dependence of adenylate cyclase, specific molecular species of phosphatidylcholine (PC) containing two *cis* double bonds, each located in Δ 9 position of the PC acyl chain, or two *cis*-9,12 double bonds located on the same chain seem to provide optimal lipid environment.[43]

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