Cardiac membrane lipid composition and adenylate cyclase activity following dietary eicosapentaenoic acid supplementation in the marmoset monkey

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The effects of dietary eicosapentaenoic acid (20:5(n-3)) on cardiac membrane phospholipid fatty acid composition and membrane-associated adenylate cyclase activity were investigated in the marmoset monkey. A 20:5(n-3) concentrate (as the ethyl ester) was tested with high fat diets formulated to give a polyunsaturated:monounsaturated:saturated (P:M:S) fatty acid ratio of either 1:1:1 (control diet, no added cholesterol) or 0.1:0.6:1.0 (atherogenic-type diet, 0.2% cholesterol) that were fed for 30 weeks. The various dietary lipid treatments did not alter the proportions of the major phospholipids present in cardiac membranes. Both 20:5(n-3)-supplemented diets resulted in an increase in the proportions of 20:5(n-3) and 22:5(n-3), but not 22:6(n-3) in membrane phospholipids and a decrease in the proportion of 18:2(n-6) and 20:4(n-6). Greater incorporation of the n-3 polyunsaturated fatty acids occurred when the level of dietary linoleic acid was reduced. These changes in fatty acid profiles were evident to a greater extent in phosphatidylcholine and phosphatidylethanolamine in comparison with cardiolipin and sphingomyelin. These diets did not influence catecholamine-stimulated adenylate cyclase activity in terms of sensitivity or responsiveness to isoproterenol. The response of adenylate cyclase to other agonists such as epinephrine, norepinephrine, sodium fluoride, and forskolin was also unaffected by the dietary lipid treatments. The results clearly show that there is extensive incorporation of dietary 20:5(n-3) and its elongation product 22:5(n-3) into cardiac membrane phosphatidylcholine and phosphatidylethanolamine. However, unlike the reported effect of altered cardiac membrane cholesterol status, alterations in cardiac membrane phospholipid fatty acid composition alone appear not to affect membrane-associated adenylate cyclase activity in the marmoset monkey.

Keywords: eicosapentaenoic acid; 20:5 n-3; cardiac membranes; membrane lipid; dietary lipid; marmoset monkey

Introduction

It is now well established that cell membrane lipid composition and membrane-associated enzyme and receptor activity are influenced by the membrane lipid composition, which in turn can be altered by dietary fat.[1-2] In this regard the activities of hormone-sensitive adenylate cyclases are known to be modulated by changes in cell membrane lipid composition and physical state,[3,4] and by dietary fats.[5-7] This influence of dietary fat extends to the beta-adrenergic receptor/adenylate cyclase system (β-AR/AC) of the mammalian heart.[8-13] Although we have recently shown that the sensitivity and responsiveness of the β-AR/AC system to catecholamines in the rat heart is not significantly altered by changes in the membrane phospholipid fatty acid composition,[10] dietary cholesterol induces a considerable increase in β-AR/AC activity

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as a result of an increase in the cardiac membrane cholesterol to phospholipid ratio.[10] In the marmoset monkey, cardiac β-AR/AC activity is also increased in concert with an increase in the membrane cholesterol-to-phospholipid ratio induced by either dietary cholesterol or a saturated fatty acid enriched diet.[11,12] Beta-adrenergic receptor affinity and number in the marmoset are also influenced by changes in cardiac membrane cholesterol status.[11,12]

The influence of dietary fat on catecholamine-stimulated adenylate cyclase activity is of particular interest in regard to the numerous effects dietary fish oils have on cardiovascular risk factors.[14] Fish oils have been reported to reduce the incidence of cardiac arrhythmia following coronary artery ligation in experimental animals.[15,16] The question of the involvement of the β-AR/AC system in cardiac arrhythmogenesis and the modulating effect of fish oil has been pursued in the marmoset monkey by feeding high-fat diets enriched in 20:5(n-3), one of two long chain n-3 polyunsaturated fatty acids (PUFAs) found in fish oil. Feeding 20:5(n-3) has been shown to lead to a significant increase in cardiac membrane 20:5(n-3) content but to have little effect on the membrane cholesterol:phospholipid ratio.[17] However, under such conditions cardiac membrane beta-adrenergic receptor affinity and number are altered.[17]

In the present study we show that despite marked compositional changes in cardiac membrane phospholipid fatty acids, catecholamine-stimulated adenylate cyclase activity is unaltered. This is in agreement with our previous results in rats and marmosets that suggest that a change in the membrane cholesterol:phospholipid ratio rather than membrane fatty acid composition is required for β-AR/AC activity to be altered by dietary lipids.[10-12,17]

Materials and methods

Marmosets

Adolescent male common cotton-eared marmosets (*Callithrix jacchus jacchus*), approximately 16–26 months of age at the start of the experiment, were divided into four groups of approximately equivalent age and weight. Marmosets were paired for optimal growth and social behavior and kept in aluminum alloy marmoset cages in a room with fluorescent light and 30 min of ultraviolet irradiation daily. The temperature was maintained at 26° C and the humidity at approximately 50%. Marmosets were maintained on the various dietary lipid regimes described below for a period of 30 weeks and were sacrificed under anesthesia using Saffan (alphaxalone, 9 mg/ml; alphadolone acetate, 3 mg/ml; Glaxo, Australia), by injection into the femoral artery at a dose of 1.5 mL per kg body weight. Body weights of marmosets (g) at the start and finish of the experiment for each dietary group were (mean ± SEM): control, (n = 8) 353 ± 7, 364 ± 10; EPA, (n = 8) 332 ± 9, 326 ± 9; ATH, (n = 10) 324 ± 8, 335 ± 12; ATH + EPA (n = 10) 313 ± 9, 307 ± 12.

Marmoset diets

Four high-fat dietary regimes of approximately equivalent energy value were used to examine the effect of 20:5(n-3) (as the ethyl ester), when administered in combination with diets differing significantly in their P:M:S fatty acid ratio. The normal colony diet for marmosets consisted of a 1:1 mixture of Arnott Harper's (Adelaide, Australia) greyhound chow and Milling Industries (Adelaide, Australia) primate meal. The overall composition of this diet has previously been described[18] and on analysis contained approximately 4.5% fat. The fatty acid composition of this normal colony diet has been reported previously.[19] This colony diet was used as the basis for the preparation of the four experimental diets described in *Table 1*. Thus, the control diet consisted of the colony diet supplemented at the time of repelletting with (by weight) 5.5% sunflower seed oil (Nuttelex, Melbourne, Australia); 1.5% olive oil (SA Olive Oil Co., Adelaide, Australia); and 3.0% sheep kidney (perirenal) fat, (Noarlunga Meat Works, Noarlunga, South Australia) a natural source of both saturated and monounsaturated fat, to achieve a final P:M:S ratio of 1.1:1.1:1.0. The 20:5(n-3) diet (EPA) was prepared by adding to the control diet 0.8% (wt/wt) of a 20:5(n-3) ethyl ester concentrate (containing (by weight) 75% 20:5(n-3) and 0.2% α-tocopherol supplied by Dr. Yasushi Tamura, Chiba University Medical School, Chiba, Japan) to give a P:M:S ratio of 1.1:1.0:1.0. The fatty acid composition of this 20:5(n-3) ethyl ester concentrate has been described previously.[17,19] The atherogenic (ATH) diet consisted of the colony diet supplemented with (by weight) 10% sheep kidney fat and 0.2% cholesterol (Ajax Chemical Co.), which achieved a P:M:S ratio of 0.14/0.6/1.0 (*Table 1*). All additions (i.e., oils, fat, cholesterol, and 20:5(n-3) concentrate), were added to the crushed colony diet and thoroughly mixed before the respective diets were repelletted. The fatty acid composition of the experimental diets are shown in *Table 2*. A sufficient amount of each diet was prepared for a 2-week feeding period and these diets were then frozen at −20° C in a N_2 atmosphere. Aliquots of these diets were thawed for each daily feeding and discarded prior to providing new diet for the next day. Animals were fed ad libitum.

Preparation of cardiac membranes

Ventricular tissue from each marmoset heart was chopped and rinsed in ice-cold isolating medium containing 250 mmol/L sucrose, 20 mmol/L Tris, 1 mmol/L EDTA, 1 mmol/L $MgCl_2$ (pH 7.4), and then homogenized in 40 mL of the above medium using a Polytron tissue homogenizer (Kinematica, GmbH, Switzerland) at setting 4 for three 30 sec bursts. The homogenate was centrifuged at 500*g* for 15 min, resuspended in the above medium, and stored at −80° C (P0-500*g* fraction) at a concentration of about 7 mg protein per mL.

Adenylate cyclase assay

Adenylate cyclase [ATP pyrophosphate-lyase (cyclizing)], EC 4.6.1.1. activity was measured in cardiac membrane preparations by methods previously described.[20] Assays were performed in triplicate by measuring the formation of [^{32}P]cyclic AMP (c-AMP) from α-[^{32}P] ATP in the presence of an ATP-generating system in addition to GTP (or other guanine nucleotide), and appropriate additions such as adrenergic agonists, NaF, forskolin, etc. The reaction was initiated by the addition of equivalent amounts of cardiac membrane protein and was carried out for 20 min at 37° C. [^{32}P]c-AMP production was linear over this time course. [^{32}P]c-AMP and a recovery marker, [^{3}H]c-AMP, were separated from the substrate, α-[^{32}P]ATP, by sequential Dowex and alumina column chromatography as previously described.[20]

Protein determination

Values for the membrane protein content were determined by the method of Lowry et al.,[21] after solubilization of the mem-

Table 1 Composition of experimental marmoset diets

Addition	Control	EPA	ATH	ATH+EPA
Sunflower seed oil (%)	5.5	5.5	nil	nil
Olive oil (%)	1.5	1.5	nil	nil
Sheep perirenal fat (%)	3	3	10	10
Cholesterol (%)	< 0.04	< 0.04	0.2	0.2
EPA concentrate (%)	nil	0.8	nil	0.8
P:M:S	1.1:1.1:1	1.1:1:1	0.14:0.6:1	0.2:0.6:1
Total fat (%)	14.3	14.3	14.8	15.3
Energy value (kj/g)	22.9	22.8	22.8	22.7

(%), percentage by weight; P/M/S, polyunsaturated/monounsaturated/saturated fatty acid ratio.

Table 2 Fatty acid composition of marmoset experimental diets

Major fatty acid (%; wt/wt)	Control	EPA	ATH	ATH + EPA
Sat.				
16:0	14.7	14.5	22.4	21.7
18:0	13.7	14.3	27.8	28.5
Mono.				
16:1(n-7)	1.6	1.6	2.9	2.6
18:1(n-9)	30.7	29.4	30.9	28.7
20:1(n-9)	tr.	0.5	0.6	tr.
22:1	n.d.	n.d.	n.d.	n.d.
n-6				
18:2	32.9	31.0	6.9	6.6
n-3				
18:3	0.9	0.9	1.1	1.0
20:5	n.d.	2.9	n.d.	3.3
Σ Sat.	31.3	31.8	56.1	55.9
Σ(n-9)	33.6	31.5	34.2	31.6
Σ(n-6)	32.9	31.0	6.9	6.6
Σ(n-3)	0.9	3.8	1.1	4.3
Σ(n-6)/Σ(n-3)	36.5	8.2	6.3	1.5
U.I.	102	112	53	65

Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The particular unsaturated fatty acid series is shown as (n-x), which refers to the first double bond counting from the terminal methyl group of the fatty acid. Fatty acid values shown below represent the average obtained from at least five separate extractions and analyses of the respective diet.
tr. (trace), present at less than 0.5%; n.d., not detected; U.I. unsaturation index.[10]
EPA, control diet plus 20:5(n-3); ATH, atherogenic; ATH+EPA, atherogenic plus 20:5(n-3).

brane preparations in 0.1 N NaOH and 1% (wt/vol) sodium dodecyl sulfate.

Lipid extraction of cardiac membranes

The P0-500*g* fraction was washed by centrifugation and resuspended in glass distilled water to a concentration of 5 mg protein/mL and lipids were extracted as previously described.[11,12] Briefly, one volume of cardiac membranes was extracted with four volumes of boiling 2-propanol containing the antioxidant butylated hydroxyanisole (0.1% of the estimated lipid weight), and the mixture boiled for 30 seconds. After cooling, eight volumes of chloroform were added and the mixture shaken. One volume of glass-distilled water was added, the samples shaken, then centrifuged, and the organic phase collected. After re-extracting the aqueous phase with a further four volumes of chloroform, the organic phases were combined and dried using anhydrous sodium sulphate to give the total lipid extract.

Separation of total membrane phospholipids and phospholipid classes

Separation of the total phospholipids from the cardiac membrane total lipid extract was performed as described[22] by thin layer chromatography (TLC) on silica gel H plates developed in petroleum ether:diethyl ether:acetic acid (90:15:1) with the phospholipids remaining at the origin. Separation of the phospholipid classes was achieved after a one-way development in a solvent system of chloroform:ethanol:water:triethylamine (30:34:8:35) on Whatman LK5D TLC plates as described.[23] Following visualization of the phospholipid spots by iodine, identification of individual phospholipids was made by comparison to phospholipid standards (Sigma). The amount of phospholipid in each spot was determined by the method of Bartlett.[24] For the measurement of the combined sphingomyelin and lysophosphatidylcholine classes, samples were pooled as indicated in *Table 3*.

Fatty acid analysis

Analyses of the fatty acids in the supplements, diets, and in the various phospholipid fractions were done using capillary gas liquid chromatography by methods previously described.[10-12] Fatty acid methyl esters (FAMEs) were analyzed using a Hewlett Packard HP 5880 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA). The column was a 50 meter glass column coated with SP2340 (Supelco Inc.) prepared by Chromolytic Technology Ltd. (Boronia, Victoria, Australia). The FAMEs were separated using a carrier gas (helium) flow of 25 cm/sec with a temperature gradient of 120° C to 200° C at 5° C/min. The injection/split temperature was 250° C and the flame ionization detector temperature was 300° C. FAMEs were identified against authentic lipid standards supplied by Nuchek, Inc. (Elysian, MN, USA).

Statistical analysis

All data are presented as the mean ± SEM for the indicated number of samples (n) for each dietary group. The statistical package Statistix Version 3.1 was used to calculate general analysis of variance (ANOVA), using the individual values from each dietary group to determine any significance between dietary groups for each parameter. Pairwise comparisons of means were determined using a Tukey test at the $P < 0.05$ (two-tailed) rejection level : this also generated the critical value (CV) for the comparisons between means.

Results

Following 30 weeks of supplementation with the experimental diets, values for plasma cholesterol (mean ± SEM for the indicated number of animals in each dietary group) were : control, 4.28 ± 0.19 mmol/L, n = 8; EPA, 4.14 ± 0.32, n = 8; ATH, 16.38 ± 1.40, n = 10; ATH + EPA, 6.64 ± 0.85, n = 10, as previously reported.[17,19]

Analyses of cardiac membrane phospholipid class distribution, the fatty acid composition of the total phospholipids, and the individual phospholipid classes were done using the P0-500*g* membrane fraction from marmoset heart. This low-speed membrane fraction has previously been shown to exhibit the greatest catecholamine-stimulated adenylate cyclase activity[20] and provides sufficient material for analyses of membrane phospholipid class distribution and fatty acid composition of most individual phospholipid classes, as well as adenylate cyclase activity, eliminating the need to pool tissues from more than one marmoset. The major phospholipid class of the marmoset cardiac membrane was phosphatidylcholine with lesser amounts of phosphatidylethanolamine and cardiolipin (*Table 3*). However, samples had to be pooled as indicated in *Table 3* to accurately measure the proportion of the combined sphingomyelin and lysophosphatidylcholine classes. Insufficient amounts of phosphatidylserine and phosphatidylinositol precluded their quantification and determination of respective fatty acid profiles in unpooled cardiac membrane lipid extracts from individual marmosets. Despite some small but significant differences in the proportion of phosphatidylcholine between some of the diet groups, no other differences were evident in the proportions of the other cardiac membrane phospholipids as a result of the various dietary lipid treatments.

The proportion of 20:5(n-3) in the total phospholipid fraction of cardiac membranes was significantly increased by dietary 20:5(n-3) with greater incorporation occurring when dietary 20:5(n-3) was combined with the ATH diet, which had a low 18:2(n-6) content (*Table 4*). The proportions of 22:5(n-3), which is the elongation product of 20:5(n-3), were also increased in the EPA (and ATH + EPA) dietary groups but this was not the situation for the delta-4 desaturase product, 22:6(n-3) which showed a slightly lower relative proportion in the two 20:5(n-3)-diet groups. The increase in the proportion of the (n-3) PUFAs was accompanied by a decrease in 18:2(n-6) and to a lesser extent 20:4(n-6). The net effect of these changes in individual fatty acids was a reduction in the n-6:n-3 PUFA ratio that was evident for the two atherogenic type diets (ATH and ATH + EPA) when compared to the control and EPA diets, respectively, and a further reduction in the n-6:n-3 ratio with the dietary EPA groups.

The fatty acid compositions of the phosphatidylcholine (PC), phosphatidylethanolamine (PE), cardiolipin (diphosphatidylglycerol [CLP]) and sphingomyelin (SPM) fractions of heart membranes from marmosets

Table 3 The effect of dietary lipid supplementation on the distribution of the major phospholipid classes in marmoset cardiac membranes

Major phospholipid (%)	Control (n = 8)	EPA (n = 7)	ATH (n = 7)	ATH + EPA (n = 8)	CV
PC	52.6 ± 0.6^{a}	54.9 ± 0.6^{ab}	55.4 ± 0.6^{b}	53.3 ± 0.5^{ab}	2.30
PE	25.5 ± 1.0^{a}	24.9 ± 0.8^{a}	24.5 ± 0.6^{a}	25.8 ± 0.5^{a}	2.93
DPG	19.2 ± 0.5^{a}	18.1 ± 0.5^{a}	17.8 ± 0.5^{a}	18.4 ± 0.6^{a}	1.89
SMP + LPC*	2.8 ± 0.1^{a}	3.1 ± 0.6^{a}	2.4 ± 0.1^{a}	2.4 ± 0.3^{a}	1.96

Results are the percent distribution (mean ± SEM) of the major phospholipid classes of the total phospholipids of the P0-500*g* membrane fraction for the number of animals in each dietary group (n).
PC, phosphatidylcholine; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol (cardiolipin); SPM, sphingomyelin; LPC, lysophosphatidylcholine.
* Values for SPM + LPC were determined from two pooled samples per diet group each representing four individual animals.
Analysis of variance and the Tukey test for pairwise comparisons of means are described in the methods.
Values without a common superscript are significantly different at $P < 0.05$ on the basis of differences in the critical values (CV) determined by the Tukey test.

Table 4 The effect of dietary lipid supplementation on the fatty acid composition of marmoset cardiac membrane total phospholipids

Major fatty acid (%; wt/wt)	Control (n = 8)	EPA (n = 8)	ATH (n = 9)	ATH + EPA (n = 10)	CV
Sat.					
16:0	8.9 ± 0.2[a]	10.6 ± 0.3[b]	11.0 ± 0.3[b]	9.3 ± 0.3[a]	1.05
18:0 DMA	2.8 ± 0.2[a]	3.8 ± 0.4[a]	2.6 ± 0.3[a]	5.8 ± 0.2[b]	1.12
18:0	21.8 ± 0.4[a]	21.2 ± 0.6[a]	22.3 ± 0.8[a]	19.5 ± 0.4[b]	2.81
Mono.					
18:1 (n-9)	7.3 ± 0.1[a]	6.1 ± 0.1[b]	10.5 ± 0.2[c]	8.0 ± 0.2[d]	0.67
n-6					
18:2	33.4 ± 0.3[a]	24.0 ± 0.4[b]	21.3 ± 0.4[c]	13.5 ± 0.3[d]	1.30
18:3	1.0 ± 0.01	n.d.	n.d.	n.d.	—
20:4	12.9 ± 0.3[a]	10.1 ± 0.2[b]	15.8 ± 0.3[c]	11.9 ± 0.3[a]	1.04
n-3					
20:5	0.7 ± 0.02[a]	9.9 ± 0.3[b]	0.8 ± 0.05[a]	14.5 ± 0.7[c]	1.55
22:5	2.6 ± 0.2[a]	6.8 ± 0.1[b]	3.8 ± 0.2[c]	8.1 ± 0.3[d]	0.87
22:6	2.9 ± 0.2[ac]	2.4 ± 0.1[a]	4.5 ± 0.2[b]	3.4 ± 0.3[c]	0.76
Σ Sat.	35.3 ± 0.3[a]	37.4 ± 0.5[b]	37.7 ± 0.8[b]	36.7 ± 0.4[ab]	1.85
Σ Poly.	55.1 ± 0.3[a]	53.7 ± 0.5[ac]	48.9 ± 0.8[b]	52.2 ± 0.5[c]	2.03
Σ Mono.	9.6 ± 0.1[a]	8.9 ± 0.1[a]	13.0 ± 0.2[b]	11.2 ± 0.2[c]	0.74
Σ (n-6)	48.8 ± 0.3[a]	34.6 ± 0.4[b]	39.4 ± 0.7[c]	25.8 ± 0.6[d]	1.97
Σ (n-3)	6.4 ± 0.5[a]	19.2 ± 0.3[b]	9.4 ± 0.3[c]	26.3 ± 0.7[d]	1.80
Σ (n-6)/(n-3)	7.6	1.8	4.2	1.0	—
U.I.	171	195	175	219	—

Fatty acids are designated as described in *Table 2*. Data are presented as the mean ± SEM for the indicated number of animals (n) in each dietary group. Only those fatty acids present at > 0.5% of the total (wt/wt) are reported.
DMA, dimethylacetal derivative; Sat., saturated fatty acids; Mono., monounsaturated fatty acids; Poly., polyunsaturated fatty acids.
Statistical analysis of data is described in *Table 3*. Statistical differences were not determined for the computational parameters Σ (n-6)/Σ (n-3) and U.I. (unsaturation index).

fed the four experimental diets are shown in *Tables 5–8*. Each phospholipid class was distinguishable by its individual array of fatty acid types. For example, when the phospholipid classes were compared within just the control dietary group, PC was characterized by having the highest proportion of 16:0; PE the highest 18:0, 20:4(n-6), 22:5(n-3), and docosahexaenoic (22:6(n-3)) acids; CLP the highest 18:2(n-6); while SPM was characterized by having high proportions of the saturated fatty acids, 20:0, 22:0, 24:0), and the monounsaturated 24:1(n-9). For marmosets fed the 20:5(n-3)-containing diets, the proportion of 20:5(n-3) increased in PC, PE, and SPM. No 20:5(n-3) could be detected in the CLP fraction isolated from any of the dietary lipid groups. The incorporation of 20:5(n-3) into PC and PE was greater for the ATH+EPA dietary animals in comparison with animals on the EPA diet. 22:5(n-3) also increased along with 20:5(n-3), however the proportion of 22:6(n-3) was not influenced by dietary 20:5(n-3). An increase in the proportion of saturated fatty acids on feeding the atherogenic diets (with or without added 20:5(n-3)) was particularly evident in the PE fraction; however, a change in the value of the unsaturation index in this phospholipid class was only evident when comparing the control and ATH diets not containing 20:5(n-3). The PC and PE fractions were by far the most responsive to 20:5(n-3) treatment, with PC showing the greatest fold increase in n-3 PUFA content and PE showing the greatest net increase in the percentage of n-3 PUFAs present.

Significant stimulation of basal adenylate cyclase activity for all diet groups was evident with isoproterenol, epinephrine, norepinephrine, NaF, and forskolin. Catecholamine-stimulated adenylate cyclase activity in the presence of the nonhydrolysable GTP analogue GMP-PNP was also greatly elevated over basal activity. Basal- and agonist-stimulated adenylate cyclase activity was not significantly affected by the nature of the dietary lipid supplement, and the specific activity under the various assay conditions employed were very similar for all four diet groups (data not shown). *Figure 1* shows the dose-response curves (plotted as specific activity) for isoproterenol stimulation of adenylate cyclase activity in cardiac membrane preparations from marmosets fed the different diet supplements. Adenylate cyclase dose response curves obtained from assays on the indicated number of animals per dietary group were virtually superimposable and there was no significant difference in the ED_{50} values, which remained between 1.26 and 2.35 × 10^{-6}M isoproterenol for all diet groups.

Discussion

The dietary supplements resulted in substantial alterations in the total phospholipid fatty acid composition as well as the fatty acid composition of the major phospholipid classes of cardiac membranes of the marmoset monkey, but did not change the proportion of the major membrane phospholipid classes nor the cholesterol:phospholipid ratio of cardiac membranes.[17]

Table 5 Effect of dietary lipid supplementation on the fatty acid composition of marmoset cardiac membrane phosphatidylcholine

Major fatty acid (%; wt/wt)	Control (n = 6)	EPA (n = 7)	ATH (n = 7)	ATH + EPA (n = 6)	CV
Sat.					
16:0	19.0 ± 1.1[a]	23.2 ± 0.9[b]	22.9 ± 0.5[b]	22.8 ± 0.6[b]	3.06
18:0 DMA	0.5 ± 0.1[a]	1.0 ± 0.2[a]	0.8 ± 0.1[a]	2.6 ± 0.2[b]	0.57
18:0	21.9 ± 0.9[a]	18.0 ± 0.6[b]	18.8 ± 0.5[b]	18.6 ± 0.9[b]	2.69
Mono.					
18:1(n-9)	10.4 ± 0.4[a]	8.8 ± 0.3[a]	18.5 ± 0.5[b]	14.5 ± 0.6[c]	1.68
n-618:2	33.9 ± 0.7[a]	20.8 ± 0.5[b]	17.4 ± 0.5[c]	6.5 ± 0.4[d]	2.02
20:4	4.7 ± 0.5[a]	6.4 ± 0.2[b]	7.6 ± 0.3[bc]	8.7 ± 0.4[cd]	1.38
n-3					
20:5	0.3 ± 0.2[a]	9.0 ± 0.5[b]	0.5 ± 0.04[a]	11.4 ± 1.0[c]	2.10
22:5	1.2 ± 0.1[a]	4.2 ± 0.3[b]	1.9 ± 0.2[a]	4.7 ± 1.9[b]	0.91
22:6	1.0 ± 0.1[a]	1.2 ± 0.1[ab]	1.7 ± 0.2[b]	1.8 ± 0.2[b]	0.58
Σ Sat.	43.4 ± 1.7[a]	44.6 ± 2.6[ab]	45.4 ± 0.6[ab]	47.6 ± 1.1[b]	4.16
Σ Poly.	42.7 ± 1.1[a]	42.3 ± 0.7[a]	31.6 ± 1.0[b]	33.5 ± 1.4[b]	3.96
Σ Mono.	13.9 ± 0.7[a]	13.1 ± 0.4[a]	23.1 ± 0.5[b]	18.6 ± 0.7[c]	2.18
Σ (n-6)	40.1 ± 1.0[a]	27.7 ± 0.4[b]	26.9 ± 0.7[b]	15.5 ± 0.5[c]	2.60
Σ (n-3)	2.6 ± 0.3[a]	14.6 ± 0.9[b]	4.5 ± 0.4[a]	18.0 ± 1.4[c]	3.18
Σ (n-6)/(n-3)	15.2	1.9	6.0	0.9	—
U.I.	121	153	118	159	—

Data are as described in Tables 2 and 4.
Statistical analysis is as described in Tables 3 and 4.

Table 6 Effect of dietary lipid supplementation on the fatty acid composition of marmoset cardiac membrane phosphatidylethanolamine

Major fatty acid (%; wt/wt)	Control (n = 5)	EPA (n = 5)	ATH (n = 7)	ATH + EPA (n = 4)	CV
Sat.					
16:0	2.5 ± 0.3[a]	2.4 ± 0.3[a]	3.2 ± 0.3[a]	2.5 ± 0.3[a]	—
18:0 DMA	3.0 ± 0.6[a]	4.6 ± 0.5[b]	3.2 ± 0.3[ab]	3.2 ± 0.5[ab]	1.56
18:0	35.2 ± 1.6[a]	34.5 ± 1.4[a]	41.6 ± 1.7[b]	40.1 ± 2.6[ab]	6.31
Mono.					
18:1(n-9)	5.9 ± 0.5[a]	3.9 ± 0.2[b]	7.2 ± 0.4[a]	4.4 ± 0.4[b]	1.50
n-6					
18:2	12.6 ± 1.3[a]	7.6 ± 0.4[b]	6.5 ± 0.5[b]	3.1 ± 0.3[c]	2.61
20:4	23.9 ± 1.2[a]	18.1 ± 0.6[c]	22.3 ± 1.1[ab]	19.6 ± 1.1[bc]	3.66
n-3					
20:5	0.3 ± 0.04[a]	10.9 ± 0.5[b]	0.7 ± 0.1[a]	12.3 ± 1.5[b]	2.08
22:5	3.7 ± 0.5[a]	9.1 ± 0.5[b]	3.8 ± 0.3[a]	8.1 ± 0.6[b]	1.49
22:6	4.4 ± 0.5[a]	3.5 ± 0.4[a]	4.5 ± 0.5[a]	4.0 ± 0.3[a]	—
Σ Sat.	43.0 ± 1.4[a]	43.8 ± 1.3[a]	50.8 ± 1.8[b]	47.7 ± 3.0[ab]	4.02
Σ Poly.	48.5 ± 1.3[a]	49.7 ± 1.1[a]	39.9 ± 2.0[b]	47.2 ± 3.3[a]	7.00
Σ Mono.	8.6 ± 0.4[a]	6.5 ± 0.3[b]	9.1 ± 0.3[a]	5.1 ± 0.4[c]	1.23
Σ (n-6)	40.1 ± 0.6[a]	26.1 ± 0.5[b]	30.7 ± 1.3[c]	22.7 ± 1.1[c]	3.51
Σ (n-3)	8.4 ± 1.0[a]	23.6 ± 1.1[b]	9.2 ± 0.8[a]	24.4 ± 2.4[b]	4.31
Σ (n-6)/Σ (n-3)	4.8	1.1	3.3	0.9	—
U.I.	191	217	141	215	—

Data are as described in Tables 2 and 4.
Statistical analysis of data is as described in Tables 3 and 4.

While cardiac β-adrenergic receptor affinity and number have also been shown to be altered in the marmoset following diet-induced changes in membrane phospholipid fatty acid composition,[11,12,17] in the present study changes in membrane fatty acid composition did not influence the sensitivity or responsiveness of adenylate cyclase to catecholamines or to other stimulators of adenylate cyclase activity such as sodium fluoride and forskolin. This, together with other data we have recently reported in which either cardiac membrane acyl fatty acid composition and/or membrane cholesterol status were altered by dietary means and the effect of adenylate cyclase activity examined,[11,12] suggests that while cardiac membrane (catecholamine-stimulated) adenylate cyclase is sensitive to changes in the membrane cholesterol:phospholipid ratio, it is relatively insensitive to diet-induced changes in the membrane phospholipid fatty acid composition.

Table 7 Effect of dietary lipid supplementation on the fatty acid composition of marmoset cardiac membrane cardiolipin

Major fatty acid (%; wt/wt)	Control (n = 6)	EPA (n = 6)	ATH (n = 7)	ATH + EPA (n = 6)	CV
Sat.					
16:0	2.4 ± 0.3[a]	2.5 ± 0.2[a]	2.7 ± 0.1[a]	2.8 ± 0.2[a]	0.75
18:0	3.4 ± 0.3[a]	3.4 ± 0.4[a]	3.3 ± 0.1[a]	3.8 ± 0.2[a]	0.92
Mono.					
18:1(n-7)	6.9 ± 0.3[a]	9.0 ± 0.4[b]	9.4 ± 0.4[b]	11.2 ± 0.5[c]	1.59
18:1(n-9)	6.1 ± 0.6[a]	5.8 ± 0.5[a]	8.4 ± 0.5[b]	11.5 ± 0.8[c]	2.28
20:1(n-15)	0.9 ± 0.1[a]	1.3 ± 0.3[ab]	1.5 ± 0.1[ab]	1.6 ± 0.2[b]	0.66
20:1(n-9)	0.3 ± 0.1	tr.	0.6 ± 0.2	tr.	—
n-6					
18:2	75.6 ± 1.1[a]	72.3 ± 1.1[a]	68.3 ± 0.8[b]	62.5 ± 1.1[c]	3.72
20:2	1.1 ± 0.1[a]	1.0 ± 0.1[a]	0.4 ± 0.05[b]	0.5 ± 0.04[b]	0.27
20:4	2.2 ± 0.2[a]	1.7 ± 0.1[b]	2.2 ± 0.1[ac]	1.7 ± 0.2[bc]	0.47
n-3					
18:3	0.2 ± 0.01[a]	0.2 ± 0.02[a]	1.2 ± 0.3[b]	1.1 ± 0.1[ab]	0.89
22:5	0.5 ± 0.05[a]	1.4 ± 0.2[b]	0.6 ± 0.04[a]	1.1 ± 0.05[b]	0.38
22:6	0.4 ± 0.05[ab]	0.4 ± 0.05[ab]	0.5 ± 0.04[a]	0.4 ± 0.04[b]	0.16
Σ Sat.	6.0 ± 0.6[a]	6.3 ± 0.6[a]	6.3 ± 0.3[a]	7.0 ± 0.4[a]	1.72
Σ Poly.	80.2 ± 1.3[a]	78.0 ± 1.2[a]	73.7 ± 0.6[b]	68.1 ± 1.4[c]	4.21
Σ Mono.	13.8 ± 0.7[a]	15.7 ± 0.7[a]	19.9 ± 0.5[b]	25.0 ± 1.1[c]	2.83
Σ (n-6)	79.2 ± 1.2[a]	75.0 ± 1.0[b]	71.6 ± 0.7[b]	64.9 ± 1.2[c]	3.79
Σ (n-3)	1.0 ± 0.1[a]	3.0 ± 0.3[b]	2.3 ± 0.3[b]	3.1 ± 0.4[b]	1.07
Σ (n-6)/Σ (n-3)	79.2	25.1	30.7	20.9	—
U.I.	182	184	173	173	—

Data are as described in Tables 2 and 4.
Statistical analysis of data is as described in Tables 3 and 4.

Table 8 Effect of dietary lipid supplementation on the fatty acid composition of marmoset cardiac membrane sphingomyelin

Major fatty acid (%; wt/wt)	Control (n = 5)	EPA (n = 4)	ATH (n = 7)	ATH + EPA (n = 5)	CV
Sat.					
16:0	15.6 ± 0.6[ac]	14.6 ± 0.5[ab]	18.0 ± 0.5[cd]	20.3 ± 1.2[d]	2.62
18:0	20.8 ± 1.0[a]	20.8 ± 0.5[a]	26.3 ± 1.1[b]	25.0 ± 1.0[b]	3.49
20:0	9.9 ± 2.0[a]	8.6 ± 1.9[a]	8.9 ± 1.4[a]	7.3 ± 0.6[a]	5.38
22:0	18.2 ± 2.1[a]	22.4 ± 1.4[a]	12.3 ± 0.7[b]	12.7 ± 0.7[b]	4.35
24:0	9.4 ± 0.8[ac]	13.0 ± 0.5[b]	8.3 ± 0.4[a]	10.9 ± 0.8[bc]	2.15
Mono.					
18:1(n-9)	5.9 ± 0.9[ab]	2.6 ± 0.3[a]	8.5 ± 1.5[b]	6.8 ± 1.2[ab]	4.43
24:1(n-9)	2.4 ± 0.3[a]	4.0 ± 0.6[a]	6.6 ± 0.5[b]	7.6 ± 0.5[b]	1.95
n-6					
18:2	15.7 ± 4.9[a]	6.7 ± 0.9[b]	7.6 ± 0.9[b]	7.0 ± 2.7[b]	6.30
20:4	2.7 ± 0.3[a]	2.0 ± 0.2[a]	4.7 ± 0.7[b]	2.2 ± 0.1[a]	1.91
n-3					
20:5(n-3)	n.d.	2.0 ± 0.7	n.d.	2.7 ± 0.6	—
22:5(n-3)	n.d.	0.8 ± 0.5	n.d.	n.d.	—
Σ Sat.	73.8 ± 2.7[a]	79.2 ± 3.4[a]	73.7 ± 2.4[a]	76.2 ± 3.3[a]	10.04
Σ Poly.	18.3 ± 2.4[a]	11.5 ± 1.6[ab]	12.3 ± 1.3[ab]	11.9 ± 1.5[b]	7.62
Σ Mono.	7.8 ± 0.8[a]	9.4 ± 2.8[ab]	14.1 ± 2.0[ab]	14.4 ± 1.3[b]	6.45
Σ (n-9)	7.8 ± 0.8[a]	9.4 ± 2.8[ab]	14.1 ± 2.0[ab]	14.4 ± 1.3[b]	6.45
Σ (n-6)	18.4 ± 2.4[a]	8.8 ± 0.8[b]	12.3 ± 1.3[ab]	8.3 ± 3.0[b]	7.11
Σ (n-3)	0	2.8 ± 1.1	0	1.1 ± 0.7	—
Σ (n-6)/Σ (n-3)	—	3.14	—	7.54	—
U.I.	48	38	48	38	—

Data are as described in Tables 2 and 4.
Statistical analysis of data is as described in Tables 3 and 4.

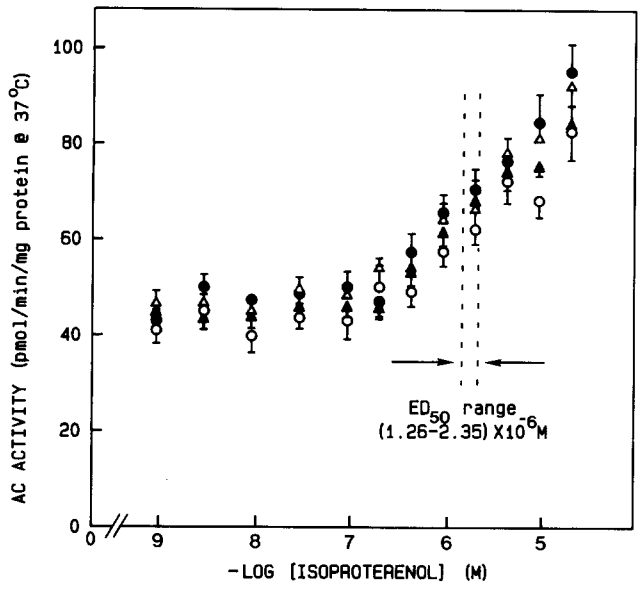

Figure 1 Isoproterenol dose response curves for cardiac adenylate cyclase activity for marmosets fed the control diet (○) n = 8; EPA diet (●) n = 8; ATH diet (△) n = 10; and the ATH + EPA diet (▲) n = 9. Data are shown as the mean value (± SEM) from the above number of animals in each dietary group. Values were not significantly different between dietary groups for the concentrations of isoproterenol tested.

This is in contrast to the results reported by others for rat heart in which adenylate cyclase activity was influenced by dietary lipid-induced changes in membrane fatty acid composition.[8,9,13] In the above studies a combination of dietary strategies including n-6 and n-3 PUFA supplementation, the feeding of coconut oil, and the effects of an essential fatty acid-deficient state were examined in relation to cardiac membrane lipid composition and the cardiac β-AR/AC system. Changes in the polyenoic:monoenoic fatty acid ratio,[8] the 20:3 (n-9): 20:4 (n-6) ratio,[9] and the n-3:n-6 PUFA ratio[13] were noted in these studies and various effects on the β-AR/AC system were reported. It is of note, however, that for one of the above mentioned studies[13] little change in membrane physical properties, as determined by fluorescence polarization techniques, accompanied these changes in adenylate cyclase activity.

Dietary administration of n-3 PUFA in various forms has been shown to alter cardiac membrane phospholipid fatty acid composition in rodents[25-28] and in the marmoset monkey.[17] Changes of the above type were also very evident in the present study, particularly for PC and PE. The incorporation of 20:5(n-3) into these phospholipid fractions, as well as the total cardiac phospholipids, was far greater when 20:5(n-3) was combined with an atherogenic-type diet, i.e., when the level of dietary 18:2(n-6) was reduced. This relationship has also been observed by us for erythrocyte membrane phospholipids of the marmoset following supplementation with these same diets.[19]

The increased incorporation of 20:5(n-3) was associated with a decrease in the proportion of 18:2(n-6) particularly in the PC fraction, and to a lesser extent 18:1(n-9). Where a decrease in 20:4(n-6) was apparent the change was relatively small and primarily associated with the PE fraction. There was little evidence of a direct substitution of 20:4(n-6) for 20:5(n-3) from the net changes in the relative fatty acid proportions. Therefore it is likely that in the marmoset 18:2(n-6) and 20:5(n-3) are competing for similar positions on various phospholipid molecules, which is in agreement with studies on other tissues in human and nonhuman primates.[29,30] Although incorporation of 20:5(n-3) into tissue phospholipids was also enhanced in the rat when dietary 18:2(n-6) was reduced,[31] it appears that for the rat an inverse relationship exists between the levels of 20:5(n-3) and 20:4(n-6) incorporation,[6] whereas in the present study this inverse relationship was apparent for 20:5(n-3) and 18:2(n-6). Although the proportions of 22:5(n-3) were elevated, particularly in the phosphatidylcholine fraction, there was little evidence that dietary 20:5(n-3) elevated 22:6(n-3) levels in any of the membrane phospholipids examined, implying that delta-4 desaturase activity may be limiting in the marmoset.

For the particular cardiac membrane fraction used in this study, PC and PE accounted for over 75% of the total phospholipids and it was in these two phospholipids that the predominant changes in acyl fatty acid composition were observed. The changes in the proportions of the various fatty acids discussed above led primarily to large changes in the n-6:n-3 ratio of PUFAs. While changes in the proportion of saturated fatty acids as a result of feeding the two atherogenic-type diets (ATH and ATH + EPA) were only apparent for sphingomyelin, they were relatively small in magnitude. The n-6:n-3 PUFA ratio did not undergo large shifts in the cardiolipin fraction and no incorporation of eicosapentaenoic acid was evident in this fraction. This may relate to the almost exclusive localization of cardiolipin to the inner membrane of the mitochondrion, particularly in heart tissue[32]; its presence in this membrane preparation representing mitochondrial contamination.

The possibility that membrane lipid dynamics and organization may be altered by a diet-induced shift in the n-6:n-3 PUFA ratio has recently been addressed by measuring lipid motional properties by fluorescence anisotropy and lifetime of the probes diphenylhexatriene and anthroylstearate.[33] For rats fed olive or fish oil, which resulted in marked changes in the proportions of membrane n-3 fatty acids, no differences were detected between the motional characteristics of intact liver microsomes or of liposomes prepared from extracted total lipids or phospholipids of liver microsomal membranes, as a result of the dietary treatment. Conroy et al.[33] conclude that changes in membrane n-3 PUFA content do not influence average or "bulk" lipid order. It is therefore clear that a change in the n-6:n-3 PUFA ratio that was the major alteration in membrane fatty acid profile observed in this study may be insufficient to alter the activity of cardiac membrane-associated adenylate cyclase activ-

ity, possibly because membrane lipid fluidity was not significantly altered.

In the context of the possible involvement of the β-AR/AC system in the generation of cardiac arrhythmias and the relationship between the nature of the dietary lipid intake and the reported susceptibility of the heart to the development of arrhythmia(s),[15,16] it appears unlikely that dietary lipids manifest their effects on arrhythmias by modulating membrane-associated catecholamine-stimulated adenylate cyclase activity. Such a conclusion can also be inferred from the findings of Reibel et al.,[34] in which the effects of dietary n-6 fatty acids (corn oil supplementation) and n-3 fatty acids (menhaden oil) on cardiac function and responsiveness to α- and β-adrenergic receptor agonists were examined in isolated perfused rat hearts. They report that cardiac inotropic responsiveness to isoproterenol (β-agonist) was not altered by menhaden oil feeding, however the fish oil diet reduced cardiac inotropic responsiveness to phenylephrine (an α-agonist) in rat hearts where membrane phospholipid fatty acid composition (particularly the n-6:n-3 PUFA ratio) was altered.[34]

While there may be a number of mechanisms whereby n-3 PUFAs affect cardiovascular function including signalling systems mediated by the eicosanoids,[14] based on the results of this and our other studies it remains unlikely that the antiarrhythmic properties of fish oils are mediated by their effects on the beta-adrenergic receptor/catecholamine-stimulated adenylate cyclase system of the mammalian heart.

Abbreviations

EPA	eicosapentaenoic acid
P:M:S	polyunsaturated:monounsaturated:saturated fatty acid
EGTA	ethylene-bis (β-aminoethylether)-N,N'-tetraacetic acid
ICYP	(−) iodocyanopindolol
β-AR/AC	beta-adrenergic receptor/adenylate cyclase
PUFA	polyunsaturated fatty acid

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