Reversible alterations in fatty acid composition of heart muscle membrane phospholipids induced by epinephrine in rats fed different fats

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Abstract The effect of epinephrine on the fatty acid composition of heart muscle phospholipids was examined in rats fed diets containing 10% by weight of butter, corn oil, or cod liver oil. Repeated administration of epinephrine caused elevation of docosahexaenoic acid in phosphatidylcholine and phosphatidylethanolamine and a corresponding decrease in linoleic acid content. Arachidonic acid was increased in phosphatidylcholine and decreased or unaltered in phosphatidylethanolamine. These alterations were qualitatively similar despite different initial levels of fatty acids due to different dietary fats. The initial level of arachidonic acid in phosphatidylcholine and phosphatidylethanolamine was more than 50% lower in the rats fed cod liver oil than in rats fed butter and was partially replaced by the (n-3) fatty acids docosahexaenoic and eicosapentaenoic acid. Dietary corn oil produced less changes in fatty acid composition than cod liver oil compared to the reference diet, 10% butter. The results demonstrate that repeated administration of epinephrine caused significant alterations in fatty acid composition of major phospholipids in heart muscle of rats fed diets enriched with either butter, corn oil, or cod liver oil. —**Benediktsdottir, V. E., and S. Gudbjarnason.** Reversible alterations in fatty acid composition of heart muscle membrane phospholipids induced by epinephrine in rats fed different fats. *J. Lipid Res.* 1988. **29:** 765–772.

Supplementary key words stress • n-3 fatty acids • arachidonic acid • dietary fat • rat heart • phospholipids

Membranes in the heart muscle are dynamic structures under constant strain and their composition is thought to play an important role in their function. Both diet and stress can change the fatty acid composition of the glycerolipids in the heart muscle (1–3).

Diets containing different fats modify the level of polyunsaturated fatty acids in phospholipids and reflect different availability of competing substrates in the biosynthesis of phospholipids. A diet high in linoleic acid leads to an increase in the arachidonic acid (20:4(n-6)) level of heart muscle phospholipids, but a fish fat diet containing a high level of (n-3)-polyunsaturated fatty acids reduces the arachidonic acid level and increases docosahexaenoic acid (22:6(n-3), DHA) and eicosapentaenoic acid (20:5(n-3), EPA) in the phospholipids of heart muscle membranes (4).

Modification of phospholipid composition in the heart muscle can affect various functions of the membranes by affecting membrane-bound enzymes and receptors directly or indirectly by change in fluidity or permeability of the membranes (5–7). In humans, modification of platelet and white cell phospholipids with a fish diet has been shown to affect cyclooxygenase and lipoxygenase products of arachidonic acid (8, 9).

Stress induced by exogenous norepinephrine markedly alters the fatty acid composition of heart muscle phospholipids. Repeated administration of norepinephrine results in a significant increase in docosahexaenoic acid in both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and a corresponding decrease in linoleic acid, whereas 20:4(n-6) is increased in PC but is decreased in PE (10). Upon cessation of stress the lipid composition returns to normal within 1 week (3). The reasons for these alterations in fatty acid composition of cardiac phospholipids in response to norepinephrine stress are not known. These changes are specific for individual phospholipid classes and are not simply a consequence of alterations in availability of fatty acids. Arachidonic acid was thus increased in PC but decreased in PE during adaptation to the catecholamine.

Stress is considered a risk factor in the development of cardiovascular diseases but the nature of such a relationship is poorly understood. The role of dietary fat in development and prevention of cardiovascular diseases is now being explored with increasing interest following studies suggesting that consumption of fish, fish oils, or (n-3)-fatty acids from other sources might reduce the incidence of cardiovascular diseases (11, 12).

This study examines the effect of repeated epinephrine administration upon the fatty acid composition of phos-

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

pholipids in heart muscle of rats fed either cod liver oil, corn oil, or butter. The purpose was to investigate how membrane phospholipids in heart muscle of rats fed different dietary fat respond to chronic stressful conditions.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats aged 2 months were divided into three groups and fed diets containing either 10% butter fat, 10% corn oil, or 10% cod liver oil. Ninety percent of the diets was a standard diet (rat and mouse maintenance diet no. 1, expanded, Scientific Diets Service, Essex). The rats fed the butter diet were used as a reference group. The three diets were isocaloric and the fatty acid composition is shown in **Table 1.** The animals were fed these diets for 4 months and then 5 rats from each group were killed by decapitation and the hearts were removed immediately and extracted for lipid analyses. The remaining 27 rats were injected subcutaneously with epinephrine daily for 15 days. The first 3 days the dose was 1 mg/kg body weight, but was increased to 2, 3, 4, and 5 mg/kg for subsequent 3-day periods. The day after the last epinephrine injection, 5 rats from each group were killed and after a 1-week recovery period 4 rats were killed. The treatment was well tolerated and there was no mortality.

Lipid extraction

One heart was used for each analysis. It was extracted with 76 ml of methanol-chloroform-water 2:1:0.8 (by vol) and homogenized with a Polytron homogenizer. After filtration a biphasic system was produced by dilution with one volume each of chloroform and 0.73% NaCl solution. The lower layer was withdrawn and concentrated under nitrogen. The antioxidant butylated hydroxytoluene was added to the extraction medium at 5 mg/100 ml. Diheptadecanoyl phosphatidylcholine was added to the extraction medium and used as internal standard to measure the recovery of phosphatidylcholine. This is a modification of the method of Bligh and Dyer (13) as described by Kates (14).

TABLE 1. Fatty acid composition of rat diets

Fatty Acid	10% Butter	10% Corn Oil	10% Cod Liver Oil
		% fatty acid of total fatty acids	
C_4-C_{10}	10.1		
12:0	4.0		
14:0	10.6		3.8
16:0	29.5	12.9	14.1
16:1	3.7	2.2	10.5
18:0	9.4	1.6	2.4
18:1	20.6	22.3	22.2
18:2	7.4	57.8	12.2
20:0 18:3			1.7
20:1			9.0
22:1			5.3
20:5(n-3)			6.9
22:5(n-3)			0.9
22:6(n-3)			7.2
Unidentified	4.7	3.2	3.8

Lipid separation

Lipids were separated by two solvent systems, each used for single development on precoated thin-layer plates (Adsorbosil-5, Applied Science Lab Inc.). Before use the plates were washed in chloroform-methanol 1:1 (v/v). The lipid extract (about 8% of the total sample) was applied as a streak 1.5 cm from the lower edge of the plate. The first solvent system consisted of chloroform-methanol-acetic acid-water 75:45:12:6 (v/v) (15). The second solvent system was petroleum ether-diethyl ether-acetic acid 80:20:1 (v/v). After brief drying with nitrogen, the plates were sprayed with 0.02% water solution of rhodamine 6-G and viewed under ultraviolet light. The lipids were identified by comparison to authentic standards.

Preparation and analysis of fatty acid methyl esters

The lipid bands from the plate were scraped into 16 × 150 mm tubes and methyl esters were prepared according to the method of Morrison and Smith (16). The methyl ester of heneicosanic acid was added to all the tubes as internal standard to quantify the phospholipid fatty acid content. The methyl esters were analyzed in a Packard model 419 gas chromatograph using SP-2330 on 100/120 chromosorb WAW (Supelco) as packing in a 180-cm column. The heater was programmed at 3°C/min from 140 to 240°C. The peaks were identified by comparison to known fatty acid methyl ester standards.

Recovery of the heptadecanoyl methyl esters from the diheptadecanoyl-PC was 75-85%. The recovery of PE was not measured, but was taken as the recovery of PC for each sample when corrections on the phospholipid fatty acid contents were made.

Cholesterol content was measured from the thin-layer plate according to the method of Veerkamp and Broekhuyse (17).

Student's *t*-test was used for statistical comparison of the results.

RESULTS

The mean initial body weights of rats were: 10% butter group, 411 ± 11 g; 10% corn oil group, 452 ± 21 g; and 10% cod liver oil group, 449 ± 19 g (mean ± SE, n = 7). The body weight difference between the groups was not significant. The rats lost weight during the epinephrine treatment and the mean body weight loss was 62 ± 2 g in the butter group, 65 ± 8 g in the corn oil group, and 66 ± 5 g in the cod liver oil group (mean ± SE, n = 7). The difference between the weights before and after treatment was significant with $P < 0.01$ in the butter group, $P < 0.05$ in the corn oil group, and $P < 0.02$ in the cod liver oil group.

The lipid content (mg/g wet weight ± SEM) of the heart muscle of rats fed butter was: cholesterol, 1.18 ± 0.1; PC fatty acid content, 7.3 ± 0.2; and PE fatty acid content, 6.0 ± 0.2. These lipid contents were not significantly different in the three diet groups and did not change with epinephrine treatment.

Table 2 shows the fatty acid composition of PC in rat heart membranes before, after 15 days of epinephrine treatment, and a week after the epinephrine treatment was stopped as described above. There were significant differences in the fatty acid profile of rats fed corn oil and cod liver oil compared to the butter-fed rats, the reference group. In the corn oil group, oleic acid, docosapentaenoic acid, and docosahexaenoic acid were lower and linoleic acid was higher than in the butter group. There were marked changes in the cod liver oil-fed group. Arachidonic acid was less than half of the level found in the butter reference group and was partially replaced by linoleic, eicosapentaenoic, and docosahexaenoic acids (Table 2). The changes in PC induced by the epinephrine treatment in the different diet groups are also shown in Table 2. These alterations were similar in all diet groups. There was a significant decrease in linoleic acid; docosahexaenoic acid was increased in all groups, whereas arachidonic acid increased significantly during epinephrine treatment only in rats fed cod liver oil.

Table 3 shows the fatty acid composition of PE in rat heart muscle at the same time points as in Table 2. In PE as in PC the corn oil- and cod liver oil fed-groups were significantly different from the butter-fed reference group before epinephrine treatment. The epinephrine treatment induced similar alterations in PE of all diet groups. In the butter-fed group a significant decrease was found in linoleic acid and arachidonic acid of PE, whereas docosahexaenoic acid was significantly increased.

In corn oil-fed rats there was a significant decrease in linoleic acid and an increase in docosahexaenoic acid, whereas the decrease in arachidonic acid was not statistically significant.

In rats fed cod liver oil the epinephrine treatment resulted in a marked decrease in linoleic acid and eicosapentaenoic acid, and an increase in docosahexaenoic acid. The arachidonic acid level of cardiac PE in these rats was less than half of that observed in butter- or corn oil-fed rats and did not change during the epinephrine treatment.

One week after cessation of the epinephrine administration the fatty acid composition of both PC and PE had returned toward normal levels for the respective diet groups.

TABLE 2. Fatty acid composition of phosphatidylcholine in the rat heart before epinephrine treatment, after 15 days of treatment, and after 1-week recovery period

Fatty Acid	Before Epinephrine Treatment			After a 15-Day Epinephrine Treatment			During Recovery, 1 Week after Epinephrine Treatment		
	10% Butter	10% Corn Oil	10% CLO	10% Butter	10% Corn Oil	10% CLO	10% Butter	10% Corn Oil	10% CLO
16:0	22.3 ± 0.2	19.3 ± 0.7	22.1 ± 1.1	20.2 ± 0.5	18.5 ± 0.7	19.6 ± 0.7	22.1 ± 0.7	20.8 ± 1.0	23.2 ± 0.5
18:0	22.7 ± 0.4	29.3 ± 0.9	25.3 ± 0.5[a,b]	29.3 ± 0.4	32.4 ± 0.9	28.4 ± 0.8[b]	27.4 ± 1.1	27.2 ± 0.7	22.2 ± 0.5[a,b]
18:1 (n-9)	9.6 ± 0.1	7.3 ± 0.1[a]	11.3 ± 0.3[a,b]	8.7 ± 0.3	6.9 ± 0.4[a]	8.2 ± 0.2[c]	10.7 ± 0.1	8.8 ± 0.5[a]	12.5 ± 0.5[a,b,d]
18:2 (n-6)	10.5 ± 0.5	13.6 ± 1.0	16.6 ± 1.1[a]	7.5 ± 0.2[c]	9.4 ± 0.5[a,c]	10.5 ± 0.6[a,c]	13.5 ± 1.1[d]	18.3 ± 0.6[a,d]	18.6 ± 0.5[a,d]
20:4 (n-6)	22.2 ± 0.7	25.0 ± 1.5	10.6 ± 0.9[a,b]	25.2 ± 1.1	26.8 ± 1.3	16.1 ± 0.7[a,b,c]	20.7 ± 1.6	21.4 ± 1.3	10.6 ± 0.2[a,b,d]
20:5 (n-3)			2.6 ± 0.3			1.6 ± 0.2			1.5 ± 0.04
22:5 (n-3)	1.7 ± 0.2	0.6 ± 0.1[a]	1.2 ± 0.1[b]	2.4 ± 0.2	0.7 ± 0.1[a]	1.5 ± 0.1[a,b]	1.5 ± 0.2	0.5 ± 0.1[a]	1.6 ± 0.1[b]
22:6 (n-3)	2.9 ± 0.2	1.9 ± 0.2	6.5 ± 0.9[b]	5.5 ± 0.2[c]	3.4 ± 0.1[a,c]	11.7 ± 1.2[a,b,c]	3.0 ± 0.1[d]	2.1 ± 0.3[d]	8.0 ± 0.7[a,b]
Others	3.1 ± 0.8	2.9 ± 0.9	3.8 ± 0.9	1.4 ± 0.1	2.0 ± 0.3	2.5 ± 0.4	1.1 ± 0.3	0.9 ± 0.3	1.9 ± 0.5

The rats were fed diets containing 10% butter, 10% corn oil, or 10% cod liver oil (CLO). The fatty acid content is expressed as % of total fatty acids ± SEM.
All differences were significant at a level of $P < 0.01$:
[a]Compared to the butter-fed group at the same time point.
[b]Between rats fed corn oil or cod liver oil at the same time point.
[c]Compared to the level before epinephrine treatment.
[d]Compared to the level after 15 days of epinephrine treatment.

TABLE 3. Fatty acid composition of phosphatidylethanolamine in the rat heart before epinephrine treatment, after 15 days of treatment, and after 1-week recovery period

Fatty Acid	Before Epinephrine Treatment			After a 15-Day Epinephrine Treatment			During Recovery, 1 Week after the 15-Day Epinephrine Treatment		
	10% Butter	10% Corn Oil	10% CLO	10% Butter	10% Corn Oil	10% CLO	10% Butter	10% Corn Oil	10% CLO
16:0	15.0 ± 0.4	12.8 ± 0.6	13.8 ± 0.7	13.8 ± 0.8	13.7 ± 0.6	14.2 ± 0.4	12.9 ± 1.0	12.0 ± 0.5	13.9 ± 0.4
18:0	30.2 ± 0.5	28.6 ± 1.1	27.5 ± 1.2	28.4 ± 1.0	28.3 ± 1.2	27.8 ± 0.6	29.6 ± 1.7	28.3 ± 1.1	27.2 ± 0.7
18:1 (n-9)	7.2 ± 0.2	8.0 ± 0.1	9.1 ± 0.4[a]	7.5 ± 0.3	8.0 ± 0.2	8.9 ± 0.2[a]	8.4 ± 0.5	8.8 ± 0.4	9.5 ± 0.5
18:2 (n-6)	5.0 ± 0.3	9.4 ± 0.6[a]	6.3 ± 0.4[b]	3.0 ± 0.1[c]	5.7 ± 0.3[a,c]	3.8 ± 0.2[a,b,c]	5.8 ± 0.1[d]	10.5 ± 0.3[a,d]	6.9 ± 0.3[b,d]
20:4 (n-6)	18.5 ± 0.2	18.2 ± 1.1	7.7 ± 0.3[a,b]	15.5 ± 0.5[c]	15.4 ± 0.7	7.3 ± 0.3[a,b]	18.2 ± 1.2	17.3 ± 0.6	8.2 ± 0.2[a,b]
20:5 (n-3)			2.1 ± 0.1			1.1 ± 0.1[c]			1.3 ± 0.05
22:5 (n-3)	3.3 ± 0.3	1.7 ± 0.1[a]	2.1 ± 0.1[a]	3.2 ± 0.2	1.7 ± 0.1[a]	1.9 ± 0.04[a]	2.7 ± 0.3	1.4 ± 0.1[a]	2.2 ± 0.1[b]
22:6 (n-3)	17.5 ± 0.8	13.7 ± 0.9	26.6 ± 1.7[a,b]	23.7 ± 1.0[c]	20.5 ± 0.8[c]	32.7 ± 1.5[a,b]	18.9 ± 0.5[d]	15.1 ± 0.9[d]	29.1 ± 1.6[a,b]
Others	3.4 ± 0.7	7.7 ± 0.6[a]	4.8 ± 1.0	4.9 ± 1.5	6.7 ± 0.3	2.5 ± 0.4[b]	3.4 ± 0.6	6.5 ± 0.9	1.7 ± 0.3[b]

The rats were fed diets containing 10% butter, 10% corn oil, or 10% cod liver oil (CLO). The fatty acid content is expressed as % of total fatty acids ± SEM. All differences were significant at a level of $P < 0.01$:
[a]Compared to the butter-fed group at the same time point.
[b]Between rats fed corn oil or cod liver oil at the same time point.
[c]Compared to the level before epinephrine treatment.
[d]Compared to the level after 15 days of epinephrine treatment.

The reversible epinephrine-induced changes in the level of polyunsaturated fatty acids in cardiac PC and PE are shown in Figs. 1, 2, and 3. **Fig. 1** shows the alterations in linoleic acid level of PC and PE in cardiac muscle after 15 days of epinephrine treatment and after a 1-week recovery period. The linoleic acid level decreased significantly in all three diet groups ($P < 0.01$) during the stress period compared to the level before the epinephrine administration. These results are qualitatively in agreement with previous findings with norepinephrine administration (3, 10).

The decrease in linoleic acid was greatest in rats that had the highest initial levels of linoleic acid. After a 1-week recovery period the linoleic acid levels had returned and increased slightly above the initial levels.

Fig. 2 shows that repeated epinephrine administration increased the arachidonic acid in PC whereas in PE the arachidonic acid remained unaltered or decreased even slightly. The increase in arachidonic acid of PC was greatest in rats with the lowest initial level, i.e., in rats fed cod liver oil. The changes in arachidonic acid of cardiac PC and PE were qualitatively similar to previous results with norepinephrine (3, 10). After a 1-week recovery period the arachidonic acid levels in both PC and PE had returned toward the normal level for the respective diet groups.

Fig. 3 shows that docosahexaenoic acid increased markedly in PC and PE during the epinephrine treatment. In PC the stress-induced increase in docosahexaenoic acid was greatest in rats with the highest initial level of this fatty acid, i.e., in rats fed cod liver oil. In PE the increase in docosahexaenoic acid was similar in all diet groups. After the 1-week recovery period the docosahexaenoic acid levels returned close to the initial levels in all three diet groups.

DISCUSSION

Adaptation to various forms of stress is essential to life. From birth and throughout life the response and adaptation to stress is important for survival. Excessive response to stimulation or impaired adaptation to stress may lead to pathological changes, and stress is considered one of the pathogenic factors in development of cardiovascular diseases.

When the central nervous system perceives a threat, it sends impulses to sympathetic nerves that release norepinephrine at target tissues, initiating rapid, localized responses. In addition, the adrenal medulla is activated causing it to secrete epinephrine and, to a lesser extent, norepinephrine into the blood.

Epinephrine raises systolic blood pressure, lowers the diastolic blood pressure, increases the heart rate, and in-

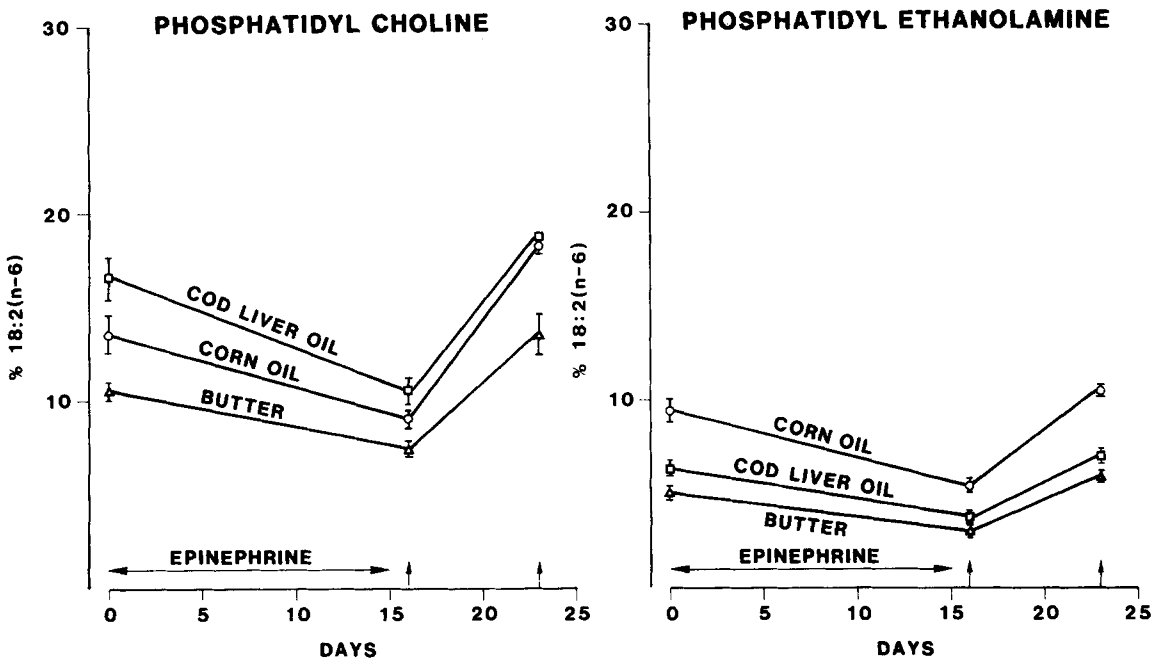

Fig. 1. Linoleic acid, 18:2(n-6), levels in phosphatidylcholine and phosphatidylethanolamine in rat heart muscle before epinephrine administration, after 15 days of treatment, and after a 1-week recovery period.

creases cardiac output, partly by increasing the force of ventricular contraction and partly by increasing the heart rate.

Norepinephrine raises both systolic and diastolic blood pressure and also increases the force of ventricular contraction, but, by slowing the heart rate, it reduces cardiac output.

The catecholamines selectively dilate or constrict blood vessels to shunt blood away from areas that are nonessential during the threat (skin, intestine, kidneys) and

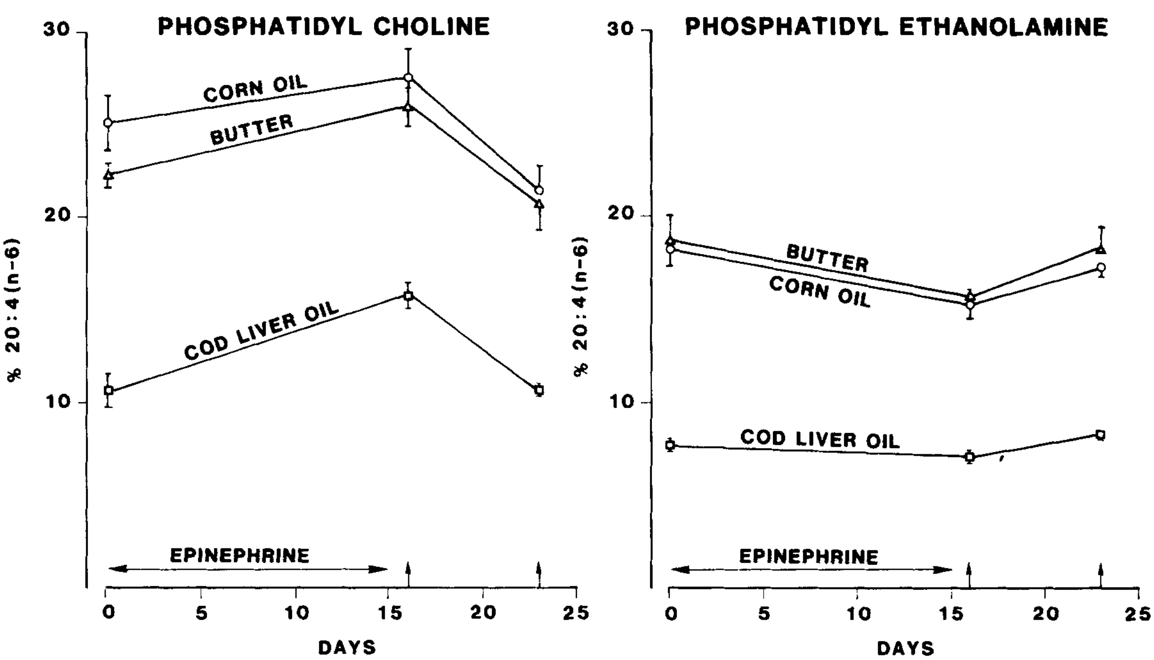

Fig. 2. Arachidonic acid, 20:4(n-6), levels in phosphatidylcholine and phosphatidylethanolamine in rat heart muscle before epinephrine administration, after 15 days of treatment, and after a 1-week recovery period.

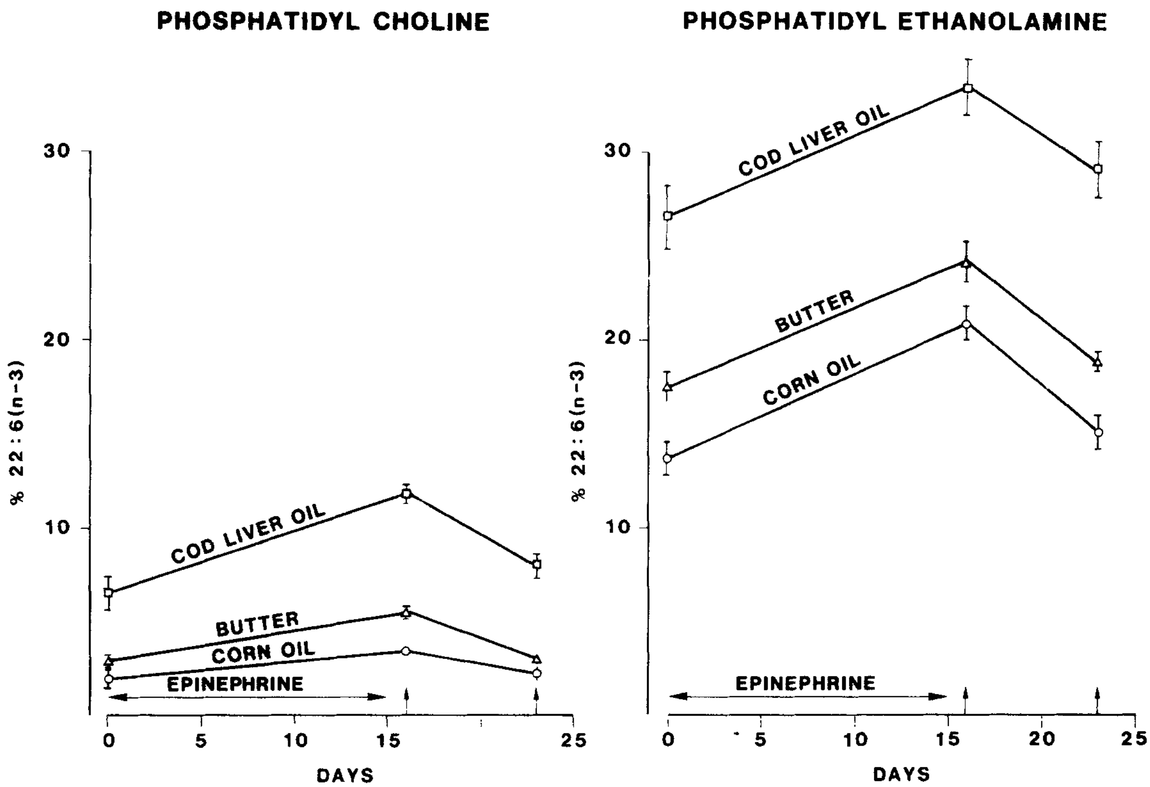

Fig. 3. Docosahexaenoic acid, 22:6(n-3), levels in phosphatidylcholine and phosphatidylethanolamine in rat heart muscle before epinephrine administration, after 15 days of treatment, and after a 1-week recovery period.

towards ones that are essential (heart, brain, skeletal muscle).

In this report we describe reversible alterations in fatty acid composition of major cardiac phospholipids during adaptation to repeated administration of epinephrine to rats fed either butter, corn oil, or fish oil. The stress caused by the epinephrine treatment was accompanied by a significant decrease in body weight, a diminution of about 15% in the three diet groups. The observed changes in fatty acid composition of heart muscle phospholipids do not resemble changes induced by starvation (3, 18), despite the decrease in body weight during the period of epinephrine administration.

The results show that the epinephrine administration induced significant changes in composition of polyunsaturated fatty acids in both PC and PE, the major phospholipids in heart muscle. These changes were similar in the three dietary groups despite large differences in initial or control levels of individual polyunsaturated fatty acids in the various groups. The changes were specific for the phospholipid class and resembled changes induced by norepinephrine (3) or neonatal stress (19). It is also noteworthy that arachidonic acid increased consistently in PC during administration of the catecholamine, whereas it decreased or remained the same in PE during the epinephrine treatment.

The repeated administration of catecholamines induced qualitatively similar changes in fatty acid composition of cardiac phospholipids in rats fed a regular, low fat diet. The stress caused by administration of norepinephrine resulted in 48% mortality and induced quantitatively greater changes in the fatty acid profile of phospholipids (3) than the more moderate stress induced by epinephrine.

The observed modification of phospholipid composition may be in response to the demands imposed upon the heart by the epinephrine administration with accompanying changes in cardiac function and metabolism. This adaptation to the epinephrine treatment requires increased deacylation and reacylation. Increased activity of phospholipase A_2, enhanced by cathecholamines (20), facilitates this remodeling of phospholipids in order to meet new requirements.

Three polyunsaturated fatty acids are of particular interest with regard to the observed adaptation to stress: linoleic acid, arachidonic acid, and docosahexaenoic acid. Linoleic acid is known primarily as a precursor to arachidonic acid but little is known about its own role in cell membranes. Arachidonic acid is derived from linoleic acid by way of desaturation and chain elongation. Arachidonic acid is an integral component of membrane phospholipids. Upon cell stimulation it is released by a calciumdependent mechanism involving phospholipase A_2 activity and is subsequently oxygenated by either cyclooxygenase or lipoxygenase pathways to the various eicosanoids. The eicosanoids are synthesized to some extent by almost every tissue and are involved in several physiological and pathophysiological mechanisms including ischemia (21).

Dietary intake of (n-3) fatty acids from fish or fish oils markedly reduces the level of arachidonic acid in membrane phospholipids where it is partially replaced by docosahexaenoic acid and eicosapentaenoic acid (Tables 2 and 3). This may influence the prostanoid production in two ways, by reducing the functional availability of arachidonic acid and by increasing the level of eicosapentaenoic acid and docosahexaenoic acid, both of which are competitive inhibitors in the conversion of arachidonic acid to prostanoids (22, 23).

The role of docosahexaenoic acid in membrane phospholipids is not known. It is present in relatively large amounts in excitable tissue such as the brain, heart, and retinal rod (24, 25). Preliminary studies suggest that replacement of arachidonic acid by docosahexaenoic acid in phospholipids of rats fed cod liver oil may reduce the incidence of left ventricular fibrillation and sudden death when these rats were subjected to a low dose of isoproterenol, 1 mg/kg (26). Further studies on the influence of dietary (n-6) and (n-3) fatty acids upon development of fatal ventricular fibrillation in rats are being conducted in this laboratory.

In conclusion it can be stated that adaptation to epinephrine stress is accompanied by significant changes in composition of polyunsaturated fatty acids in phospholipids of heart muscle. The fatty acid profile of dietary fat markedly influences the fatty acid composition of cardiac phospholipids, but the qualitative response to stress is similar regardless of dietary fat. The levels of polyunsaturated fatty acids in major cardiac phospholipids may thus differ markedly depending upon dietary fat and stress.

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